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Par

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ETUDE DU ROLE DES RECEPTEURS GABAB DANS L'ANXIETE, LA DEPRESSION ET L'ADDICTION : APPROCHE PHARMACOLOGIQUE ET GENETIQUE.

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Résumé

L'acide gamma aminobutyrique (GABA) étant le principal neurotransmetteur inhibiteur du cerveau, il joue un rôle clé dans la modulation de nombreux processus physiologiques et psychologiques. Il existe deux classes de récepteurs GABAergiques : les récepteurs GABAergiques ionotropiques, comprenant les récepteurs $GABA_A$ et $GABA_C$, et les récepteurs GABAergiques metabotropiques, incluant uniquement les récepteurs $GABA_B$. Les récepteurs $GABA_B$ sont des hétérodimères composés de deux sous-unités, la sous-unité $GABA_{B(1)}$ et la sous-unité $GABA_{B(2)}$. La présence de chacune de ces sous-unités est requise pour que le récepteur soit fonctionnel. De nombreuses études ont montré que les récepteurs $GABA_B$ étaient impliqués dans de nombreuses psychopathologies, comme l'anxiété, la dépression et l'addiction. Le but de la présente thèse a été d'examiner, chez l'animal, la contribution des récepteurs $GABA_B$ dans ces trois différentes pathologies, prenant avantage du récent développement de souris transgéniques $GABA_{B(1)}$ et $GABA_{B(2)}$ knock-out et d'un modulateur positif allostérique du récepteur $GABA_B$ (GS39783).

Dans la première partie de notre travail, nous avons montré que l'ablation du gêne codant l'une ou l'autre de ces sous-unités abolissait l'hypothermie induite par le deux $GABA_B$ agoniste (baclofen, GHB). Ces résultas confirment que l'hétérodimérisation de ces deux sous-unités est nécessaire au bon fonctionnement du récepteur $GABA_B$.

La seconde partie de notre étude avait pour but d'examiner le rôle des récepteurs $GABA_B$ dans l'anxiété. Pour cela, nous avons évalué l'impact de l'inactivation du gène codant pour l'une des deux sous-unités du récepteur $GABA_B$ dans des modèles animaux d'anxiété. Nous avons ainsi observé que les souris $GABA_{B(1)}^{-/-}$ et $GABA_{B(2)}^{-/-}$ présentaient un phénotype hyper-anxieux et étaient insensibles à l'action d'anxiolytiques tels que les benzodiazépines. Afin de confirmer la contribution de l'hétérodimère $GABA_B$ dans l'anxiété, nous avons examiné les effets du GS39783 dans ces mêmes tests. Ainsi, nous avons mis en évidence que le GS39783 possédait des propriétés anxiolytiques. Etant donné que ces modulateurs allostériques sont dépourvus des effets secondaires associés aux benzodiazépines, comme la sédation et la tolérance, ils représentent une nouvelle approche thérapeutique dans le traitement des troubles anxieux.

Dans une troisième partie, nous avons entrepris l'étude de l'implication des récepteurs $GABA_B$ dans les syndromes dépressifs. L'approche génétique, nous a montré que la suppression du gène codant pour l'une ou pour l'autre des deux sous-unités du récepteur $GABA_B$ induit un effet antidépresseur dans le test de la nage forcée, mais pas dans le test de tail suspension. Corroborant ce phénomène, nous avons mis en évidence que le blocage pharmacologique de ces récepteurs induisait aussi avoir des effets antidépresseurs dans ce test, suggérant un rôle des récepteurs $GABA_B$ dans les syndromes dépressifs. Toutefois les processus sous-tendant les propriétés antidépressives des antagonistes des récepteurs $GABA_B$ sont à ce jour encore mal connus. Supposant une possible interaction entre les systèmes sérotoninergiques et les récepteurs $GABA_B$, nous avons étudié les effets des ligands du récepteurs $GABA_B$ sur l'effet antidépresseur de la fluoxétine (un inhibiteur de la recapture de la sérotonine) puis leurs impacts sur la downrégulation des récepteurs sérotoninergiques 5-HT_{1A} dans l'hippocampe induite par le stress chronique. Nous avons observé que les antagonistes $GABA_B$ ne modifiaient pas les propriétés antidépressives de la fluoxétine dans le test de la nage forcée, mais bloquaient la downrégulation des récepteurs 5-HT_{1A} induite par un stress chronique. Ces résultats suggèrent un role clé du système sérotoninergique dans les propriétés antidépressives des antagonistes $GABA_B$. Inversement, nous avons mis en évidence que la fluoxétine administrée de manière chronique atténuait la réponse hypothermique induite par le baclofen, montrant que le système sérotoninergique participerait aussi à la modulation des fonctions physiologiques liées aux récepteurs $GABA_B$.

Etant donné que de nombreuses études précliniques et cliniques ont suggéré que les récepteurs $GABA_B$ pourraient moduler les comportements associés aux systèmes de récompenses, nous avons entrepris d'évaluer l'effet de la stimulation des récepteurs $GABA_B$ dans des modèles associés aux comportements addictifs et sur les adaptations moléculaires induites par l'administration aiguë et prolongée de psychostimulants ou de nicotine. Au niveau comportemental, nous avons montré que le GS39783 atténuait l'hyperlocomotion induite par l'administration aiguës de cocaïne, et également la sensibilisation locomotrice à la cocaïne induite par administration répétée de cocaïne. Par ailleurs, nous avons observé que le GS39783 s'opposait à l'établissement d'un conditionnement de place induite par la nicotine. Au niveau moléculaire, le GS39783 réduit l'induction de c-fos dans le noyau accumbens induite par l'administration aiguë de cocaïne et diminue l'activation du CREB et du DARPP-32 provoquée par l'administration chronique de cocaïne. De plus, le GS39783 atténue l'accumulation de ΔfosB dans le noyau accumbens et le striatum dorsal causée par l'administration de cocaïne et de nicotine. Ces observations suggèrent que la stimulation des récepteurs $GABA_B$ par un modulateur allostérique inhibe à la fois la perception des effets réenforçants des substances appétitives mais aussi les adaptations moléculaires associées à celles-ci.

Ainsi, ces études nous permettent de conclure que les récepteurs $GABA_B$ pourraient représenter une cible potentielle dans l'élaboration de nouvelles pharmacothérapies des troubles anxieux, de la dépression et des comportements addictifs.

Summary

Although there is much evidence for a role of the inhibitory neurotransmitter γ-aminobuytric acid (GABA) in the pathophysiology of neuropsychiatric disorders, the role of $GABA_B$ receptors in behavioral processes related to these disorders has not yet been fully established. Indeed, further progress in the field has been largely hampered by the lack of appropriate tools. The recent development of new pharmacological and genetic tools offers a novel opportunity to investigate the contribution of $GABA_B$ receptors in anxiety, depression and addiction. Consequently, the studies in the present thesis are focus on addressing a broad hypothesis that $GABA_B$ receptors play a key role in the manifestation of psychiatric disorders. Thus, using recently generated $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice, which lack functional $GABA_B$ receptors, and pharmacological tools; we assessed the role of $GABA_B$ receptors these three neuropsychiatric disorders.

Firstly, we demonstrated that targetted deletion of either of $GABA_B$ receptor subunit induced an exacerbate anxiety in several animal model of anxiety. Indeed, both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice were more anxious than their wildtype littermates (less time spent in the light; reduced number of transitions) in the light-dark box paradigm. Conversely, we also demonstrated that pharmacological activation, via administration of a novel $GABA_B$ receptor positive modulator GS39783 decreased anxiety in the light-dark box and elevated zero maze tests. Altogether, these data support an involvement of $GABA_B$ receptors in the modulation of anxiety-related behaviors.

Secondly, we also demonstrated that $GABA_B$ receptor might contribute to the modulation of depressive-related behavior. Thus, we showed that genetic inactivation of either of $GABA_B$ receptor subunit induced an increase in the time spent in immobility in the forced swim test, but not in the tail suspension paradigm, suggesting that targetted deletion of either of $GABA_B$ receptor subunit produce an antidepressant-like effect in mice. These behavioral effects are unrelated to alterations in locomotor activity. In confirmation of the genetic data, acute and chronic treatment with CGP56433A, a selective $GABA_B$ receptor antagonist also decreased immobility in the FST, whereas GS39783 did not alter this behavior. In effort to gain a better understanding of processes underlying the antidepressant properties of $GABA_B$ antagonist, we also explored the interaction between $GABA_B$ and serotoninergic system. Thus, we demonstrated that pharmacological blockade or genetic inactivation of serotonin transporter affect $GABA_B$ -related function, using baclofen-induced hypothermia. Conversely, we also demonstrated that acute, sub-chronic and chronic treatement with $GABA_B$ ligands affected the expression of $5\text{-}HT_{1A}$ receptor in the hippocampus. Together, these data confirm both the putative involvement of $GABA_B$ receptor in depressive disorder and the strong interaction between 5-HT and $GABA_B$ system.

Finally, the last part of the present thesis addressed the issue of the role of $GABA_B$ receptors in addiction. Indeed, we showed that activation of $GABA_B$ receptor counteract both behavioral and molecular changes associated with a single adminsitration of cocaine. More specifically, both baclofen, a $GABA_B$ receptor agonist, and GS39783 attenuate both hyperactivity and accumbal c-fos induction elicited by a single administration of cocaine. Moreover, we also demonstrated that GS39783 attenuates the acquisition of locomotor sensitization triggered by chronic cocaine administration. GS39783 also differentially attenuated the accumulation of ΔfosB in the CPu, and inhibited CREB and DARPP-32 activation and upregulation in the Nac, suggesting that $GABA_B$ activation counteracted both molecular and behavioral changes triggered by repeated administration of cocaine.Finally, in a nicotine place conditioning paradigm, we showed that repeated treatment with GS39783 inhibits the acquisition of nicotine place preference acquisition. $GABA_B$ positive modulation also inhibited the accumulation of ΔfosB in the NAc, supporting a role for this transcription factor in reinforcing properties of nicotine. Taken altogether, these results show that $GABA_B$

receptor modulation is effective in attenuating reinforcing properties of drugs of abuse and interferes with drug-induced modulation of several signaling pathways.

To conclude, our data support the role of $GABA_B$ receptor in anxiety, depression and addiction and that $GABA_B$ receptor might be considered as one of the most promising therapeuthic targets for treating these disorders.

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LIST OF PAPERS.

This thesis is based on the papers listed below:

- Kaupmann, K., Cryan, J.F.**,** Wellendorph, P. **Mombereau, C.** Sansig, G. Klebs, K., Schmutz, M., Froestl, W. van der Putten, H., Mosbacher, J., Bräuner-Osborne, H., Waldmeier, P., and Bettler, B. Specific γ-Hydroxybutyrate (GHB) binding sites but loss of pharmacological effects of GHB in GABA-B(1) receptor deficient mice ***Eur. J Neurosci.*** 18, 2722-2730, 2003.

- Gassmann, M., Barbieri, S., Sansig, G. Humeau, Y., Schuler, V., Müller, M., Klebs, K., Schmutz, M., Froestl, W., Heid, J., Kelly, P.H. Gentry, C., Jaton, A.L., Fox, A., Van der Putten, H., **Mombereau, C**., Mosbacher, J., Cryan, J.F., Lüthi, A., Kaupmann K., and Bettler, B. Functional postsynaptic $GABA_B$ receptors in the absence of the $GABA_{B(2)}$ subunit ***J. Neurosci.*** 24 6086-6097, 2004.

- **Mombereau, C.**, Kaupmann, K. Froestl, W. Sansig, G. van der Putten H. and Cryan, J.F. Genetic and pharmacological evidence of a key role for $GABA_B$ receptors in the modulation of anxiety and antidepressant-like behaviour. ***Neuropsychopharmacol.*** 29, 1050-1062, 2004

- **Mombereau C,** Kaupmann K, van der Putten H, and Cryan, JF Altered response to benzodiazepine anxiolytics in mice lacking $GABA_{B(1)}$ receptors.. ***Eur J. Pharmacol.*** *497, 119-20, 2004*

- Cryan, J.F., Kelly, P.H., Chaperon, F., Gentsch, C., Froestl, W., **Mombereau, C.**, Lingenhoehl, K, Bettler, B. Kaupmann, K. and Spooren, W.P.J.M. Behavioural characterization of the novel GABAB receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. ***J. Pharmacol. Exp. Ther.*** 310, 952-63, 2004

- **Mombereau, C.**, Kaupmann, K., Gassman, M., Bettler, B., van der Putten, H., Cryan, J.F. Altered anxiety and depression-related behaviour in mice lacking $GABA_{B(2)}$ receptor subunits ***NeuroReport*** 16:307-10, 2005.

- **Mombereau, C**., Kuntzelmann, G., Loetscher, E., J.F. Cryan. Chronic antidepressants and $GABA_B$ receptor ligands counteract handling-stress induced downregulation of 5-HT_{1A} receptor mRNA In the hippocampus of BALB/c Mice. Manuscript submitted.

- Lhuillier, L., **Mombereau, C.**, Cryan, J.F. and Kaupmann, K.$GABA_B$ receptor positive modulation decreases selective molecular and behavioural effects of acute and repeated cocaine administration. Manuscript submitted.

- **Mombereau, C.**, Lhuillier, L., Kaupmann, K. and Cryan, J.F. $GABA_B$ receptor positive modulation blocked reinforcing properties of nicotine and its associated accumbal ΔfosB accumilation. Manuscript in preparation.

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CHAPTER 1: GENERAL INTRODUCTION.

1.1 γ-AMINOBUTYRIC ACID (GABA).

1.1.1 Historical perspective.

GABA is the major inhibitory neurotransmitter in brain. Although the chemical structure of γ-aminobutyric acid (GABA) was first synthesized as far back as 1883 (see (Cooper et al., 2002), GABA was independently identified and reported to be present in the vertebrate brain only in 1950. Indeed, three different groups (Awapara et al., 1950; Roberts and Frankel, 1950; Udenfriend, 1950) discovered a large amounts of GABA in the mammalian brain and suggested that this substance could have some metabolic function. Although subsequently, several groups suspected the existence of a inhibitory neurotransmitter, which was ascribed to an unknown "factor I" (Florey, 1954), the abundance of GABA and its structural analogy with glutamate supported the idea that it seemed to play a role in glutamate metabolism. Thereafter, several key studies demonstrated that GABA could actually function in the inhibition of central neurotransmission.. In 1958, two groups demonstrated that the action of GABA and synaptic inhibition could be quite similar, resulting in a selective increase in membrane Cl^- conductance (Boistel and Fatt, 1958; Kuffler and Edwards, 1958). Although several groups suggested that GABA could be a inhibitory transmitter during the following decade (Hayashi, 1959; Kravitz et al., 1963; Otsuka et al., 1966), GABA had been only been shown to satisfy all the classical criteria of neurotransmitter, in the early 1970s (Krnjevic, 1974; Roberts, 1986a).

Growing interest in GABAergic neurotransmission, during the last four decades, results from evidence of a direct or indirect involvement of the GABAergic system in numerous psychiatric and neurologic disorders; most interestingly in relation to anxiety

disorders. GABA has been shown to be the principal neurotransmitter involved in the action of benzodiazepine drugs (Haefely et al., 1975), historically, the most used class of anxiolytic family. In addition, more recent studies had pointed a role of GABA system in the pathology of mood disorders (Brambilla et al., 2003), drug dependence (Markou et al., 2004) and schizophrenia (Coyle, 2004).

1.1.2 Distribution.

Since its discovery in 1950, it has been shown that GABA is present in high concentration in the mammalian brain. GABAergic neurons in the brain are primarily interneurons, and it has been estimated that 30-40% of all CNS neurons utilize GABA as their primary neurotransmitter (Roberts, 1986b; Hendry et al., 1987). In contrast, GABA seems to be absent or present in only trace amounts in peripheral tissue, such as liver, spleen and heart; or in peripheral nerve tissue such as sciatic nerve, splenic nerve and sympathic ganglia.

1.1.3 Metabolism, storage and release.

GABA is formed by a closed metabolic pathway known as the GABA shunt (Fig.1.1). The GABA shunt begins with α-ketoglutarate from the Kerbs cycle being changed to the excitatory neurotransmitter glutamate by GABA-transaminase (Blanton et al., 1987).

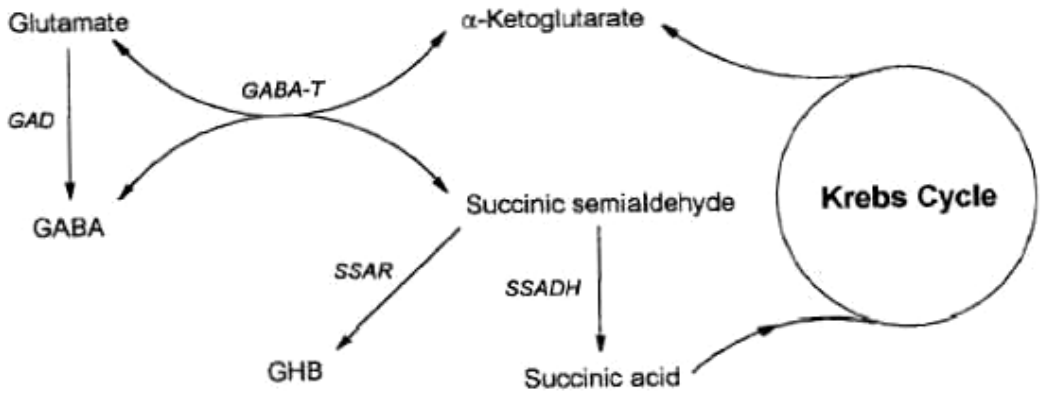

Fig.1.1 GABA metabolism pathway.
GABA-T: GABA transaminase; GAD: Glutamic acid decarboxylase, GHB: γ -hydroxybutyric acid, SSADH: succinic semialdehyde deshydrogenase, SSAR: Succinic semialdehyde reductase.

Fig.1.1 Voies métaboliques de GABA. *GABA-T: GABA transaminase; GAD: Glutamic acid decarboxylase, GHB: γ -hydroxybutyric acid, SSADH: succinic semialdehyde deshydrogenase, SSAR: Succinic semialdehyde reductase.*

GABA is formed through the decarboxylation of glutamate by glutamic acid decarboxylase (GAD) within neurons (Olsen and DeLorey, 1999). GABA in glial cells can be metabolized to succinic semialdehyde by GABA-T if α-ketoglutarate is available to accept the amino group and thus form glutamate. This creates the closed loop preventing the depletion of GABA. Excess succinic semialdehyde can be oxidized to succinic acid or γ -hydroxybutyric acid (GHB) by succinic semialdehyde dehydrogenase (SSADH). Succinic acid re-enters the Krebs cycle to complete the loop. The rate limiting step in GABA formation is the enzymatic action of GAD. GAD is only found in GABAergic neurons and has two isoforms in most vertebrates, GAD65 and GAD67, named for their molecular weights (in kilodaltons), which derive from two distinct genes (Erlander et al., 1991). GAD will decarboxylates glutamate to form GABA only when it is bound to its cofactor, pyridoxal phosphate (PLP; (Miller and Walters, 1979)). Studies in the rat have shown that GAD65 is usually not associated with PLP while GAD67 is nearly saturated with this cofactor (Kaufman et al., 1991). The amount of

inactive GAD65 and its location at the nerve terminal suggests it is utilized to respond to short-term increases in demand for GABA (Martin and Rimvall, 1993).

Following its synthesis, GABA is stored in synaptic vesicles and its release occurs by the classical Ca^{2+}-dependent mechanism upon depolarization of presynaptic membrane.

1.1.4 GABA receptors subtypes.

Once released into the synaptic cleft, the inhibitory neurotransmitter GABA acts through specific receptors located in both pre-and postsynaptic membranes. In vertebrates , there are two major types of GABA receptors: the ionotropic receptors, including $GABA_A$ receptors and $GABA_C$ receptors and the $GABA_B$ metabotropic receptors(Bormann, 2000).

1.1.4.1 $GABA_A$ receptors.

$GABA_A$ receptors are the most prevalent GABA receptors in the vertebrate brain. They are expressed in the CNS but also in the peripheral nervous system. $GABA_A$ receptors are ionotropic, and mostly postsynaptic. Their activation induces a fast inhibitory postsynaptic activation potential (IPSP, (Eder et al., 2001)).Regarding their structure, they are transmembrane hetero-oligomeric protein composed of five subunits which are organized into a channel. Each subunit comprises for transmenbrane domains (TM1-4), a large intracellular loop between TM3 and TM4 containing protein kinase and tyrosine kinase phosphorylation sites and a short C-terminus. By now, seven distinct classes of subunits (α, β, γ, δ, ε, π and ρ) have been cloned and several isoform of these subunits were identified (See Fig.2). In the mammalian brain, the major subunit composition of $GABA_A$ receptor is an assembly of one α, two β and two γ subunits (Rudolph et al., 2001). $GABA_A$ receptor activation by GABA allows the chloride ion channel (Cl^-) to open, increasing the conductance of Cl^- (Bormann, 1988). This activation induces an hyperpolarisation of neuronal membranes, reducing the cell excitability.

In terms of pharmacology, $GABA_A$ receptors have a binding site for GABA, as well as sites for agonists, such as muscimol and isoguvacine and competitive antagonists such as bicuculline. In addition, several ligands have been shown to modulate $GABA_A$ receptor activation. Benzodiazepines are allosteric modulators of $GABA_A$ receptors, they increase the probability of channel openings, where barbiturates and neurosteroids increase the period of pore opening. Further, $GABA_A$ receptors present also some bindings sites for picrotoxin (non competitive antagonist) and alcohol (Rudolph et al., 2001).

During the last decade, localization of various binding sites on $GABA_A$ receptor has been investigated. For example, GABA has been shown to bind at the interface of α and β-subunits (Zezula et al., 1996). Benzodiazepines binding site is localized at interface of α and γ-subunits (Skolnick et al., 1997). More recently, genetically engineered mice have added new tools for the dissection of specific pharmacologic function of $GABA_A$ subunits (Rudolph et al., 2001).

Although it is now clear that $GABA_A$ ligands and especially benzodiazepines represent a relevant pharmacotherapy for neuropsychiatric disorders such as anxiety, their associated side-effects, including sedation, muscle-relaxation, and amnesia has led the scientific community to investigate other possible therapeutic targets.

1.1.4.2 $GABA_C$ receptors.

$GABA_C$ receptor structure is relatively analogous to $GABA_A$ receptors. They are also ionotropic receptors, composed of five subunits organized in Cl^- channel. In contrast to $GABA_A$ receptors $GABA_C$ receptors can assemble as homoligomers composed exclusively of ρ_1-3 subunits (See Fig.1.2.). There has been much debate as to whether they represent a novel receptor subunit family distinct from $GABA_A$ receptors (Bormann, 2000), however the current IUPHAR classification feels that it is more appropriate to classify them as part of the

$GABA_A$ superfamily. $GABA_C$ receptors are also characterized by their insensitivity to bicuculline and benzodiazepines. $GABA_C$ receptors are expressed exclusively in retina, spinal cord, superior colliculus and intestinal tract. Thus, their localization limits their relevance as target for the treatment of neuropsychiatric disorders.

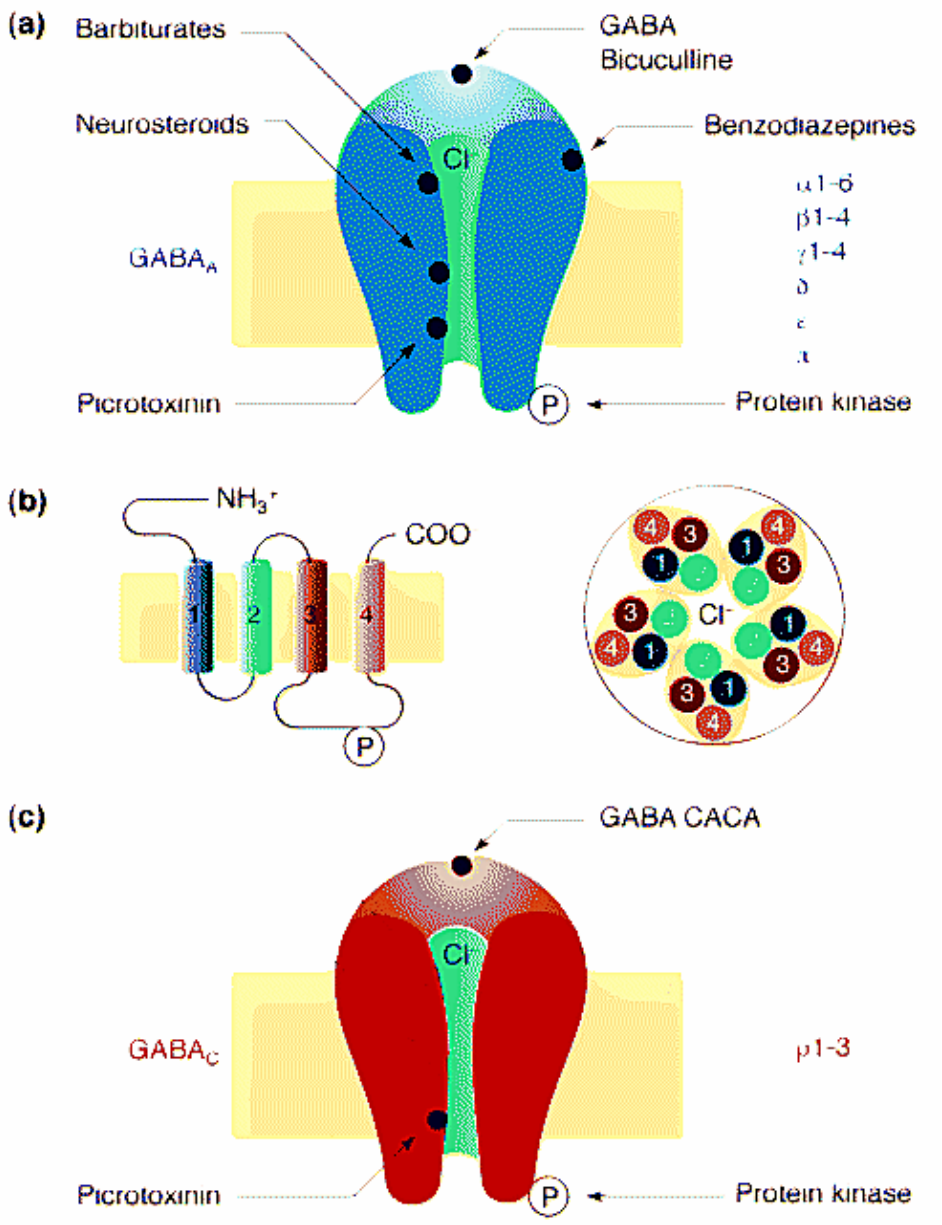

Fig.1.2. $GABA_A$ and $GABA_C$ Receptors
(a) $GABA_A$ receptors and its associated modulatory binding sites. (b) $GABA_A$ receptor subunit structure. Organisation of $GABA_A$ receptor subunits. (c) $GABA_C$ receptors and its associated modulatory binding sites. Adapted from (Bormann, 2000).

Fig.1.2. Les récepteurs $GABA_A$ et $GABA_C$
(a) Le récepteur $GABA_A$ et ses sites de modulation. (b) La structure des sous-unités des récéepteurs $GABA_A$. Organisation des sous-unités des récepteurs $GABA_A$. (c) Le récepteur GABAC et ses sites de modulation.

1.2 $GABA_B$ Receptors

1.2.1 $GABA_B$ receptors structure.

1.2.1.1 General structure.

Initially, $GABA_B$ receptor was identified as a GABA receptor insensitive to the $GABA_A$ receptor antagonist (bicuculline) but sensitive to a GABA analog, baclofen (Bowery et al., 1981). Although a considerable amount of pharmacological tools were developed during the decade subsequent to the discovery of $GABA_B$ receptors (Kerr and Ong, 1995) the cDNAs for $GABA_B$ receptors were not cloned until 17 years later (Kaupmann et al., 1997) a decade after that of $GABA_A$ receptor (Schofield et al., 1987).

Using high-affinity radioligand antagonist, Kaupmann and colleagues isolated two different splice variants of $GABA_B$ receptor, $GABA_{B(1a)}$ and $GABA_{B(1b),}$ which differ only at their two different N-terminus (Kaupmann et al., 1997). $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor splice variants are protein of 960 and 844 amino acids respectively. $GABA_{B(1)}$ recombinant protein are composed of three main regions: the N-terminal extracellular domain (ECD), a region comprising seven transmenbrane domains (TMD) and intracellular C-terminal domain (ICD)(Kaupmann et al., 1997). Their molecular structure exhibits some similarities with family 3 G-protein coupled receptors; this family includes metabotropic glutamate receptor, Ca^{2+} sensing, vomeronasal and taste receptors. Surprisingly, in the same study, Kaupmann and collaborators demonstrated that both $GABA_{B(1)}$ proteins exhibited 100 to 150-fold lower binding affinities for agonist compared to native $GABA_B$ receptors. In addition, they also demonstrated that $GABA_{B(1)}$ protein failed to couple to their effector systems. Ultimately,

$GABA_{B(1)}$ proteins have shown to be retained in the endoplasmatic reticulum and not transported to the membrane (Couve et al., 1998). Taken together, these data suggested that $GABA_{B(1)}$ subunit may need a missing factor which traffics it to the membrane and consequently renders them functional.

One year after the discovery of $GABA_{B(1)}$ subunit, six different groups identified a novel subunit, the $GABA_{B(2)}$ receptor subunit (Jones et al., 1998; Kaupmann et al., 1998a; White et al., 1998; Kuner et al., 1999; Martin et al., 1999; Ng et al., 1999). This second $GABA_B$ receptor subunit exhibits many of structural features of $GABA_{B(1),}$ such as high molecular weight, seven transmenbrane domain and a long extracellular chain at the N terminus. Furthermore, $GABA_{B(2)}$ subunit shows 35% of homology and 54% similarity to $GABA_{B(1)}$ receptor subunit. In contrast to $GABA_{B(1)}$ subunit, $GABA_{B(2)}$ is expressed at the surface of plasmatic membrane. Interestingly, this $GABA_{B(2)}$ receptor was shown to be coexpressed with $GABA_{B(1)}$ subunit and transport to the plasma membrane.

1.2.1.2 Heterodimerization : A prerequisite for $GABA_B$ receptors function?

As stated previously, $GABA_{B(2)}$ receptor subunit has been shown to be coexpressed with $GABA_{B(1)}$ receptor subunit. However, the six research groups which contributed to $GABA_{B(2)}$ discovery, suggested that this subunit may play a key role in $GABA_B$ function. Indeed, the co-expression of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit results in increase in agonistic potency compared to $GABA_{B(1)}$ alone (Kaupmann et al., 1998a). Further, recombinant hetoromeric $GABA_{B(1,2)}$ has been shown to couple to all effector system associated to native $GABA_B$ receptor, such as adenylate cyclase, K^+ channels and Ca^{2+} channels. Moreover, the $GABA_{B(2)}$ receptor subunit facilitates proper trafficking to the cell membrane. Indeed, $GABA_B$ receptor trafficking depends on interaction between $GABA_{B(1)}$ and $GABA_{B(2)}$ at the C-terminal coil-coil domain by masking the retention motif RXRR present at the C terminus of $GABA_{B(1)}$ receptor subunit (Margeta-Mitrovic et al., 2000;

Pagano et al., 2001). However, an exclusive role of this C-terminal coil-coil domain in the heterodimerization process of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunits has not been determined.

Taken together, these data led to the supposition that heterodimerization may be a prerequisite for $GABA_B$ receptors function and offer a putative canvas for $GABA_B$ receptor activation (See Fig.1.3.).

It has been shown that GABA or $GABA_B$ receptor agonists binds to a component of $GABA_{B(1)}$ subunit. Indeed, the ligand site is located within the ECD of the $GABA_{B(1)}$ (Malitschek et al., 1999), named the Venus flytrap modules (VFTMS) (Galvez et al., 1999). Although $GABA_{B(2)}$ also possesses a similar VFTMS, it does not bind any ligands (Kniazeff et al., 2002). The binding of $GABA_B$ receptor ligand to $GABA_{B(1)}$ receptor subunit thus produces a conformational change in $GABA_B$ receptor subunit protein that allows $GABA_{B(2)}$ subunit to engage and activate G protein –coupled signaling system (Marshall et al., 1999)

1.2.1.3 $GABA_B$ receptors isoforms.

Originally, Kaupmann and collaborators described only two different isoforms of the $GABA_{B(1)}$ receptor subunit, but subsequently several $GABA_{B(1)}$ isoforms were identified. To date, nine different isoforms were isolated : $GABA_{B(1a)}$, $GABA_{B(1b)}$, $GABA_{B(1c)}$, $GABA_{B(1c\text{-}a)}$, $GABA_{B(1c\text{-}b)}$, $GABA_{B(1d)}$, $GABA_{B(1e)}$, $GABA_{B(1f)}$ and $GABA_{B(1g)}$ (Bettler et al., 2004). These isoforms differ by their ECD (presence or absence of sushi's motifs), the presence or the absence of ER-retention signal, their transmenbrane domain and their coiled-coil domain. In addition, certain of these variants are not conserved from the human to the mice (ie. $GABA_{B(1c)}$ or $GABA_{B(1c\text{-}a)}$); and certain isoforms are absent in both human and mouse (ie, $GABA_{B(1d)}$). However, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ appears to be the major isoform in the brain (Bettler et al., 2004).

These two isoforms have been shown to be conserved in several studies :human, rat, mouse, chicken, frog and zebrafish. As previously mentioned, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ differs in their ECD (Kaupmann et al., 1997). Thus, the first 147 aminoacids of the mature $GABA_{B(1a)}$ subunit are replaced by 18 amino acids in the $GABA_{B(1b)}$ isoform. More specifically, $GABA_{B(1b)}$ subunit does not exhibit repeated sushi motif sequences which exhist in the $GABA_{B(1a)}$ receptor subunit (Bettler et al., 1998; Hawrot et al., 1998). Although repeated sushi' motif have been shown to be involved in protein-protein, its role in $GABA_{B(1a)}$ receptor subunit remain elusive.

In addition these two isoforms have been shown to exhibits differences in their temporal and spatial expression patterns. In a elegant study, Fritschy and collaborators have demonstrated that $GABA_{B(1a)}$ receptor subunits are the predominating isoform during early development, whereas $GABA_{B(1b)}$ receptor subunits are generally more expressed in the adult brain (Fritschy et al., 1999). Regarding their respective distribution, it appears that $GABA_{B(1a)}$ receptor subunits would be more located pre-synaptically whereas $GABA_{B(1b)}$ receptor subunits would be more post-synaptic. Indeed, in the cerebellum, the $GABA_{B(1a)}$ receptor subunit is mostly confined to the granular cell and molecular layer whereas $GABA_{B(1b)}$ transcripts are abundant in Purkinje cells that are postsynaptic (Kaupmann et al., 1998b). However, there is no evidence for this pre-post synaptic distinction in other structures to date.

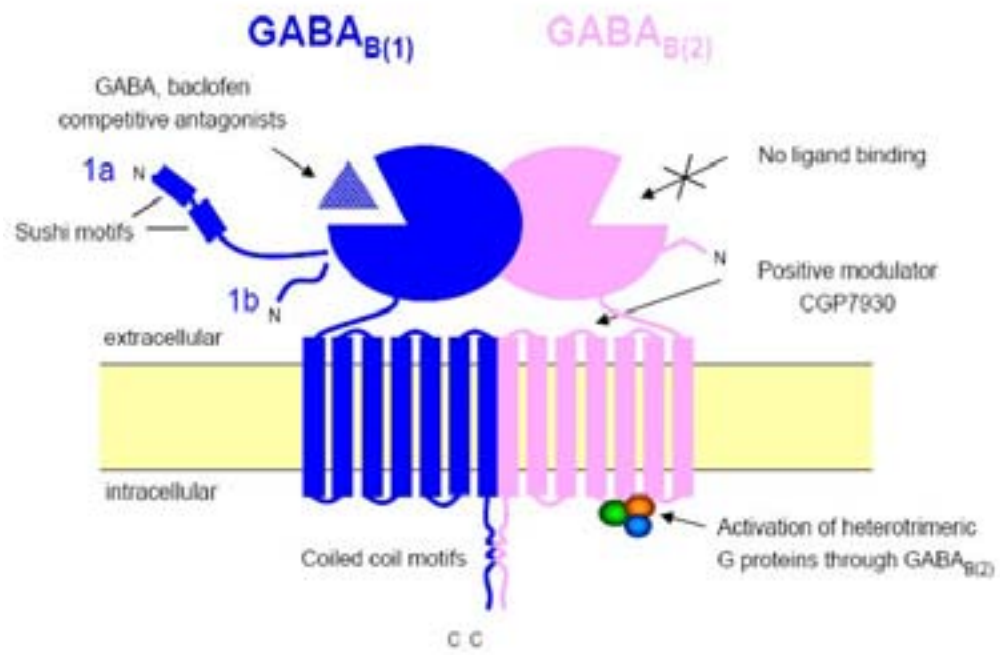

Fig.1.3. The native $GABA_B$ receptor.
$GABA_B$ receptors is an heterodimer composed of two subunits., $GABA_{B(1)}$ and $GABA_{B(2)}$. Each subunit have N-terminal extracellular domain (ECD), a region comprising seven transmenbrane domains (TMD) and intracellular C-terminal domain. $GABA_B$ receptors ligands bind to the $GABA_{B(1)}$ subunit only., inducing a conformational change. This latter process allows $GABA_{B(2)}$ receptor subunit to initiate G protein activation is facilitated. In the brain two predominant $GABA_{B(1)}$ isoforms (1a, 1b) are expressed which differ only at their very N-terminal sequence. $GABA_{B(1a)}$ discriminates from $GABA_{B(1b)}$ by the presence of two 'Sushi domain' motifs. The binding site of the positive allosteric modulator CGP7930 has recently been localized to the transmembrane region of $GABA_{B(2)}$ (Binet et al., 2004). Adapted from (Cryan and Kaupman, 2005).

Fig.1.2 le récepteurs $GABA_B$.
Le récepteur $GABA_B$ est un hétérodimère composé de deux sous-unités. Chacune de ces deux sous-inutés possèdent un domaine extracellulaire, une région transmenbranaire et un domaine intracellulaire. Les ligands se fixent à la sous-unité $GABA_{B(1)}$ induisant un chamgement dans la conformation du récepteur autorisant la sous-unité $GABA_{B(2)}$ à initier l'activation des proteine G. Dans le cerveau, les isoforms prédominantes sont $GABA_{B(1a)}$ et $GABA_{B(1b)}$.

1.2.2 $GABA_B$ receptors distribution and localization.

The distribution of $GABA_B$ receptors in the vertebrate brain has been described using in situ hybridization, immunohistochemistry and autoradiography. In the rat, $GABA_{B(1)}$ receptor mRNA is expressed in most neuronal cell populations with the highest levels in the hippocampus, thalamic nuclei and cerebellum (Bischoff et al., 1999). Moderate levels of $GABA_{B(1)}$ receptor mRNA are found in the olfactory bulb, amygdala, pallidum, septum, hypothalamus and preoptic area (Bischoff et al., 1999). Very low levels of $GABA_{B(1)}$ receptor mRNA are expressed in the molecular layer of the cerebellum and in glial cells (Bischoff et al., 1999). Interestingly, $GABA_B$ receptor mRNA have been shown to be very abundant in the raphé nuclei, including median and dorsal raphé nuclei. In dorsal raphé nucleus (DRN), $GABA_{B(1)}$ transcripts have been shown to be colocalized with the serotonin transpoter mRNA: 85% of $GABA_{B(1)}$ labeled cells exhibited serotonin transporter transcript. In contrast; only 5% of $GABA_{B(1)}$ labeled cells expressed GAD mRNA. Thus, it appears that $GABA_{B(1)}$ transcript may be located more on serotoninergic neurons than GABAergic interneurons in this structure (Serrats et al., 2003). Furthermore, the distribution pattern of $GABA_{B(1)}$ transcript and $GABA_B$ receptor binding sites overlap in the majority of brain structure (Bischoff et al., 1999).

Regarding $GABA_{B(2)}$ subunit, its transcripts are abundant in the piriform cortex, hippocampus and habenula. The regional distribution of both $GABA_{B(1)}$ and $GABA_{B(2)}$ is mostly similar, suggesting that heterodimerization is crucial for signaling. However, in some brain areas such as caudate putamen, $GABA_{B(2)}$ transcript are not detectable, even though $GABA_{B(1)}$ transcripts are expressed (Bischoff et al., 1999).

However, the lack of $GABA_{B(2)}$ mRNA expression in the striatum/caudate putamen region is not in agreement with immunohistochemical data which shows high levels of

$GABA_{B(2)}$ protein expression in that region (Kaupmann et al., 1998a; Durkin et al., 1999; Clark et al., 2000; Berthele et al., 2001). High levels of $GABA_{B(1)}$ and $GABA_{B(2)}$ protein expression were also found in the neocortex, hippocampus, thalamus, cerebellum and habenula (Charles et al., 2001).

Autoradiography in rat brains has shown that generally, $GABA_B$ receptors have lower expression levels than $GABA_A$ receptors with exceptions in a few distinct brain regions (Bowery et al., 1987; Chu et al., 1990). In one study, $GABA_B$ receptors in the rat were at higher levels than $GABA_A$ receptors in the globus pallidus, lateral posterior thalamus, lateral amygdaloid nucleus, habenula, and the molecular layer of the cerebellum (Bowery et al., 1987). Another other study found $GABA_A$ receptors had higher numbers of binding sites than $GABA_B$ receptors in nearly every brain region (Chu et al., 1990). Thus, $GABA_B$ receptors are widespread in the rat brain, but the ratio between $GABA_A$ and $GABA_B$ receptors may vary between brain regions.

At the cellular level, electron microscopy studies have demonstrated that $GABA_{B(1)}$ protein is abundant in the cytoplasm of neurons (Sloviter et al., 1999). This observation confirms that $GABA_{B(2)}$ is the limiting factor for heterodimer expression at cell surface. In addition, there is no clear evidence for a presence of $GABA_{B(1)}$ protein in the absence of $GABA_{B(2)}$ receptor subunit (Charles et al., 2001). Furthermore, numerous electrophysiological studies demonstrated that $GABA_B$ receptor are located pre- and post-synaptically (Dutar and Nicoll, 1988; Potier and Dutar, 1993). More recently, electron microscopy studies confirmed colocalization of $GABA_{B(1)}$ and $GABA_{B(2)}$ protein at both synaptic and extrasynaptic sites (Kaupmann et al., 1998a; Fritschy et al., 1999; Kulik et al., 2002); see figure.1.4).

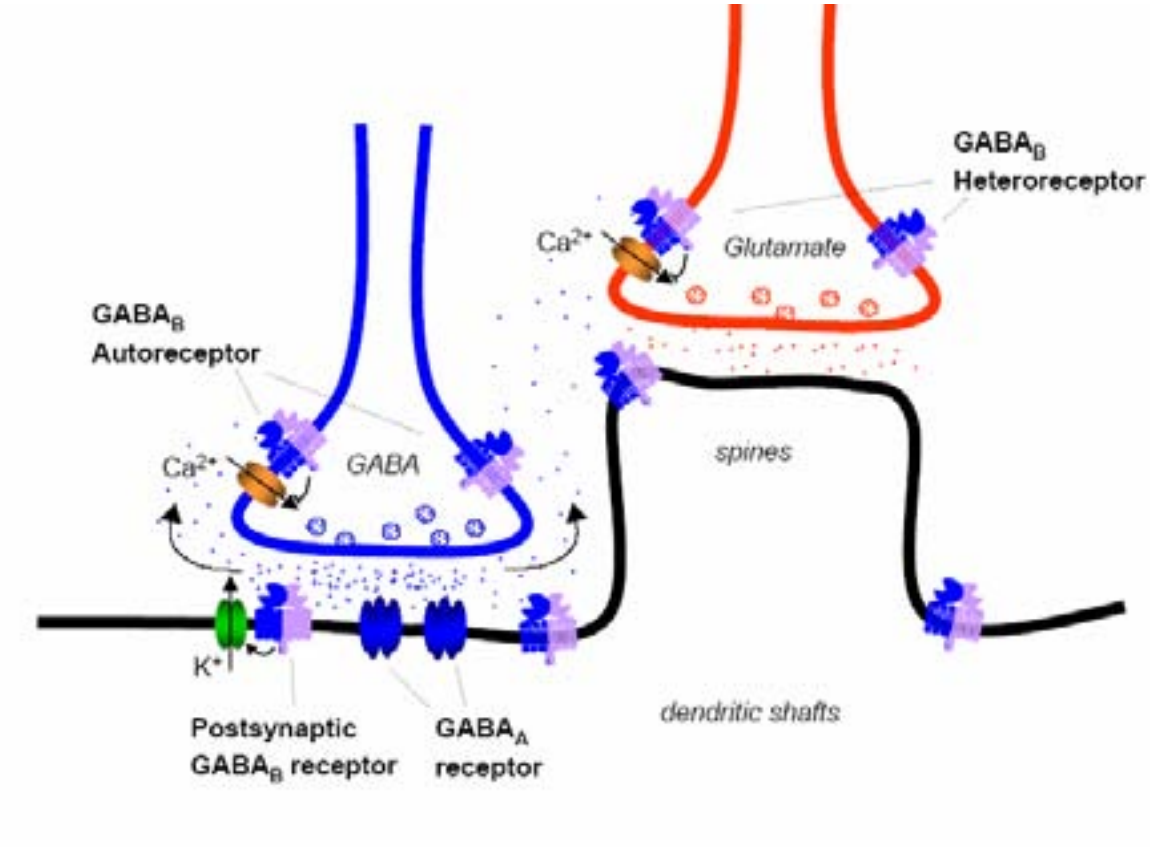

Fig.1.4. Cellular localization of $GABA_B$ receptors in the hippocampus .
$GABA_B$ receptors are located both pre- and postsynaptically as well as on extrasynaptic membranes. Presynaptic $GABA_B$ autoreceptors located on GABAergic terminals inhibit the release of GABA whereas $GABA_B$ heteroreceptors inhibits the release of several other neurotransmitters (e.g. glutamate) as well as of bioactive peptides. Postsynaptic $GABA_B$ receptors activate potassium channels and induce slow inhibitory postsynaptic potentials (IPSPs), the fast component of which is mediated through $GABA_A$ receptors. Extrasynaptic receptors are likely activated via GABA spill-over from adjacent synapses. Adapted from (Cryan and Kaupman, 2004).

Fig 1.4. Localisation cellulaire des récepteurs $GABA_B$ dans l'hippocampe.
Les récepteurs $GABA_B$ sont à la fois pré et post synaptique. Les récepteurs $GABA_B$ présynaptique localisés sur les cellules GABAergique inhibent la libération de GABA alors que les hétérorécepteurs inhibent la libération d'autres neurotransmetteurs. Les récepteirs post-synaptiques, quant à eux, active les cannaux potassiques et induisent une IPSP.

1.2.3 $GABA_B$ receptor effector mechanism.

Downstream effects of $GABA_B$ receptor activation are mediated by G-proteins, although G protein independent effects have been described as well (Harrison, 1990). Initially, evidence for a coupling to G proteins came from studies showing binding that at $GABA_B$ receptors was inhibited by GTP analogs (Hill and Bowery, 1981; Asano et al., 1985).

Using various techniques, several groups showed that $GABA_B$ are mainly couple to $G_i\alpha$ – and $G_o\alpha$-type G proteins (Asano and Ogasawara, 1986; Morishita et al., 1990; Campbell et al., 1993; Menon-Johansson et al., 1993; Greif et al., 2000). These G proteins then interact with Ca^{2+} and K^+ channels as well as adenylyl cyclase to elicit specific intracellular responses to agonist binding.

1.2.3.1 Coupling to Ca^{2+} channels.

Presynaptic $GABA_B$ receptors can be divided into autoreceptors or heteroreceptors depending on whether or not they control GABA release or a different neurotransmitter (Bettler et al., 2004). $GABA_B$ receptors inhibit neurotransmitter release by blocking Ca^{2+} influx through voltage dependent Ca^{2+} channels via the Gβγ subunits of the G-protein complex (Dascal, 2001; Zamponi, 2001). Ca^{2+} channels of both N- and P/Q-type have been implicated in presynaptic neurotransmitter release (Wu and Saggau, 1997), and inhibition of both of these types of channels by $GABA_B$ receptors has been demonstrated (Menon-Johansson et al., 1993; Mintz and Bean, 1993; Amico et al., 1995; Herlitze et al., 1996; Ikeda, 1996; Poncer et al., 1997; Takahashi et al., 1998; Shen and Slaughter, 1999). This inhibition is voltage-dependent and can be overcome by a high frequency action potentials (Herlitze et al., 1996; Ikeda, 1996) (Brody and Yue, 2000). In addition, both L- and T-type Ca2+ channels can either be inhibited or facilitated by $GABA_B$ receptors (Scott and Dolphin, 1986; Scott et al., 1990; Crunelli and Leresche, 1991; Matsushima et al., 1993) dependent on the input site. $GABA_B$ receptors have also been shown to inhibit postsynaptic N- and P/Q-type Ca^{2+} channels in the rat supraoptic nucleus (Harayama et al., 1998).

1.2.3.2 Coupling to K^+ channels.

Postsynaptically, $GABA_B$ receptors activate inwardly rectifying K^+ channels (GIRK), via the Gβγ subunits, which creates a slow inhibitory postsynaptic current (Newberry and Nicoll, 1985; Luscher et al., 1997). This IPSP is distinguishable from the faster IPSP of the

$GABA_A$ receptor because of its longer latent period and shorter decay time (Lacaille, 1991; Otis et al., 1993). These IPSCs are stimulated by baclofen, can be inhibited by the GIRK channel blocker barium or $GABA_B$ receptor antagonists and display an inhibitory potential of similar magnitude to the K^+ equilibrium potential (Lacaille, 1991) (McCormick, 1989; Thompson and Gahwiler, 1992; Pitler and Alger, 1994). There is also evidence that $GABA_B$ receptors may act through K^+ channels other than GIRKs, such as fast inactivating, voltage-gated K^+ channels (Saint et al., 1990) and Ca^{2+}-sensitive K^+ channels (Blaxter et al., 1986; Gerber and Gahwiler, 1994). In addition, $GABA_B$ receptors also activate presynaptic K^+ channels (Thompson and Gahwiler, 1992), although they are likely made up of different subunits than the postsynaptic channels (Luscher et al., 1997).

1.2.3.3 Coupling to adenylyl cyclase.

$GABA_B$ receptors also modulate postsynaptic receptor activity via adenylyl cyclase. There are nine known isoforms of adenylyl cyclase and all of them are expressed in the brain (Simonds, 1999). $GABA_B$ receptors have been shown to both inhibit and stimulate cAMP formation and this is dependent upon the specific G-protein as well as the type of adenylyl cyclase present in the neuron (Bowery et al., 2002; Calver et al., 2002). Adenylyl cyclase types I, III, V and VI are inhibited by $G_i\alpha$- and $G_o\alpha$-type proteins, the predominant targets of $GABA_B$ receptors (Bettler et al., 2004). $GABA_B$ receptors can also stimulate adenylyl cyclase types II, IV and VII via $G\beta\gamma$ proteins when $G_s\alpha$-type proteins from another G protein coupled receptor (GPCR) are activated by norepinephrine, isoprenaline, histamine or vasoactive intestinal peptide (Tang and Gilman, 1991; Simonds, 1999; Bettler et al., 2004). The results of in vitro studies have been confirmed using in vivo microdialysis in rats (Hashimoto and Kuriyama, 1997). $GABA_B$ receptors have been shown to modulate the activity of protein kinase A (PKA). PKA targets many ion channels, and K^+ channel activation is reduced when $GABA_B$ receptors modulate PKA formation by inhibiting cAMP formation (Gerber and

Gahwiler, 1994). PKA activity can also be reduced by $GABA_B$ receptors in presynaptic neurons which prevents neurotransmitter release (Kubota et al., 2003). Cross-talk also occurs with protein kinase C, but the mechanism is unknown (Kubota et al., 2003).

1.2.3.4 Other Effectors

In addition to their conventional effector mechanism, it was also postulated that $GABA_B$ receptors interact with several proteins. Indeed, three laboratories demonstrated that the coiled coil domain of $GABA_{B(1)}$ receptor subunit interacts with transcriptional factors from ATF/CREB family: ATF4 and ATFx (Nehring et al., 2000; White et al., 2000). It appears that $GABA_B$ receptors and ATF4 are colocalized in the soma and dendrites of cultured neurons and in retinal cells. Further, $GABA_B$ receptor activation induces an increase in ATF4 transcriptional activity. Interestingly, $GABA_B$ agonists has also been shown to both stimulate (Ito et al., 1995) and inhibit (Barthel et al., 1996) transcription via CREB pathways.

1.2.4 Pharmacology of $GABA_B$ receptors.

1.2.4.1 $GABA_B$ receptors agonists.

β-p-chlorophenyl-GABA, named baclofen, is the prototypical $GABA_B$ receptor agonist. It was synthesized first in 1962 and introduced to the market in ten years later (Bettler et al., 2004) for the treatment of spasticity. Although it has been widely used as pharmacological tool in elucidating the role of $GABA_B$ receptor in several disorders including epilepsy, cognition, pain, its associated side-effects, including sedation and hypothermia, limit its relevance in the context of behavioural pharmacology. Its activity is stereospecific with l-baclofen approximately 3-5 times more potent than the racemic mixture in the rat (Bowery et al., 1983; Bowery et al., 2002). Ten years after its discovery, several groups initiated synthesis of new generation of $GABA_B$ receptor agonist, including CGP 35024 (Froestl et al., 1995). This generation of agonist appears to be 5-7 fold more potent than the active form of baclofen

(Froestl and Mickel, 1997). These ligands served also for the development of radioligands such as CGP27492. Ultimately, a third generation of $GABA_B$ receptor agonist, including CGP44532, was developed in the 1990s.

1.2.4.2 $GABA_B$ receptors antagonists.

The first described $GABA_B$ receptor antagonists were phaclofen, saclofen and 2-hydroxysaclofen in the late 1980s (Kerr et al., 1987; Kerr et al., 1988). These compounds were instrumental in characterizing the $GABA_B$ receptor despite the requirement of doses in the high mircromolar range to antagonize baclofen induced hyperpolarizations (Dutar and Nicoll, 1988; Karlsson et al., 1988). The next advance in antagonist development was the creation of compounds that cross the blood-brain barrier after intraperitoneal (CGP35348) (Olpe et al., 1990) or oral (CGP36742) administration (Olpe et al., 1993). The latest generation of $GABA_B$ receptor antagonist, including CGP56433A, in the late 1990s. These ligands now reached nanomolar affinities at $GABA_B$ receptor (Froestl and Mickel, 1997).

1.2.4.3 $GABA_B$ receptors and γ -hydroxy butyric acid (GHB).

γ -hydroxy butyric acid (GHB) is a short-chain fatty acid derived metabolically from GABA (see Fig. 1.1). However, widespread interest in this compound has arisen only in the past 5-10 years, primarily as a result of the emergence of GHB as a major recreational drug and public health problem. Indeed, GHB is currently one of the most frequently used agents for pharmacological-assisted sexual assault. Its administration induces disinhibition, muscle relaxation and lasting anterograde amnesia in the victim. In addition, GHB is also present at micromolar concentration in the brain where high-affinity [^{3}H]GHB binding-sites are located (Bernasconi et al., 1999). GHB is also used medically in the treatment of narcolepsy (Black and Guilleminault, 2001).

Although, there is large body of evidence that GHB may act via an independent GHB-specific receptor site in the brain (Maitre, 1997; Bischoff et al., 1999), it has also been suggested that $GABA_B$ receptors could mediate at least some effects of exogenous GHB. Indeed, GHB has been shown to bind to native and recombinant $GABA_B$ receptors, although with significantly lower affinity than its cognate high-affinity [^{3}H]GHB binding-sites in the brain (Lingenhoehl et al., 1999). In addition, several studies reported that $GABA_B$ receptor antagonists could block GHB-induced effects. For example, CGP35348, a $GABA_B$ receptor antagonist, blocks the effect of GHB and baclofen on locomotor activity and dopamine level in forebrain (Nissbrandt and Engberg, 1996). Together, these data suggested that the involvement of $GABA_B$ receptor in GHB-mediated function remain elusive.

1.2.5 Novel pharmacological and genetic tools.

1.2.5.1 $GABA_B$ receptors positive modulator.

Over the years, $GABA_B$ receptor agonists have been extensively used to assess the role of $GABA_B$ receptors in several pathologies. Nevertheless, as mentioned previously, baclofen exhibited a numerous number of side-effects. Given the above considerations a new strategy to modulate $GABA_B$ receptor function was initiated. Indeed, allosteric modulators of GPCRs have become a major research topic due to their ability to affect a receptor's response to the endogenous agonist (Bettler et al., 2004). Recently, three synthetic allosteric modulators of $GABA_B$ receptors have been described (Urwyler et al., 2001; Kerr et al., 2002; Urwyler et al., 2003). Application of $GABA_B$ receptor positive modulators in the presence of an agonist shifts the concentration-response curve to the left, as the modulators increase the potency of GABA (Urwyler et al., 2001; Kerr et al., 2002; Urwyler et al., 2003). These modulators (CGP7930, CGP13501, GS39783) enhance both the potency and the maximal efficacy of GABA at $GABA_B$ receptors in both native and recombinant (Urwyler et al., 2001; Kerr et al., 2002; Onali et al., 2003; Urwyler et al., 2003). All of these compounds are

hydrophobic, suggesting that they interact with the $GABA_B$ receptors in the TMD, a hypothesis that has recently been verified for CGP7930 (Binet et al., 2004). Allosteric positive modulation of metabotropic receptors is a recently identified phenomena, providing novel means for the pharmacological manipulation of G-protein-coupled receptors acting at a site apart from the orthosteric binding region of the receptor protein (Soudijn et al., 2002). Such properties suggest that allosteric modulators may offer a number of potential pharmacological improvements over the use of conventional agonists as has been demonstrated for modulators acting at ligand-gated ion channels (Costa, 1989). In the case of $GABA_A$ receptors, such modulation has been therapeutically utilized with the benzodiazepines, which amplify the action of the endogenous neurotransmitter GABA. Therefore, we hypothesized that $GABA_B$ receptor positive modulators will be superior drugs, devoid of the side effect profile associated with full agonists such as baclofen.

1.2.5.2 $GABA_{B(1)}$ Knock-out mice.

Recently, three different groups generated mice lacking $GABA_{B(1)}$ receptor subunit (Prosser et al., 2001; Schuler et al., 2001; Queva et al., 2003). Interestingly, these mice exhibit a strong downregulation of $GABA_{B(2)}$ subunit confirming that the expression of this subunit is intricately dependant to the presence of $GABA_{B(1)}$ receptor subunit. Furthermore, these mice were generated on two different genetic backgrounds. Schuler and collaborators used mouse on a BALB/c genetic background while the others groups used a 129Sv/J or a C57BL/6. Although, mice generated on BALB/c background are viable, those on other genetic backgrounds die within 3-4 weeks after birth, limiting their utility in the context of behavioural analysis in adult animals.

Regarding their phenotype, Schuler and collaborators have shown that targeted deletion of $GABA_{B(1)}$ receptor induced spontaneous seizures, hyperalgesia and memory impairment in mice. In addition, they also demonstrated that baclofen, the prototypical

$GABA_B$ receptor agonist, failed to induce its typical muscle relaxant and hypothermic effects in $GABA_{B(1)}^{-/-}$ mice. In line with the behavioural data, this group also demonstrated that $GABA_{B(1)}^{-/-}$ mice exhibited a loss of all biochemical and electrophysiological $GABA_B$ receptor responses. Taken together, these data lead to suggest that $GABA_{B(1)}$ receptor subunit is essential for $GABA_B$ receptor function and that heterodimerization is clearly a prerequisite for $GABA_B$ receptor function.

1.2.5.3 $GABA_{B(2)}$ Knock-out mice.

To date, only one group has generated mice with targeted deletion of $GABA_{B(2)}$ receptor subunit (Gassmann et al., 2004). The original goal of this study was to assess the role of $GABA_{B(2)}$ receptor subunit in brain and also to clarify whether $GABA_{B(1)}$ receptor subunit can participate in functional $GABA_B$ receptor in the absence of $GABA_{B(2)}$ subunit. This group generated the mice using BALB/c genetic background in accordance to previous studies showing reduction of viability in other strain after targeted deletion of $GABA_{B(1)}$ subunit (Prosser et al., 2001; Schuler et al., 2001; Queva et al., 2003). In line with phenotype observed in $GABA_{B(1)}^{-/-}$ mice , $GABA_{B(2)}$ receptor subunit deficient mice exhibit also spontaneous seizures, hyperalgesia and memory impairment. In addition, Gassmann and collaborator observed a strong downregulation of $GABA_{B(1)}$ receptor subunit protein in these mice. In contrast to $GABA_{B(1)}^{-/-}$ mice, mice lacking $GABA_{B(2)}$ receptor subunit exhibit atypical electrophysiological $GABA_B$-receptor mediated responses in hippocampal slices. Furthermore, the genetic ablation of $GABA_{B(2)}$ subunit induce a relocation of $GABA_{B(1)}$ receptor subunit protein from the distal neuronal sites to the soma and proximal dendrites compared to wild-type animals. Thus, it appears that $GABA_{B(2)}$ receptor subunit is essential for receptor localization. However, it is conceivable, regarding atypical electrophysiological response observed in these mice, that $GABA_{B(1)}$ receptor subunit could be functional in neurons that naturally lack $GABA_{B(2)}$ receptor subunit. Nevertheless, the recent development

of transgenic mice in which the endogenous $GABA_{B(2)}$ gene has been mutated in order to express a C-terminally truncated version of protein contradict this theory (Thuault et al., 2004). Indeed, both pre- and post-synaptic $GABA_B$ functions are abolished in these mice. Therefore, $GABA_{B(1)}$ receptor subunit does not reach to the cell surface in these mice. To conclude, further studies are required in order to understand the exact role of each $GABA_B$ receptor subunits in $GABA_B$ function and to confirm or not a putative $GABA_B$ receptor function in neuron lacking naturally $GABA_{B(2)}$ receptor subunit.

1.3 Involvement of $GABA_B$ receptors in neuropsychiatric disorders.

1.3.1 Role of $GABA_B$ receptors in anxiety.

1.3.1.1 Anxiety disorders..

Anxiety is a common human emotional reaction that occurs in response to a threatening situation. At mild level anxiety is considered “normal” and resulting in a multiplicity of adaptative changes, including increase of heart-rate, blood pressure or arousing states. However, anxiety is considered pathological when it interferes with every day life, or when it becomes persistent, excessive or inappropriately triggered by little or no external stressful stimuli. To date, the diagnostic and statistical manual of mental disorders- Fouth edition (DSM-IV) classification of anxiety describes several forms of anxiety disorders, which currently include: generalized anxiety disorders, obsessive-compulsive disorder, phobias, panic disorder, and post traumatic disorder (American Psychiatric Association.). Each type of anxiety disorder exhibits a unique combination of symptoms that in some cases overlap (see Table 1). Together, these disorders affect over 20 % of the population at some point in their life time, with an annual estimated cost of $44 billion in the United States (Greenberg et al., 1999). A recent pan-European study demonstrated that anxiety disorders are

the most prevalent medical disorder across E.U. members states (Andlin-Sobocki and Wittchen, 2005).

Disorder	Symptoms	Lifetime prevalence %	Treatments.
Generalized anxiety disorders	Excessive anxiety and worry, a difficulty in controlling the worry, sleep disorder, hyperaurosal.	5	Benzodiazepines, SSRIs, Buspirone
Panic disorder	Occurrence of spontaneous panic attacks, presence of anticipatory anxiety and the presence of phobic avoidance.	3	SSRIs, benzodazepines
Post-traumatic stress disorder	Recurrent episodes of inappropriate fear resulting of a initial trauma. The traumatic event is persistently reexperienced. Sleep disorders. Irritability.	3	SSRIs
Social phobia	Marked and persistent fear of one or more social or performances situation. Marked avoidance of these situation interefering with life.	13	SSRIs, benzodiazepines
Specific phobia	Specific aversion to an element (animals, blood…)	11	Mainly behavioural therapy.
Obsessive-compulsive disorder	Recurrent obsessions and compulsions: Obsession are persistent, intrusive or inappropriate thoughs that cause anxiety. Compulsion are repetitive acts that the sufferer feels driven to perform to cope with anxiety.	2	SSRIs

Table.1. Classification of anxiety disorders, their prevalence and commonly used treatments. Adapted from (Barlow, 2001)

Tableau.1. Classificatio des troubles anxieux, leur prévalence et leurs traitements.

To date, barbiturates, benzodiazepines, many classes of antidepressants and 5-HT_{1A} receptor agonist have been used as anxiolytics. However, benzodiazepines and selective serotonin reuptake inhibitor (SSRIs) are considered first line treatments for anxiety disorder. Benzodiazepines are shown to be effective in generalized anxiety disorders, social phobia and panic disorders (See Table, 2), whereas SSRIs , including fluoxetine, paroxetine, sertraline fluvoxamine and citalopram, appears to be more effective in the majority of anxiety disorders. It is important to note that these treatments differ in their onsets. Indeed, benzodiazepines have a rapid onset of action, consequently they are mainly used to treat acute anxiety episode or panic attack. Nevertheless, many factors limit their used for chronic anxiety such as dependence, alcohol interactions, cognitive impairment and sedation (Nemeroff, 2003). Concerning the SSRIs, it has been shown that they have slow onset of action 2 to 8 weeks before benefit may be noticed ((Nemeroff, 2003). However, several groups reported that SSRIs may be anxiogenic during the early phase of treatment (Gorman et al., 1987). Currently, the combination of both families of drug seems to be the most effective treatment for panic disorders or social phobia. It has to be noted that buspirone, a partial 5-HT_{1A} receptor agonist, is also currently used in certain anxiety disorders, suggesting that serotoninergic might be involved in these pathologies. Although, this strategy still remain effective, side-effects associated with these families, including sedation, tolerance, cognitive impairment for benzodiazepines, sexual dysfunction for SSRIs, has propelled efforts in the scientific community to explore extensively the neurophysiology of anxiety disorder, in attempt to develop new agents.

1.3.1.2 Modeling anxiety in animals.

Given the heterogeneity of symptoms and the multiplicity of anxiety disorders, it appears difficult to recapitulate all of them in animals and particularly in rodents. Nevertheless, several DSM-IV criteria have been successfully modeled in rodents (Rodgers

et al., 1997; Belzung and Griebel, 2001; Cryan and Holmes, 2005). For example, the avoidance of places from which escape, observed in agoraphobia, could be observed in certain rodents model of anxiety. Separation anxiety could be modeled measuring ultrasonic vocalizations in pups separated from their mother (Miczek et al., 1995). However, it is essential not too anthropomorphize emotional human behaviours to mice because “Aberrant behaviours symptomatic of human mental illness, therefore, most of pathological behaviours observed in human can not occur in a recognizable form in rodents”(Crawley, 2000). Indeed, some symptoms of schizophrenia such as hallucination or delusion or some symptoms of depression such as recurrent thoughts of death will be impossible to observe in rodents. Consequently, several authors have proposed criteria for evaluating whether paradigm has validity as a model of psychiatric disease. McKinney and Bunney suggested four major criteria to evaluate animal model of depression, but these criteria are also valid to the others neuropsychiatric disorders (McKinney and Bunney, 1969):

1) It has to be “reasonably” analogous to the human disorder in its manifestation or symptomatology.

2) Behavioural change observed can be objectively monitored.

3) The pharmacological treatments effective in the model have to be similar than observed in humans.

4) The system should be reproducible between investigator.

Other groups would prefer three different criteria including: “Predictive validity“ allied with the second criterion proposed by MacKinney; “Face validity”, similar to the first criterion proposed above; “Construct validity”, related to similarities of neurobiology and etiologically between the model and human disorder (Willner and Mitchell, 2002). Regardless

of the differences between these proposals, it appears a real difficulty to satisfy all this criterion, but they provide a useful tool to modeling neuropsychiatric disorders. One pragmatic proposal by Geyer and Markou is that the only criteria that are necessary and sufficient for *initial* use of an animal model, are that the paradigm has strong predictive validity, and that the behavioural readout be reliable and robust in the same laboratory and between laboratories (Geyer and Markou, 1995; Geyer and Markou, 2000). Nevertheless, the term predictive validity does not have not the same meaning for Willner and Mitchell as it does for Geyer and Markou. For the latter investigators, the predictive validity is defined as the ability to make the accurate predictions about human phenomenon of the interest based on the performance of the model. This last definition includes Willner's definition, but extends it to the identification of any variables that influence both experimental preparation and the modeled phenomenon in similar ways.

Regardless of theses semantic consideration, many authors have suggested different classification of animal models of anxiety. Thus, Rodgers described two categories of paradigms. (Rodgers et al., 1997). The first includes tests based on conditioned fear whereas the second regroups paradigms based on unconditioned fear. More recently, Cryan and Holmes proposed an alternative classification (Cryan and Holmes, 2005). They distinguished model based of fear-related behaviour from the exploratory-based approach-avoidance test.

Conditioned anxiety paradigms

Using Rodgers classification, conditioned anxiety paradigms assess the ability of an animal to emit or suppress responses induced by the delivery of an unavoidable form of punishment. This category can be further categorized as conflict or non/conflict based paradigms (See Table 2.). In non-conflict based tasks the rodent is re-exposed to an environment or stimuli that result in fear or anxiety related behaviour. This subcategory

includes fear-potential startle (Davis et al., 1993), fear conditioning, conditioned ultrasonic vocalization or defensive burying (Blampied and Kirk, 1983). In contrast, the second subcategory (i.e. Conflict models) involves punishment in response to innate behaviour, including eating or drinking. Classically, conflict paradigms largely comprised Vogel test (Vogel et al., 1971), Geller-Seifter conflict (Geller et al., 1962). Although, conditioned paradigms allow for experimental control over behavioural baselines, they exhibit a huge learning and memory component. Thus, amnesic agent could be considered as falses positives in these paradigm. For example, scopolamine has been shown to be active in Geller-Seifter paradigm (Ketelaars and Bruinvels, 1989). Regarding their predictive validity, benzodiazepines appears to be effective (Geller et al., 1962; Vogel et al., 1971) in most part of these models. However, there is only limited number of studies which reported an anxiolytic effect of chronic SSRIs in these test (Borsini et al., 2002; Millan, 2003).

Unconditioned anxiety paradigms

In the present dissertation, the majority of studies were carried out using unconditioned animal model of anxiety. This category includes exploratory-based approach-avoidance conflicts tests described by Cryan and Holmes, social tests (File and Hyde, 1978) and several other paradigms based on unconditioned behaviours (see Table 2). The first subcategory exploits the natural tendencies of rodents to avoid a potentially dangerous area. In the elevated-zero mazes, the aversive area is the open quadrants might (see, material and methods). Thus, anxiolytics tend to decrease both the entries into and the time spent in these quadrants. In addition, several ethological marker of anxiety are monitored during this test, such as stretch-attempt posture, rearings or head dips. Similarly, the light-dark box test use spontaneous tendency of mice to avoid the light area of a test chamber (see material and methods). In this model, anxiolytic activity is characterized by a increase in the latency to enter in the safe (dark) compartment, an increase in the number of transitions between dark

and light compartment and an increase in the time spent in light compartment (Bourin and Hascoet, 2003). The staircase test is based on the fact that mice tend to avoid height (Simiand et al., 1984; Pick et al., 1996; Weizman et al., 1999). Anxiolytic, including benzodiazepines, have shown to increase the ratio of the number of the steps climbed compared with number of rearings. Similarly to the elevated zero maze, some behavioural indicators of anxiety are measured in this test such as rearing. This subcategory comprises also the mirrored chamber and the open-field hole-board paradigm. Regarding their predictive validity, classical benzodiazepines and several other anxiolytics have been shown to decrease in avoidance of aversive/threatening areas. Conversely, anxiogenic drugs, such as beta carbolines or yohimbine, often potentiate the anxiety-like avoidance response in these tests (Rodgers et al., 1997). However, chronic SSRIs appear also to be less effective or ineffective in these tests (Borsini et al., 2002). More recently, a novel approach has been shown to be more sensitive to this last treatment, Dulawa and collaborators demonstrated a effect of chronic fluoxetine in the novelty-suppressed feeding (Dulawa et al., 2004; Dulawa and Hen, 2005). Indeed, BALB/c mice have been shown to decrease their food intake when they are in a novel environment. In this study, it has been demonstrated that chronic, but not acute treatment with SSRIs reversed this suppression.

Although, this category of paradigm is widely used in behavioural pharmacology research, there are several potential caveats associated with their used. For example, psychostimulant or sedative effect of a drugs have the potential to cause a false positive anxiety-related behaviour in these tests (Pollard and Howard, 1986).

Conditioned response tests	Unconditioned response test.
Conflict tests • Geller-seifter paradigm. • Vogel paradigm.	***Exploratory-based approach-avoidance conflicts tests*** • Elevated plus-maze. • Elevated zero-maze. • Light-dark box • Mirrored Chamber. • Staircase test. • Novelty-suppressed feeding.
Non-conflict tests • Active/passive avoidance. • Conditioned emotional response. • Shock probe defensive burying. • Fear potentiated startle. • Fear conditioned paradigm. • Conditioned ultrasonic vocalization.	***Social tests*** • Separation-induced ultrasonic vocalizations. • Social competition • Social interaction
	Others • Stress-induced hyperthermia. • Acoustic startle. • Hot-plate. • Mouse defensive test battery. • Shock-induced ultrasonic vocalizations • dPAG-induced flight. • Predator odor induced • Lactate or cystokinin administration induced panic-like behaviour.

Table.2. Classification of animal model of anxiety disorders. Adapted from (Rodgers et al., 1997).

Tableau 2. Classification des modéles animaux d'anxiété.

Quantification of general measures of locomotor activity in a test (e.g., distance traveled in the light/dark test; closed arm entries in the elevated plus-maze) can increase confidence in the anxiety-related specificity. Alternatively, these psychostimulant or sedative effect can be assessed on formal tests of locomotor activity (e.g., open field; (Hascoet and Bourin, 1998) and motor function (e.g., rotarod coordination); however, caution should be taken to minimize the stressful nature of such tasks to avoid cross-contamination with anxiety.

In addition, this category comprises also tests measuring innate response to aversive stimulus or situation, such as maternal separation, electric shock or predator presentation

(Blanchard et al., 1997; Blanchard et al., 2003). The response measure can be a behaviour, including flight (Blanchard et al., 1997; Blanchard et al., 2003) and ultra-sonic vocalization (Miczek et al., 1995), or physiological parameters, such as autonomic arousal or body temperature. For example, the stress-induced hyperthermia (SIH) is mainly based on the increase of core temperature observed after stress. This procedure involves two sequential rectal temperature measurements, spaced 10 min apart. The first rectal temperature measurement (T_1) reflects the basal body temperature, whereas the second measurement, 10 min later reflects the stress-induced temperature (T_2) due to the rectal procedure 10 min earlier. The difference, ΔT ($=T_2-T_1$) is the measure for stress-induced hyperthermia. This parameter appears to be an indicator of anticipatory anxiety. Benzodiazepine, including diazepam, chlordiazepoxide; 5-HT_{1A} receptor agonists such as flesinoxan, buspirone are active in blocking stress-induced hyperthermia (Van der Heyden et al., 1997).

Although pharmacological studies have provided a tremendous amount of information regarding the neurobiology of anxiety, the recent development of genetically engineered mice have added innovative new tools to the armamentarium of researchers (Finn et al., 2003) (Cryan and Holmes, 2005). Thus, genetic manipulations, including overexpression or knock-down, allow to examine novel targets for the anxiolytic activity for whicsh few established pharmacological tool exist. Recently, Cryan and collaborators demonstrated that targeted deletion of mGlur7 receptor induced anxiolytic-like behaviour, despite an absence of pharmacological studies (Cryan et al., 2003c). Additionally, genetic manipulation will enable better testing of the validity of current molecular theories of a anxiety disorders. Thus, the anxiogenic effect of targeted deletion of 5-HT_{1A} receptor supports the role of serotoninergic system in the neurophysiology of anxiety (Parks et al., 1998; Ramboz et al., 1998; Sibille and Hen, 2001). At this time more that 40 different mutant lines have been reported to have phenotypes interpreted as abnormal anxiety related behaviour in these test and this technology

must be considered as the primary technological advance in neuroscience during these last decades.

1.3.1.3 Neurobiology of anxiety.

The recent development of non-invasive brain imaging techniques generated a large number of studies on the involvement of brain structure in anxiety disorders. In a recent review, Douglas Bremner at Emory University school of medecine, proposed a diagram of key structures involved in the majority of anxiety disorders. Thus, several authors demonstrated that the amygdala, a region generally associated with fear responses, is remarkably overactive in PTSD patients (Bremner, 2004). Similarly, increased amygdala activation was also observed with fMRI during symptom provocation in phobic patients. In addition, some structure such as medial prefrontal cortex or anterior cingulate cortex might be affected in anxiety disorders. PTSD patients exhibit a decrease in medial prefrontal activity. It has been proposed that this structure, including the anterior cingulate, could be involved in the extinction of fear (Maren, 2005), and consequently in the reminiscence of initial trauma observed in PTSD. Regarding obsessive compulsive disorders, studies have reported an increase on orbitofrontal cortex, anterior cingulate cortex, and caudate nucleus function in OCD patients compared to normal subjects, that could be normalized with antidepressant treatments (Bremner, 2004). This data support the theory that OCD disorder could be characterized by abnormal processing within the lateral orbitofrontal loop, including caudate, cingulate and orbitofrontal cortices (Chamberlain et al., 2005).

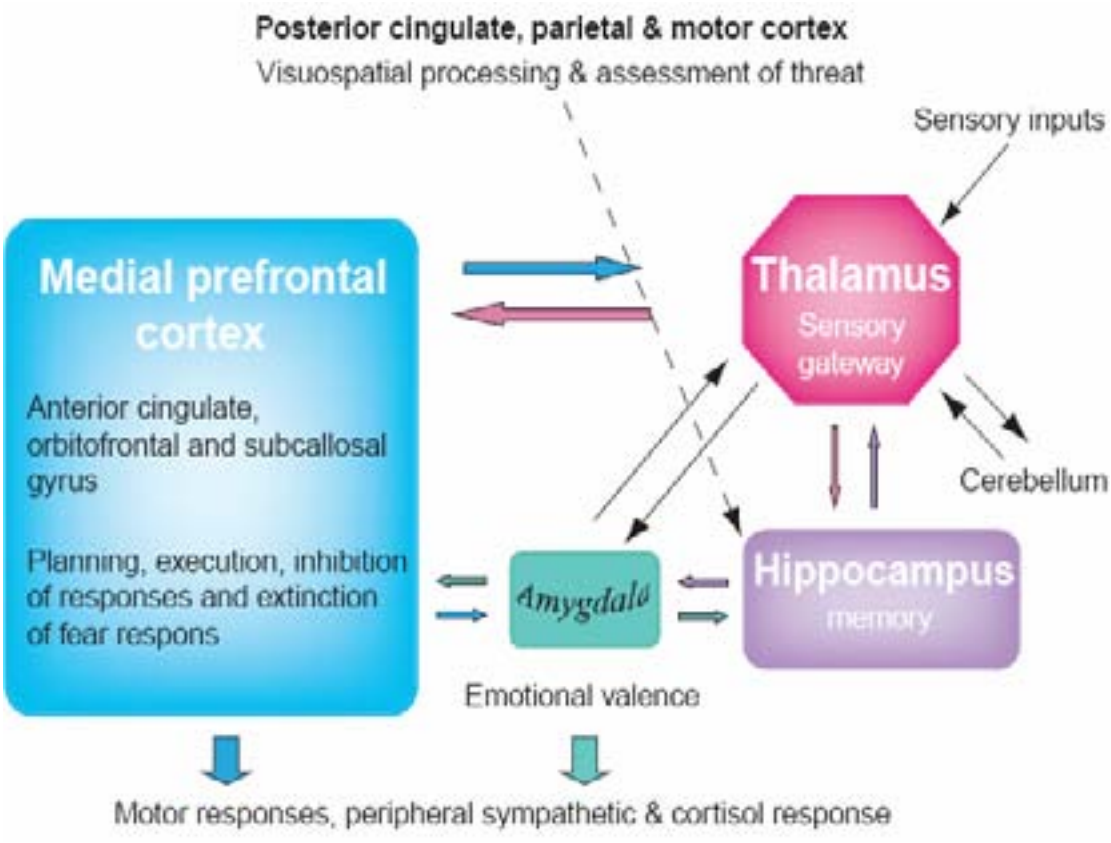

Fig.1.5. Schematic diagram of structures involved in anxiety disorders. Adapted from (Bremner, 2004)
Fig.1.5. Diagram des structures impliquées dans les troubles anxieux.

In addition to this structures, most of the work on the neurobiology of anxiety has focused on monoamines, including serotonin and norepinephrine, and on the GABAergic system (Millan, 2003). Although serotoninergic, noradrenergic and GABAergic system appears to be the major system involved in anxiety disorders, there is emerging evidence for the role of glutamatergic system (Spooren et al., 2003), vasopressin, galanin (Holmes et al., 2003a) or other in the neurobiology of anxiety disorders.

Noradrenaline (NA).

It is now generally accepted that noradrenaline (NA) play a key role in anxiety. Indeed, several studies reported that stressful stimuli such as immobilization, foot-shock, and tail pinch have increase NA turnover in the locus coeruleus, hypothalamus, hippocampus,

amygdala and cortex (Bremner et al., 1996). Clinically, yohimbine, a α2-receptor antagonist has been shown to increase anxiety in normal patients (Goldberg et al., 1983) and to increase panic frequency in panic patients (Charney et al., 1984). Moreover, propanolol, a β-adrenoreceptor antagonist is also used to treat some form of anxiety, particularly when physical symptoms are severe (e.g., social phobia; performance anxiety (Noyes, 1985).

Serotonin (5-HT).

Regarding the serotoninergic system, the well-established effectiveness of SSRIs and buspirone in the treatment of anxiety (Feighner and Boyer, 1989; Lydiard, 1998) support an eventual role of this system in anxiety disorders. In addition challenge studies have demonstrated that single dose of 5-HT receptor agonist, such as mCPP, is anxiogenic in patient with panic disorders and in control subjects (Charney et al., 1987). In animals, exposure of a variety of stressors produces an increase in 5-HT release in the medial prefrontal cortex, nucleus accumbens and lateral hypothalamus (Inoue et al., 1994). In addition, local injection of 5-HT_{1A} agonist has been shown to affect anxiety behaviour in several animal paradigms (Menard and Treit, 1999). More recently, mice with targeted deletion of 5-HT_{1A} receptor have been shown to exhibit high level of anxiety (Ramboz et al., 1998; Sibille and Hen, 2001). Ultimately, Neumeister and collaborators demonstrated that patients suffering of panic disorder exhibit a marked reduction of cerebral 5-HT_{1A} receptor binding in the anterior and posterior cingulate cortices compared to normal subjects (Neumeister et al., 2004). Regarding the other receptors, some studies have also reported an anxiolytic-like action of 5-HT_2 receptor agonists, an anxiogenic-like effect of 5-HT_{2A} receptor antagonism in mice (Nic Dhonnchadha et al., 2003).

GABA.

As mentioned previously, alterations in the GABAergic system have been linked to the pathophysiologiy of anxiety disorders. It is widely accepted than patients with anxiety disorders, including panic disorder, PTSD and generalized disorder; have reduced benzodiazepine binding sites in various brain region, in comparison with healthy subjects (Tiihonen et al., 1997; Malizia et al., 1998; Bremner et al., 2000a). Consistent with theses observations patient with panic disorder were found to exhibit lower brain levels of GABA than controls (Goddard et al., 2001). Ultimately, the widespread used of benzodiazepines in the treatment of anxiety disorder corroborated the predominant role of GABA in these disorders.

Preclinically, several pharmacological or genetic manipulations of GABA receptor or GABA levels have been reported to modulated anxiety. Briefly, the majority of preclinical model of anxiety disorders have been validated with classical benzodiazepines, such as diazepam or chlordiazepoxide (Rodgers et al., 1997; Millan, 2003). Interestingly, benzodiazepines receptor inverse agonists, beta-carbolines, have been reported to be anxiogenic in most of animal model of anxiety (Guidotti et al., 1980). In addition, a large number of studies demonstrated also that $GABA_A$ receptor activation, via agonist, have been reported to induce anxiolytic-like effects in several models of anxiety (Higgins et al., 1988). Moreover, several agents acting on GABA transaminase (GABA-T) has widely been found to produce anxiety reduction in several animal model of anxiety. For example, GABA-T inhibitors, such as vigabatrin appear to be anxiolytic in the social interaction or elevated plus-maze tests (Corbett et al., 1991). Although $GABA_A$ receptor activation or GABA level elevation seems to be clearly involved in the pathophysiology of anxiety, there is no clear preclinical literature, to date, on the possible involvement of $GABA_B$ receptor in anxiolytic-

like activity. To date, there are only a few studies assessing the effect of $GABA_B$ receptors ligands in anxiety paradigm.

1.3.1.4 Role of $GABA_B$ receptors in anxiety: Clinical studies.

To date, clinical data on the specific role of $GABA_B$ receptors are limited. Breslow and collaborators were the first to use baclofen to treat anxiety disorders. In their studies, they treated nine medication-free panic subjects with oral baclofen (30 mg/day for 4 weeks) in a double-mind, placebo-controlled crossover trial. Interestingly, they also reported that baclofen reduced significantly the number of panic attacks and score in Hamilton anxiety scale (Breslow et al., 1989). Correspondingly, baclofen has been also reported to be effective in the treatment of PTSD. Indeed, Drake and collaborators demonstrated that 8-weeks of treatment with baclofen significantly reduced the score in clinician-administered PTSD Scale. Nevertheless, this study was performed in a open-label trial and a double-blind, placebo controlled studies are required to support the efficacy of baclofen in the treatment of PTSD (Drake et al., 2003). In addition, it has been also reported that baclofen reverses the anxiety associated with spinal chord injury. Using a double-blind design, Hinderer reported that decreased level of their Beck Inventory-A anxiety scale scores with 40 mg/day of baclofen, and a further level reduction with 80 mg/day of baclofen (Hinderer, 1990).

Finally, several studies reported that baclofen is effective in the treatment of anxiety associated with alcohol dependence. Indeed, alcohol withdrawal results in several different symptoms, including autonomic hyperactivity, tremor, and nausea or vomiting, hallucinations, psychomotor agitation, grand mal seizures and anxiety. Addolorato and collaborators, using double-blind design assessed the effect of baclofen on alcohol intake, alcohol craving and associated anxiety symptoms (Addolorato et al., 2002b). They showed a significantly decrease of alcohol intake and craving in patients treated with baclofen, but also a decrease in

state anxiety. These data are supported by a recent a self-case reported showing that baclofen relieved anxiety associated with alcohol abstinence (Ameisen, 2005).

At this point, baclofen has been shown to be effective in the treatment of 4 different anxiety disorders; however its use is confined to marginal clinical studies. Largely, this is because baclofen have many unwanted sides effects, including sedation, hypothermia. Moreover, the absence of post-mortem and imaging data supports the idea that more studies are needed to elucidate the role of $GABA_B$ receptor in human populations.

1.3.1.5 Role of $GABA_B$ receptors in anxiety: Preclinical studies.

In the Vogel conflict test, only two studies reported an anxiolytic effect of baclofen. Ketelaars and collaborators demonstrated that 0.46 mg/kg and 1 mg/kg of baclofen increased significantly the number of punished-drinking in rats (Ketelaars et al., 1988). Subsequently, Shephard and collaborators confirmed these effects using higher doses of baclofen (0.5-2 mg/kg, i.p; (Shephard et al., 1992). Although these studies supported a potential anxiolytic effect of baclofen, there is a potential caveat regarding their results. Indeed, baclofen has been shown to induce memory impairment in several animal models. For example, it is now clear that baclofen induces memory deficits in the passive avoidance test (Swartzwelder et al., 1987). In addition, few studies failed to observed anxiolytic-like effect of baclofen in this paradigm (Agmo et al., 1991; Umezu, 1999).

In accordance with clinical data, baclofen has also been shown to counteract anxiety associated with alcohol or benzodiazepines withdrawals in rats. File and colleagues demonstrated that the day after 21 days of treatment with diazepam, animals exhibited decrease in social interaction (File et al., 1991; File et al., 1992). At physiological level, they also reported that baclofen reverses increased in K^+ evoked release of [^{3}H] 5-HT in hippocampus associated with benzodiazepine withdrawal (File and Andrews, 1993).

Similarly, the same group have shown that baclofen (1.25 and 2.5 mg/kg) reversed also anxiety associated with alcohol withdrawal, using social interaction and elevated plus maze as behavioural readout. Nevertheless, these authors reported also some sedative effect of baclofen at higher dose.

In a more ethological animal model of anxiety, Nastiti and collaborators demonstrated that baclofen (0.5 and 1 mg/kg) decrease significantly ultrasonic calling of mouse pups induced by maternal separation (Nastiti et al., 1991). Surprisingly, they reported that these effects are not due to a potential sedative effect of baclofen.

The most conflicting results, regarding the effects of $GABA_B$ ligands in anxiety paradigm, were observed using the elevated plus maze. In a first study, Dalvi and Rodgers observed that baclofen at 3 mg/kg decreases dramatically the ratio between open/closed arm entries suggesting anxiogenic effect of $GABA_B$ receptor activation in mice. In contrast, they reported that at this dose, baclofen increase the time spent in the central area and decrease the total number of arm entries, suggesting that sedative effect could be contribute to anxiogenic effect observed (Dalvi and Rodgers, 1996). Interestingly, they also observed that the $GABA_B$ receptor antagonist, CGP35348, failed to affect any behavioural parameters in these test. Conversely, a recent study reported that baclofen (0.05, 0.1 and 0.2 μg/rat i.c.v. or 1, 2, 4 mg/kg i.p) failed to affect the locomotor activity and behaviour in rat elevated plus maze. More surprisingly, these authors observed an anxiolytic-like effect of CGP35348 in the same test, when is injected i.c.v (Zarrindast et al., 2001).

Given the discrepancy of results observed in all these studies, it is currently hard to conclude to a conclusive role of $GABA_B$ receptor in anxiety disorders. The major limiting factor of these observation is that the majority of studies use the prototypical $GABA_B$ receptor agonist, baclofen, which has been reported to have a wide constellation of side-effects,

including motor impairment, amnesic effect and hypothermia (Bowery et al., 2002). Thus, the discovery of $GABA_B$ receptor allosteric positive modulators and genetic modified mice might help in the investigation of the involvement of $GABA_B$ receptors in anxiety disorders.

1.3.2 Role of $GABA_B$ receptors in Depression.

1.3.2.1 Depression: Definition.

Depression is one of the most serious disorders in today's society (Wong and Licinio, 2001). The World Health Organization predicts that unipolar depression will be the second most prevalent cause of illness-induced disability by 2020 (Murray and Lopez, 1997) and recently published data suggest that the current lifetime prevalence for depression is as high as 16.2% in the US adult population (Kessler et al., 2003). Further, with the economic burden of depression estimated to be as high as $44 billion per year in lost productive work time (Stewart et al., 2003), the imputes has never been greater to gain better understanding of the underlying pathophysiology and to develop superior treatment strategies for depression. Since the 1960s, depression as been diagnosed as "major depression" based on symptomatic criteria set in the DSM-IV: Symptomatology of the depression (see Table 3.). Moreover, depression should not be considered as a single disease, but a heterogeneous pathology comprised of numerous diseases, including melancholic depression, psychotic depression and dysthymia.

The first antidepressants were discovered, entirely by serendipity, about fifty years ago. Indeed, imipramine, a tricyclic antidepressant (TCA) (Kuhn, 1957) and iproniazid, a monoamine oxidase inhibitor (MAOI) (Loomer et al., 1957), were derived from work on antihistamine research and antitubercular drugs respectively. These two classes of antidepressant have been demonstrated to dramatically improve the symptom of depression (Frazer, 1997). Nevertheless, they have several side-effects: MAOI are associated with dangerous hypertensive episodes above, which are caused when patients eat food rich in

tyramine. Similarly, TCA have been shown to block sodium channels, which can cause cardiac arrhythmias and cardiac arrest in overdose (Frazer, 1997). Consequently, today psychiatrists generally prefer newer antidepressants such as SSRI or norepinephrine-selective reuptake inhibitors. These new categories share with the TCA the ability to inhibit monoamine reuptake, but they are reported to be more selective and lacking the undesirable binding properties of TCAs. Regarding their onset of action, it is now well accepted that all antidepressants start to be fully effective only after 4 to 6 weeks of treatment (Frazer, 1997). These data support the idea that, although all antidepressant medications elicit their primary pharmacological effects by altering monoamine (serotonin, noradrenalin or dopamine) concentrations in the brain, their mood elevating effects would parallel long term adaptive changes.

1.3.2.2 Animal models of depression-related behaviour and antidepressant activity.

Like anxiety disorders, depression is a heterogeneous disorder with symptoms manifested at the psychological, behavioural and physiological level which leads to difficulty in attempting to mimic the disorder in the laboratory (Table 3). Indeed, many of the human symptoms of depression as described in the DSM-IV (such as recurring thoughts of death or suicide or having excessive thoughts of guilt) are impossible to model in rodents. However, it is conceivable to think that some core symptom of major depression could be modeled in animals. Indeed, the loss of interest or pleasure observed in depressed patient, called anhedonia, has been translated in rodents (see Table 3). Thus, environmental or pharmacological manipulations have been reported to decrease the sucrose consumption in (Willner et al., 1992b) or intracranial self stimulation (Moreau et al., 1992). To date, there is a huge variety of animal models of depression (see Table 3), differing in terms of their predictive validity, face validity and their reliability.

Depression Symptom in humans	How it is modeled in rodents?	Ref.
Depressed mood most of the day	Not applicable	
Markedly diminished interest or pleasure in all or most activities most of the day	Intracranial Self-Stimulation (ICSS) and progressive ratio responding in response to rewards such as sucrose can assess anhedonia	(Kokkinidis et al., 1986; Gilliss et al., 2002)
Large changes in appetite or weight gain	Easily measured	(Nonogaki et al., 1998; Karolyi et al., 1999)
Insomnia or excessive sleeping	Sleep architecture can be measured using EEG	(Boutrel et al., 1999; Boutrel et al., 2002)
Psychomotor agitation or slowness of movement	Can be assessed in terms of ease of handling. Activity can be measured in novel environment And motor co-ordination assessed using rotarod	(Mizoguchi et al., 2002; Kafkafi et al., 2003; Wahlsten et al., 2003)
Fatigue or loss of energy	Social withdrawal Energy Expenditure Treadmill/Running wheel Swimming Nesting Behaviour Active Waking in EEG	(Dixon et al., 1994) (Nonogaki et al., 2003) (Grippo et al., 2003) (Dunn and Crnic, 1993) (Ballard et al., 2002) (Cheeta et al., 1997)
Indecisiveness or diminished ability to think or concentrate	Animal models of cognition Working memory Spatial Memory Attention	(Crawley, 2000) (Estape and Steckler, 2002) (Contarino et al., 1999) (van Gaalen et al., 2003)
Recurrent thoughts of death or of suicide	Not applicable	
Feelings of worthlessness or excessive or inappropriate guilt	Not applicable	

Table.3. Symptoms of depression and their associated model in rodent. Adapted from (Cryan and Mombereau, 2004).

Tableau 3. Les symptomes de la dépression et leur modèles animaux associés.

Forced swim test.

The forced swim test (FST) is probably the most widely and most frequently used experimental paradigm for detecting antidepressant activity, largely due to its relative reliability across laboratories and its ability to detect activity a broad spectrum of clinically effective antidepressants (Cryan et al., 2002a). The test is based on the observation that rodents, following initial escape-oriented movements, develop an immobile posture in an inescapable cylinder filled with water. If antidepressant treatments are given prior to the test, the subjects will actively persist engaging in escape-directed behaviours for longer periods of time than after vehicle treatment. Recently, the rat FST has been further modified and demonstrated that the test reveals specific behavioural components of active behaviours, namely swimming, which is sensitive to serotonergic compounds such as the selective serotonin (5-HT) reuptake inhibitors and 5-HT receptor agonists, and climbing (a.k.a. struggling), which is sensitive to tricyclic antidepressants and drugs with selective effects on catecholaminergic transmission (Cryan et al., 2002a; Cryan et al., 2002b). In addition, Alcoro and colleagues (Alcaro et al., 2002) have shown that chronic treatment with the serotonergic antidepressant clomipramine increased swimming behaviour whereas the catecholaminergic drugs desipramine and amphetamine primarily increased struggling behaviour in a mouse version of the test. This dissection of active behaviours in the mouse FST has also recently been extended to unveil depressive-like behaviours in mice lacking the corticotrophin releasing factor (CRF) receptor 2 (Bale and Vale, 2003).

Tail suspension test.

The tail suspension test is theoretically similar to the FST, briefly mice are suspended by their tails for six minutes, and the amount of time they spend immobile is recorded (Steru et al., 1985; Cryan et al., 2005). Acute antidepressant treatments will decrease these

immobility scores. Advantages of this test include its ability to detect a broad spectrum of antidepressants; it is inexpensive, methodologically unsophisticated and easily open to automation. This automation enables the assessment of additional parameters such as power and energy of movement (Porsolt et al., 1987; Steru et al., 1987). Furthermore, the TST also circumvents the need of the mouse to swim which may be relevant for examining the effects of certain genetically modified animals where motor co-ordination may be compromised. A relevant example of this is mice of the 129 strain which have problems keeping afloat when tested in the FST following treatment with SSRIs (Lucki, 2001) are suitable for testing in the TST.

Considering the different validity criteria of these tests, both the FST and TST have strong validity. Initially, Porsolt, for the FST, and Steru, for the TST reported that most of clinically effective antidepressants are active in this test (Porsolt et al., 1977; Steru et al., 1985). In addition, non-pharmacological treatments, such as electroconvulsive shock are effective in both of them (Porsolt et al., 1977; Teste et al., 1993). Interestingly, etiological factor of major depression, such as stress or drugs of abuse-induced withdrawal, have been shown to affect the immobility in these test. Thus, withdrawal states induced by chronic administration phenylcyclidine, “angel dust” or amphetamine decreased significantly time spent in immobility in the FST and TST, respectively (Noda et al., 1995; Cryan et al., 2003b). While these tests seem to have remarkable predictive validity, there are major caveats associated with their use. For one, because these tests respond to acute treatment, thus they do not reflect the slow onset of antidepressant in the clinic. However, recent studies demonstrated that sub-effective dose of antidepressant, observed in acute study, is effective when it is administrated chronically (Dulawa et al., 2004). Another major issue is the relevance of immobility behaviour in the context of depression. Indeed, several authors debated of the signification of this behaviour (Cryan and Mombereau, 2004; Petit-Demouliere

et al., 2005). Finally, some authors have pointed the major lack of selectivity of these tests. Indeed, the original studies, Steru and Porsolt demonstrated that drugs, such as amphetamine or cocaine non-selectively decrease immobility behaviour in these test. Thus, in order to exclude eventual false positive, it seems to be fundamental to assess locomotor activity, susquently these test (Porsolt et al., 1977; Steru et al., 1985).To conclude, while FST and TST appears to have low and questionable construct validity, they assert them-self as a powerful animal model of antidepressant activity more than animal model of depression.

Chronic mild stress.

In contrast to these to tests, the chronic mild stress might be the model of depression with the strongest construct validity (Willner et al., 1992b; 1992a; Willner, 1997). This test consists of exposing rodents to series of mild unpredictable stressors during a prolonged period. Interestingly, the regimen has been demonstrated to induce anhedonia-like behaviour in animals. Thus, chronically stressed animals exhibit decrease in the consumption for sucrose solution and brain reward function as assessed using ICSS. Although anhedonia-like behaviours has generally, been shown to be reversed by chronic, but not acute, treatment with several classes of antidepressants (Moreau et al., 1992; Willner, 1997), this paradigm appears to have a poor inter-laboratory reliability in rat (Reid et al., 1997; Cryan and Mombereau, 2004). In mice, recent studies interesting modification of the chronic mild stress model, using a 1-3 point scale for the assessment of the physical state of the animals fur. Animals subjected to chronic stress do not groom themselves or take interest in the state of their fur. There is certainly some analogy between this stress-induced state and the observations that depressed patients have a reduced efficiency with which even the smallest tasks are accomplished, leading to the inability to maintain minimal personal hygiene (Griebel et al., 2002b; Santarelli et al., 2003). Further, it has been shown that chronic treatment with the antidepressant fluoxetine and novel antidepressant candidates improved the physical state index of the mice.

In addition to this behavioural readout, several studies demonstrated that chronic mild stress could induce neuroimmune, neuroendrocrine or physiological changes observed in patients. For example, chronically stressed animals exhibits high plasma corticosterone analogous to hypercorticolism observed in major depressed patient (Froger et al., 2004). Consequently, this paradigm should be considered as a powerful tool to elucidate the involvement of stress in the etiology of depression.

Learned helplessness.

Similarly, the learned helplessness is also based on exposure to unpredictable stressors. This paradigm is mainly based on the observation that repeated exposure to inescapable and uncontrollable electric shock induce escape deficit in animals. Although, originally observed in dogs (Seligman, 1972), this model was subsequently translated in rodents, including rat (Seligman et al., 1975) and mice. In these test, antidepressant have been reported to reverse escape deficits usually after short-term treatment (Anisman et al., 1978; Anisman et al., 1979; Leshner et al., 1979; Sherman and Petty, 1982; Martin et al., 1990a). One important caveat that must be considered with the learned helplessness paradigm is that alterations in pain sensitivity caused by pharmacological or genetic manipulation will influence the behaviour of the animals. Indeed, a recent example has pointed the difficulty to distinguish the helplessness from hypoalgesia in this paradigm (MacQueen et al., 2001).

Olfactory bulbectomy model.

The last major animal model of depression is the olfactory bulbectomy model. As the name suggests this paradigm consists in bilateral removal of the olfactory bulbs of rodents. At behavioural level, this manipulation induced an hyperactive response in a novel brightly lit open field apparatus, which is reversed almost exclusively by chronic, but not acute, antidepressant treatment and at doses which do not compromise the performance of sham-

lesioned control animals (Kelly et al., 1997; Cryan et al., 1998). Moreover, olfactory bulbectomy have been shown to induce neurochemical, neuroendocrine and neuroimmune alterations many of which are comparable with changes seen in patients with major depression (Jesberger and Richardson, 1988; van Riezen and Leonard, 1990; Lumia et al., 1992; Kelly et al., 1997). Indeed, studies in the rat have shown that following olfactory bulbectomy there are marked changes in the serotonergic (Zhou et al., 1998; Connor et al., 1999; Watanabe et al., 2003), noradrenergic (van Riezen and Leonard, 1990) and GABAergic (Dennis et al., 1993) systems.

Like in anxiety, several genetic manipulations have been assessed in the majority of test. Nevertheless, some the FST and the TST seem to share the exclusivity of research based on transgenic. Indeed, more than 30 studies, for the FST and 13 studies, assessed the effect of genetic manipulations on antidepressant-like activity (Cryan and Mombereau, 2004).

Behavioural Model	Readout	Comments	Ref
FST	Immobility	Quick, Easy, robust, Sensitive to both acute and chronic treatments	(Porsolt et al., 1977; Lucki et al., 2001)
Modified FST	Immobility Swimming Climbing	Sensitive to acute and chronic antidepressant treatments; Differentiates antidepressants from different classes including SSRIs in rat, needs to be further characterized in mouse	(Alcaro et al., 2002; Bale and Vale, 2003)
TST	Immobility	Quick, Easy, robust. Sensitive to acute treatments. Certain strains climb their tail	(Porsolt et al., 1987; Bai et al., 2001; Mayorga et al., 2001)
Olfactory Bulbectomy	Hyperactivity in a novel environment Passive avoidance deficits	Behavioural effects evident only following chronic treatment; mechanism of action poorly understood	(Otmakhova et al., 1992) (Hozumi et al., 2003)
Learned helplessness	Number of escape failures Latency to escape	Sensitive to short-term antidepressant treatments; ethical restrictions in some countries	(Anisman et al., 1979; Shanks and Anisman, 1993)
Chronic Mild Stress	Sucrose Preference ICSS Fur State	Behavioural effects reversed in temporal fashion to that seen in depressed patients	(Harkin et al., 2002) (Griebel et al., 2002a; Griebel et al., 2002b; Ducottet et al., 2003)
Drug-withdrawal induced anhedonia	ICSS Sucrose Preference FST TST Learned helplessness Progressive ratio (rat)	Requires further validation; cannot easily assess baseline strain differences using ICSS Is probably dependent on regimen of administration of drug	(Kokkinidis et al., 1986; Barr and Phillips, 1999; Anraku et al., 2001; Barr et al., 2002; Cryan et al., 2003b) (Russig et al., 2003)
Prenatal Stress	Immobility (FST) Endocrine Parameters (rat)	May not be specific to depression, also proposed as model of schizophrenia	(Koenig et al., 2002)
Neonatal Clomipramine	Immobility (FST) Circadian disturbances Endocrine parameters	Only limited testing of antidepressants have been conducted in rat and none in mouse	(Vogel et al., 1990; Velazquez-Moctezuma and Diaz Ruiz, 1992; Hansen et al., 1997)
Maternal Deprivation	HPA axis ICSS (RAT)	Only limited testing of antidepressants have been conducted in rat and none in mouse	(Schmidt et al., 2002) (Matthews and Robbins, 2003)
DRL 72	Response rate Reinforcement rate	Sensitive to short-term antidepressant treatments in rat	(Wong et al., 2000) (Seiden et al., 1985)
Resident Intruder	Agonistic behaviour	Distinguishable behavioural effects only following chronic treatment; Requires further validation in other laboratories	(Mitchell and Redfern, 1997)
LPS-induced immunological activation	Temperature responses Cytokines production Endocrine parameters Sickness behaviour	Requires further validation for mouse	(Dunn and Crnic, 1993; Shen et al., 1999; Yirmiya et al., 2001)

Table 4: Comparative analysis of rodent depression models (adapted from Cryan and Mombereau, 2004)

Tableau 4: Analyse comparative des modèles animaux de dépression.

1.3.2.3 Neurobiology of depression.

Like in anxiety disorders, several brain imaging studies have reported some changes in blood flow or related measures in several brain areas. For example, Drevets and colleagues reported an increased in regional metabolism and cerebral blood flow in the amygdala, orbital cortex and medial thalamus, and decreased in dorsomedial/dorsale anterolateral prefrontal cortex and anterior cingulated cortex in major depressed subjects (Drevets, 2000). As previously reported, these structures have been shown to be particularly involved in emotional behaviour. Therefore, several studies have also investigated the morphological of brain structure in depressed patients. Indeed, it has been observed an enlargement of amygdala during the first episode of depression (Frodl et al., 2002; Frodl et al., 2003). In contrast, several studies demonstrated decrease of hippocampus volume in depressed patients (Sheline et al., 1996; Bremner et al., 2000b; Steffens et al., 2000).

Regarding the neurochemistry of depression, the monoamine hypothesis was first formulated forty years ago (Bunney and Davis, 1965; Coppen, 1968; Schildkraut, 1995). This hypothesis proposes that depression could be a consequence of deficiency of the monoamine neurotransmitters norepinephrine and/or serotonin in the brain. On the basis on this hypothesis, various classes of antidepressant agents have been developed that act in increase levels of monoamines within the synaptic clef.

Noradrenaline (NA).

Originally, the relationship between NA and depression began with the observation that reserpine-induced depletion of monoamines (including, NA), leads to depressive symptoms in some vulnerable subject (Bernstein, 1957). Subsequently, several post-mortem studies demonstrated alterations in adrenoceptor functions in depressed patients and suicide victims. Thus, it has been shown that major depressed patients exhibit elevated α_2-adrenoceptor density and affinity in the locus coeruleus compared to healthy subjects

(Ordway et al., 1994; Ordway et al., 2003). Moreover, it has also been reported alteration increase in β-adrenoceptors in frontal cortex of suicide victim (Zanko and Beigon, 1983). Finally, Klimeck and collaborators demonstrated reduced levels of norepinephrine transporter binding in the locus coeruleus of depressed patients (Klimek et al., 1997). Preclinically, it has been reported that chronic treatment with desipramine decreases dramatically the responses induced by α_2-adrenoceptor agonists, such as clonidine or dexmetdetomidine (Guo et al., 1998; Sacchetti et al., 2001). These data suggest that chronic antidepressant treatment might induce a desensitization of α_2-autoreceptor. Together, it could be safe to conclude that α_2-autoreceptors are hypersensitive in depression that could be counteracted by the progressive desensitization induced by chronic treatment with antidepressant.

Serotonin.

Like for NA, the fact that reserpine-induced depletion of monoamines caused depressive symptoms in subjects, lead also to initiate the investigation of the role of serotonin in depression (Coppen, 1968). Concerning 5-HT receptors, it has been demonstrated that 5-HT_{1A} autoreceptors seems to be supersensitized in the midbrain of depressed subject (Stockmeier et al., 1998). Therefore, two different studies, have also demonstrated a decrease of serotonin transporter binding in the midbrain of depressed patients (Pirker et al., 1995; Tauscher et al., 1999). Preclinically, several studies demonstrated that chronic SSRI treatement or targeted deletion of serotonin transporter induced a desensitization of somatodentric 5-HT_{1A} receptor (Fabre et al., 2000; Le Poul et al., 2000). Conversely, it has been shown a hypersensitive response of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, in helpless mice, mice model of depression developed via selective breeding (El Yacoubi et al., 2003). Regarding postsynaptic 5-HT_{1A}, there is no conclusive effect of chronic antidepressant on this receptor. However, some studies demonstrated a supersensitization of postsynaptic 5-HT_{1A} receptor after chronic SSRI treatment (Mongeau et al., 1997). Together, these data suggest

that hypoactivity of serotoninergic system could be induced via the hypersensivity of 5-HT_{1A} autoreceptors in raphé nucleus. Although, the 5-HT_{1A} receptor appears to be the primary 5-HT receptor most implicated in depression, several studies have also shown involvement of 5-HT_{2A} or 5-HT_{1B} in the physiopathology of depression (see review, (Cryan and Leonard, 2000).

Other neurotransmitters and cell survival pathway.

Like anxiety, several other major systems seem to play an important role in depression, such as glutamatergic system (Krystal et al., 2002) or hypothalamic-pituitary-adrenal axis (Nemeroff, 1989). For example, anhedonia, one of the core symptoms of depression, could be related to dysfunction of dopaminergic system (Willner, 1983b; 1983a; 1983c). However, there is emerging evidence for a role of cell survival pathway dysfunction in the pathophysiology of depression. This hypothesis is mainly based on the observation that depressed patients exhibit a reduction of hippocampal volume and increase of amygdala volume (see above). These results lead to the hypothesis that proteins involved in the neuroplasticity, such as neurotrophic factors could be also affected in depression (Altar, 1999). Indeed, brain derived neurotrophic (BDNF), the most widespread growth factor in the brain, have been shown to be upregulated by chronic, but not acute, antidepressant treatment in the dentate gyrus and pyramidal cell layer of hippocampus of rodents (Nibuya et al., 1996). Conversely, it has been reported that chronic stress decreases levels of BDNF in the same region (Smith et al., 1995; Vollmayr et al., 2001). More recently, Shirayama and collaborators demonstrated that injection of BDNF in hippocampus induced antidepressant-like effect in two animal model of depression. The most compelling evidence comes from a recent study , where antidepressants increase level of hippocampal BDNF level in depressed patient (Chen et al., 2001).These data suggest that drugs which can lead to upregulated BDNF levels have the potential to have antidepressant activity. Another approach to affect BDNF is

to act on the transcription factor CREB (cAMP responsive element binding protein). Indeed, BDNF gene is induced in vivo and in vitro by CREB (Tao et al., 1998; Conti et al., 2002).Thus, it has been demonstrated that chronic antidepressant increases levels of CREB expression in several brain structure, including hippocampus (Thome et al., 2000). Clinically, Levels of CREB are reportedly reduced in temporal cortex of depressed patients (Dowlatshahi et al., 1998; Dowlatshahi et al., 1999). Although, there is currently an extensive literature on these hypotheses, no direct pharmacological approaches to enhance BDNF concentrations have been developed at this time.

GABA.

It is now generally agreed that GABAergic system plays a key role in the pathophysiology of depressive disorders. Indeed, a vast amount of studies have implicated a GABAergic function in the depression (Brambilla et al., 2003). Thus, it has been shown that depressed patient exhibited reduced plasma (Petty et al., 1990) and cerebrospinal GABA levels (Gold et al., 1980). Further, post-mortem studies described a decrease of GABA cortical levels negatively correlate with the severity of depression (Honig et al., 1988). As early observations predicted, studies, using imaging techniques, also demonstrated GABAergic dysfunction in this pathology. Sanacora and collaborators demonstrated reduced GABA concentration in occipital cortex of drug free depressed patients (Sanacora et al., 2002), with a possible normalization after treatment with SSRI or electroconvulsive therapy (Sanacora et al., 2003; Sanacora et al., 2004).

1.3.2.4 Role of $GABA_B$ receptors in depression: Clinical studies.

Paradoxically, only a sporadic number of studies have investigated the role of $GABA_B$ receptor in depression. The majority of these studies used baclofen induced release of growth hormone as an in vivo index of hypothalamic $GABA_B$ receptor function. In healthy subjects, administration of baclofen induced increases in growth hormone concentrations, cortisol and a

lowering of body temperature. It has been reported that depressed patients exhibits a blunted growth hormone response to baclofen (Marchesi et al., 1991; O'Flynn and Dinan, 1993). Marchesi and collaborators demonstrated a significantly lower response to baclofen in depressed compared to healthy subjects. In line with these findings, O'Flynn and Dinan reported also a blunted growth hormone response to baclofen in depressed patient particularly in dexamethasone non-suppressor patients. However, two different studies demonstrated that these parameters are not affected in major depressed subject (Monteleone et al., 1990; Davis et al., 1997). In addition, Monteleone and collaborators also showed that chronic treatment with SSRIs or tricyclic antidepressant failed to affect growth hormone response to baclofen.

In addition, only one study investigated the effects of baclofen on the affect of depressed patient (Post et al., 1991). In this study, the authors reported that baclofen exacerbates depressive symptoms. However, they examined the effect of baclofen only in five patients.

Thus, although GABAergic system seems to play an important role in depressive disorder, no conclusive picture of the potential involvement of $GABA_B$ receptor in depression has emerged to date. Nevertheless, the development of more potent ligands (Froestl et al., 1999) and the cloning of $GABA_B$ receptor (Kaupmann et al., 1997) offer to the scientific community a new opportunity to assess the role of $GABA_B$ receptors in the pathophysiology of depression.

1.3.2.5 Role of $GABA_B$ receptors in depression: Preclinical studies.

Although clinical studies failed to demonstrate a clear picture of the role of $GABA_B$ receptor in depression, numerous preclinical studies have investigated the effect of $GABA_B$ ligands in large number of animal model of depression

Nakagawa and collaborators, using learned helplessness paradigm, reported that systemic baclofen, but not bicuculline, administration exacerbates the escapes failures to the inescapable shocks (Nakagawa et al., 1996a; 1996b). Furthermore, this group also demonstrated that $GABA_B$ activation, via systemic administration of baclofen, counteracted the antidepressant-like effect of desipramine in this paradigm, whereas muscimol appeared to be ineffective at modifying the actions of antidepressant drugs (Nakagawa et al., 1996a). On the contrary, the same group reported that CGP36742, a high potent $GABA_B$ receptor antagonist, dose-dependently improved the escapes failures induced by inescapable shocks (Nakagawa et al., 1999). On the other hand, these studies support an eventual involvement of $GABA_B$ receptors in depression.

Subsequently, Kram and collaborators investigated the impact of learned helplessness paradigm on the expression of $GABA_B$ and $GABA_A$ receptors in several brain structures. Using autoradiography, they demonstrated a downregulation of $GABA_B$ receptors in the medial and lateral septum of rats which did not develop helpless behaviour and the helplessness rat (Kram et al., 2000). However, this group did not find any changes in the $GABA_B$ receptor in the frontal cortex among groups, as it was previously reported by Martin and collaborators (Martin et al., 1990b). In this study, a decreased $GABA_B$ receptor binding density in the frontal cortex of rats with learned helplessness was observed. Moreover, this group also demonstrated that chronic, but not acute, treatments with antidepressants normalize the lower $GABA_B$ receptor binding observed in helpless rats. Regardless of these discrepancies, $GABA_B$ receptor seems to play an important role in coping behaviour.

In the forced swim test, another paradigm involving the ability of animals to cope with stress, baclofen appears to counteract also the antidepressant-like effect induced by desipramine, mianserin and buspirone (Nakagawa et al., 1996c) in rat. Interestingly, baclofen has been shown to decrease the time spent in immobility in the mice paradigm (Aley and

Kulkarni, 1990). Slattery and colleagues recently demonstrated that $GABA_B$ receptor antagonists but not the $GABA_B$ receptor positive modulator GS39783 had an antidepressant-like profile similar to SSRIs in the modified rat FST (Slattery et al., 2005a).

In effort to clarify the effect of $GABA_B$ ligands in animal model of depression, Bittiger and collaborators assessed the effect of CGP 51176A, a highly potent $GABA_B$ receptor antagonist in the chronic mild stress paradigm (Willner, 1997). Indeed, they observed that chronic $GABA_B$ receptor blockade (four weeks of treatment) blocked the reduction of sucrose consumption induced by chronic mild stress in rat (Bittiger et al., 1996).

In accordance with the data observed in learned helplessness model, bulbectomized animals have been shown to exhibit a downregulation of $GABA_B$ receptors in frontal cortex (Lloyd et al., 1989). In this paradigm, animals underwent a surgical ablation of the olfactory bulb which induced hyperactivity in the open-field test and a deficit in the passive avoidance paradigm (Kelly et al., 1997). Initially, baclofen has been reported to be able to block the hyperactive response in this model (Dennis et al., 1993). However the sedative properties of baclofen could interfere with any potential antidepressant-like effect in this paradigm. In contrast, a recent study demonstrated that $GABA_B$ receptor antagonist has an antidepressant like effect in this model (Nowak et al., 2004).

Taken together, results obtained in these different animals model of depression, supported the idea that $GABA_B$ receptor antagonist can be viewed as a potential targeted to treat depressive disorder.

Finally, several groups have also assessed the impact of antidepressant treatment on $GABA_B$ receptor binding and expression in animals' brain. In the majority of these studies, chronic antidepressant treatment appears to up-regulated $GABA_B$ receptors in the frontal cortex and hippocampus. Indeed, the majority of binding studies, demonstrated that

antidepressant, including SSRI, tricyclic antidepressant and MAO inhibitors, increased $GABA_B$ receptor density in the frontal cortex (Gray and Green, 1987). In addition, it has also been shown that antidepressants upregulated $GABA_{B(1a)}$ receptor subunit transcript in hippocampus without affecting $GABA_{B(1b)}$ transcript (Sands et al., 2004). In addition, various studies investigated $GABA_B$ function in vivo after chronic antidepressant treatment. Borsini and colleagues demonstrated that 18 days of treatment with desipramine failed to affect hypothermia response induced by single administration of baclofen (Borsini et al., 1986, 1988). In support of this, MacManus and colleagues reported also absence of effect of chronic antidepressant treatment, using motor-suppressant effect of baclofen as an index of $GABA_B$ receptor function (McManus and Greenshaw, 1991). In contrast, it has been shown that chronic antidepressant treatments enhance baclofen induced hypothermia in mice (Gray et al., 1987). Concerning these data, the differences between the protocols used, including treatment regimen, routes of administration and the species used could explain these discrepancies. Therefore, it has been postulated that motor-suppressant and hypothermic effect of baclofen recruit different subpopulation of neurons (Jacobson and Cryan, 2005). Consequently, we can presume that chronic antidepressant may affect differentially these two subpopulations.

Despite these discrepancies, the recent development of $GABA_B$ receptor engineered mice (Schuler et al., 2001; Gassmann et al., 2004) will provide a useful tool for a better understanding of $GABA_B$ receptor function in antidepressant action. Moreover, further studies are required in order to dissect the multiplicity of physiological function underlied by $GABA_B$ receptors.

1.3.3 Role of $GABA_B$ receptors in addiction.

1.3.3.1 Addiction: Definition.

Drugs dependence is one of the major public health issues in today's society. Recent surveys such as the National household survey on drug abuse revealed that the prevalence of drugs abuse among US adults aged 12 years or older is 29.5% for nicotine abuse, 5.9 % for heavy alcohol abuse and 7.1% for illicit drug abuse (see www.drugabusestatistics.samhsa.gov/nhsda/2k3nsduh/2k3Results.htm). A study prepared by The Levin Group for the National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism estimated the total economic cost of alcohol and drug abuse to be $245.7 billion for 1992 (see www.whitehousedrugpolicy.gov/publications/index.html). Finally, addiction has recently been proposed to be categorized as a chronic medical illness (McLellan et al., 2000).

Addiction can be defined as a chronic disorder that manifests as a psychological compulsion for the affected individual to maintain drug administration without being able to control or reduce intake. Clinically, psychiatrists distinguish at least two different disorders associated with drug intake, the "drug dependence" that is often used synonymously with addiction and "substance abuse" (DSM-IV). In addition to compulsive use of drugs, "drug dependence" include several other symptoms including the tolerance and the withdrawal syndrome observed following chronic use and abstinence respectively (see Table 5.). Tolerance is defined by the necessity to administer a drug in increasing doses in order to achieve the same effect. Concerning withdrawal, it represents the physical aspects of drug dependence and can be defined by the repertoire of aversive symptoms when the abused drug is suspended, including agitation, tremor, insomnia and anxious states Although it is currently not described as a required criterion for drug dependence in the DSM-IV, sensitization has

been proposed as key element of drug dependence (Robinson and Berridge, 1993). Sensitization can be defined as the increased response to drug that follows its repeated, intermittent presentation (Robinson and Berridge, 1993). Conversely, “substance abuse” is simply excessive use of a drug or use of a drug for purposes for which it was not medically intended (see Table 5.). In addition, “substance abuse” doesn’t require the development of tolerance or withdrawal (see Table 5.). However, it is important to note that substance abuse may lead to addiction or substance dependence.

Substance abuse	**Drug dependence.**
A maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by one of more of the following occurring over the same 12-month period. 1. Recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home. 2. Recurrent substance use in situations in which it is physically hazardous. 3. Recurrent substance-related legal problems. 4. Continued substance use despite having persistent or recurrent social or interpersonal problems caused by substance.	1. Tolerance. 2. Withdrawal. 3. The substance is often taken in larger amount over a longer period than was intended. 4. Any unsuccessful effort or persistent desire to cut down or control substance use. 5. A great deal of time is spent in activities necessary to obtain the substance or recover from its effects. 6. Important social, occupational, or recreational activities given up or reduced because of substance use. 7. Continued substance despite the knowledge of or having had a persistent or recurrent physical or psychological problem that is likely to be caused or exacerbated by the substance. 8 Three or more symptoms occurring during the last year.

Table.5. Diagnostic criteria for substance abuse and drug dependence (DSM-IV, the American Psychiatric association).

Tableau 5. Critère de diagnostique pour les abus de substances et la dépendence.

Regarding the pharmacological treatment of addictive disorders, the multiplicity of primary sites of action of drugs of abuse has led to the thinking that it would be difficult to try to treat all different drug dependences with a common treatment (Heidbreder and Hagan, 2005). Thus, during the last two decades, several medications have been developed for specific drugs of abuse. Currently, there are at least three different approaches for the pharmacotherapeutic treatment of addiction. The first strategy consists of treating the disorder by mimicking drug action. This strategy includes methadone and buprenorphine for heroin addiction and nicotine replacement, such as patch or gum, for nicotine addiction. The first and the best established example of this approach is methadone, a slow-onset, long-acting μ-opioid receptor agonist Thus, at a specific dose, addicts on methadone have a low level of sustained activation of opioid receptors limiting the craving and consequently allowing the patient to reengage in social activities (Although, the patient can be maintained for many years using appropriate doses, the major caveats of this approaches is that the patient is still being exposed to opiates). The second approach consists in the blockade of drugs target, such as μ-opioid receptor for heroin addiction. Thus, naltrexone, an antagonist with high affinity for μ-opioid receptor, blocks the ability of opiates to produce their many effects including addiction. Nevertheless, its efficacy is relatively limited and its use is usually confined to the "white collar " addicted, including physicians or nurses addicted to opiates (Roth et al., 1997). The last approach consists in the blockade of the addiction process. Thus, naltrexone as been also shown to be effective in alcoholism-related disorders (Volpicelli et al., 1992). Indeed, preclinical studies have demonstrated that alcohol's addictive action might be mediated by opiod receptor (Modesto-Lowe and Fritz, 2005). Despite the relative wide amount of medications against addictive disorders, a better understanding of adaptative physiological changes involved in subsequent to chronic drug exposure, of individual genetic susceptibility

and for the environmental factor underlying intake of abused drugs might be helpful in the development of new pharmacological therapeutic.

1.3.3.2 Animal models of addiction.

Although drug addiction might be, as compared to other psychiatric diseases, relatively easy and reliably measurable in laboratory animals, it seems to be difficult to reproduce the entire symptomatology of this pathology within a single animal model. Thus, several models have been developed to investigate a single pattern of symptoms, such as withdrawal, tolerance, sensitization or relapse behaviour. However, the majority of paradigms, including place preference, self-administration or intracranial self-stimulation, are mainly designed in order to appreciate the reinforcing properties of drugs (see below), they can't be considered as model of addiction per se. Nevertheless, these models provides interesting tools in order to investigate the capacity of potential anti-addictive therapeutic to attenuate reinforcing properties of drugs.

Locomotor Activity

In addition, to enhancing cerebral dopamine release, drugs of abuse increase the locomotor activity of animals (for review see (Kalivas and Stewart, 1991). Indeed, it has been speculated that mesolimbic dopaminergic system mediate both the rewarding and the locomotor activity stimulating effects of various abused drugs (for reviews see (Wise and Bozarth, 1987; Wise and Rompre, 1989). Lesion based studies demonstrated that selective 6-OHDA-induced destruction of mesolimbic dopamine neurons results in abolition of psychostimulant-induced locomotor activity in rats (e.g. (Kelly et al., 1975)) suggesting that locomotor activity might be considered as a behavioural index of activation of the dopaminergic system. Thus, an extensive number of studies have assessed the effects of potential anti-addictive agents on the hyperactivity elicited by drugs of abuse. For example, Tessari demonstrated that MPEP, a metabotropic glutamate 5 receptor antagonist, blocked the

hyperlocomotion elicited by a single administration of nicotine (Tessari et al., 2004). Nevertheless, there are several caveats in using this paradigm as a surrogate for the rewarding aspects of abused drugs. Pharmacological agents with muscle relaxant properties can be considered as “false positive” in this test. One approach to overcome such issues is to combine such locomotor paradigms with neurochemical analysis of brain dopamine release, via microdialysis (Kuczenski et al., 1995), or analysis of activity of mesolimbic system, via c-fos analysis (Dalia and Wallace, 1995). Finally, this test is not specific to addictive disorders but to all pathologies relative to hyperdopaminergic alterations. Indeed, locomotor based assays have been extensively used in schizophrenia-related research (Beninger, 1983).

Locomotor sensitization

As mentioned previously, tolerance and sensitization phenomenon are both key components of the behavioural repertoire of addictive disorders. In laboratory animals, repeated treatment with opioids or psychostimulants, including cocaine and amphetamine, has been shown to elicit these of two phenomenons, depending of the dose and the regimen used (Stewart and Badiani, 1993). Classically, intermittent repeated administration of drugs elicits sensitization whereas continuous administration of drugs results in tolerance phenomenon. A behavioural manifestation of sensitization to many drugs of abuse is progressive and enduring enhancement in motor stimulant effects elicits by repeated administration of drugs. Although the relevance of behavioural sensitization as a model of addiction is matter of debate, Robinson and Berridge have proposed that sensitization may play a role in some of persistent features of drug abuse, such as drug craving and compulsive drug-seeking behaviour (Robinson and Berridge, 1993). Regardless of these considerations, behavioural sensitization paradigms is, currently, used in order to investigate long-lasting molecular changes induced by repeated administration of drugs abuse (Nestler and Aghajanian, 1997; White and Kalivas, 1998). Using this paradigm combined with molecular analysis, Nestler and collaborators have

demonstrated that repeated administration of cocaine elicit both, pointed mutation of DARPP-32 attenuated both behavioural sensitization to cocaine and its associated accumulation in nucleus accumbens, suggesting that DARPP-32 might be involved in behavioural and molecular adaptation induced by repeated administration of cocaine (Zachariou et al., 2005). Thus, this behavioural sensitization might be an elegant approach to assess the effects of potential anti-addictive medications on long-lasting changes induced by repeated treatment with drugs of abuse. For example, γ-vinyl gaba (Vigabatrin), an irreversible inhibitor of the enzyme GABA transaminase, have been shown to alter the acquisition and expression of cocaine-induced sensitization (Gardner et al., 2002).The major limitation of this approach is that drugs are passively administrated, limiting his construct validity. In addition, several studies have been shown that passive vs active repeated administration may elicited different molecular and physiological adaptation (Jacobs et al., 2003).

Place conditioning

Another commonly used paradigm to evaluate rewarding effects of drugs of abuse is place conditioning (see review (Tzschentke, 1998). The place conditioning paradigm is based on the principal that was first introduced by Pavlov, that environmental cues that originally possess no incentive salience could acquire such salience by virtue of being repeatedly paired with affective stimuli. In this case, animals would learn to approach or avoid environmental stimuli which have been repeatedly paired with rewarding or aversive events. These responses would be named conditioned place preference (CPP) or conditioned place aversion (CPA). Typically, a conditioned place preference apparatus for rodents consists of at least two compartments, one of which is paired with the administration of a drug of interest, whereas the other associated to vehicle. After the conditioning the animals will be allowed to choose between the two compartments (see review (Tzschentke, 1998). If the animals spend more time in previously drug-paired compartment the animals displays CPP, if they spend more

time in vehicle associate compartment the animals displays CPA. In the context of addiction, the CPP paradigm has been extensively used to appreciate the rewarding properties of drugs (Carr and White, 1983). Thus, most drugs of abuse, such as, morphine, heroin, cocaine, amphetamine, nicotine, alcohol and cannabinoid agonist, have been described to elicit CPP (Stewart and Grupp, 1981; Mucha et al., 1982; Spyraki et al., 1982; Fudala et al., 1985; Lepore et al., 1995). Consequently, several groups have investigated the effect of potential anti-addictive compound on the CPP-elicited by drugs. For example, a recent study demonstrates that ultra-low doses of naloxone blocked the CPP to morphine (Olmstead and Burns, 2005). In addition, the place conditioning model provides also an interesting tool to investigate withdrawal phenomenon. Thus, buprenorphine, a treatment for opiate dependence, has been shown to block the opiate withdrawal-induced conditioned place aversion in rats (Stinus et al., 2005). Recently, this model was also adapted in order to investigate relapse behaviour by the reinstatement of CPP triggered by stress or priming administration of drugs of abuse (Kreibich and Blendy, 2004; Romieu et al., 2004; Popik et al., 2005; Ribeiro Do Couto et al., 2005). Although place conditioning might be viewed as a powerful paradigm to investigate addictive disorders, there are several potential caveats associated with its used. Firstly, this paradigm is devoid of construct validity, mainly because it has not been validated as protocol for measuring rewarding properties of drugs in human (Bardo and Bevins, 2000). In addition, this paradigm has been shown to have a huge learning component, mainly amygdala dependent (White and McDonald, 1993). Thus, NMDA antagonist have been shown to alter the acquisition of ethanol and morphine induced conditioning preference (Biala and Kotlinska, 1999; Ribeiro Do Couto et al., 2005), suggesting that agent with amnesic properties would be considered as a false positive in this paradigm.

ICSS (Intracranial self stimulation)

As mentioned previously, ICSS was the first experimental tools to investigate brain reward system and provides a direct measure of brain reward function (Olds and Milner, 1954; Phillips et al., 1989; Wise et al., 1992). In this paradigm, animals are trained to perform an operant (press a lever, nose poke, turn a wheel, head-dip) in order to obtain electrical stimulation in the so-called “pleasure centers” (see above). As ICSS directly activates the brain reward pathways, there is no satiation, tolerance, or sensitization to its rewarding effects (Phillips et al., 1989; Markou and Koob, 1991). The other main advantage of this paradigm is that it provides a quantitative measure of reward that was demonstrated to be extremely stable over periods of months under baseline conditions (see (Phillips et al., 1989; Wise et al., 1992; Kornetsky, 2004). Different drugs of abuse, including cocaine, morphine, heroin, amphetamine, cocaine, phencyclidine, all reduce the threshold current for ICSS (Kornetsky, 2004). Thus, several groups have investigated the effects of potential anti-addictive drugs on these parameters. For example, the reduction of intracranial self stimulation reward thresholds induced by cocaine have been demonstrated to be counteracted by metabotropic glutamate 5 receptor antagonist (Kenny et al., 2005) or α7 nicotinic receptor antagonist (Panagis et al., 2000). Interestingly, low dose of bupropion, an antidepressant use in the treatment of nicotine addiction, have been also shown to block the reduction of intracranial self stimulation reward thresholds induced by acute nicotine injection (Cryan et al., 2003a). In addition, ICSS paradigm have been widely use in the investigation of withdrawal symptoms. Indeed, ICSS has provided investigators with a reliable behavioural readout to assess such alterations in brain reward after cessation of drug administration (Leith and Barrett, 1976; Markou and Koob, 1991; Cryan et al., 2003b; Spielewoy and Markou, 2003). For example, it has been shown that withdrawal from chronic nicotine induces a dramatic decrease in brain reward function as reflected by elevations in brain reward thresholds, these elevation has been have

been shown to be a great model of anhedonia, a core syndrome of withdrawal(Epping-Jordan et al., 1998; Harrison et al., 2001; Semenova and Markou, 2003). More recently, Cryan and collaborators, using ICSS, demonstrated that bupropion counteracted the anhedonic states induced by cessation of chronic nicotine administration (Cryan et al., 2003a).

Self administration

The most elaborated model in experimental research is self-administration paradigm. In this case rats, monkeys and more recently mice earn an intravenous or intracerebral infusion of the drug of interest by execution of instrumental response, i.e. lever pressing, nose pokes or head dipping. This approach is considered to be one of the best measures of reinforcing properties of a drug in the brain because it appears to be analogous to self-administration observed in humans. Thus, the majority of drugs of abuse , such as cocaine, morphine, heroin, alcohol, nicotine, and cannabinoid receptor agonists; have been shown to elicit self-administration in rats (Pettit et al., 1984; Vaccarino et al., 1985; Corrigall and Coen, 1989; Koob, 1992; Martellotta et al., 1998b).Initially, several groups investigated the reinforcing properties of drugs used a "fixed ratio" schedule of self administration. In this approach, the number of responses required for an infusion of drug is set at fixed number. However, "fixed ratio" paradigm might be viewed as measuring the fact of reinforcement, but not the degree of reinforcing properties (Arnold and Roberts, 1997). In other terms this schedule indicates the presence of reinforcing properties of drugs without measuring its rewarding properties per se. In addition, the other strategy for measuring the degree of reinforcing efficacy is drug self-administration under "progressive ratio" reinforcement (Stretch et al., 1971). In this case, animals are required to increase the number of response for each successive reinforcement. The reinforcing efficacy of the drug is determined by the measure of the breaking point, defined by the largest ratio of responses: reinforcements that the subjects complete or the number of responses: reinforcements ratios completed by the

subject per session. Interestingly, it has been shown that breaking point vary dose-dependently (more the dose is high, more the breaking point is elevated). Consequently, the paradigm was extensively used in order to assess the effects of potential anti-addictive drugs on reinforcing properties of drugs of abuse. For example, MPEP, a metabotropic glutamate 5 receptor antagonist, has been shown to decrease the breaking point maintained by nicotine and cocaine, suggesting that this compound might attenuate reinforcing properties of drugs of abuse (Paterson and Markou, 2005). More recently, self-administration schedules have been adapted to investigate the phenomenon of relapse. After a behavioural extinction of drug-taking habit, the reinstatement of drug administration can be triggered in laboratory animals by stimulus, including drug, stress or drug paired-environmental cues (Shalev et al., 2002). This last approach is analogous to the behavioural aspects observed during human relapse (Epstein and Preston, 2003). To conclude, the self-administration paradigm could be considered as the most powerful tool to investigate addictive disorder, mainly because it exhibit a high face and construct validity and a particularly high reliability. However, its main disadvantage is largely technical, because it requires survival surgery and sophisticated testing apparati.

1.3.3.3 Neurobiology of addiction.

Despite their diverging primary sites of action, all drugs of abuse can lead to dependence, suggesting that all of there may be a common biological substrate underlying their mechanism of action i.e. the brain reward system. Initially , Olds and colleagues demonstrated that rodents work to electrically stimulate relatively discrete areas of brain, which demonstrates the existence of brain-reward reward system. In the rat, several brain structure have been shown to elicit intracranial self stimulation including, septal nucleus, the medial forebrain bundle and the mesolimbic dopaminergic system (Porrino et al., 1984; Phillips et al., 1989).

Indeed, it is now generally agreed that dopaminergic system play a key role in reinforced behaviours and consequently in addictive disorders. Briefly, the midbrain dopamine system is composed of two major projections: the nigrostriatal system, which projects from the substantia nigra to the corpus striatum, and the mesocorticolimbic dopamine system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens, olfactory tubercle, frontal cortex, and amygdala. It is the mesocorticolimbic system that has been primarily implicated in the reinforcing actions of drugs of abuse. Virtually all drugs of abuse, including psychostimulant, opioids and nicotine share the common ability to increase the release of dopamine in nucleus accumbens and in the caudate putamen (Bassareo et al., 1996; Pontieri et al., 1996; Tanda et al., 1997; Cadoni and Di Chiara, 1999). Nevertheless, the effects of addictive drugs on brain dopaminergic activity seem to be stronger in the nucleus accumbens than in caudate putamen (Imperato et al., 1987; Imperato et al., 1988). In addition, several groups have performed selective dopaminergic lesion of mesolimbic system in order to confirm the key role of this system in drug reinforcement/reward. Using 6-OHDA, Roberts and collaborators demonstrated that dopaminergic lesions of the nucleus accumbens or VTA decreased cocaine-maintained self-administration (Roberts et al., 1977; Roberts and Koob, 1982).In line with these findings, 6-OHDA lesions of the nucleus accumbens have also been shown to decrease amphetamine self-administration (Lyness et al., 1979).

Although these data support the role of VTA and nucleus accumbens in the reinforcing action of drugs of addiction, these structures also participate in the motivation to seek the drug and in the reinstatement of drug administration. Indeed, inactivation of VTA and the core compartment of the nucleus accumbens using the GABA agonists' baclofen and muscimol prevented cocaine-primed drug-seeking behaviour in an animal reinstatement model of relapse (McFarland and Kalivas, 2001). In addition to these structures, several others brain areas have been implicated in relapse phenomenon. Thus, MacFarland and collaborators have

demonstrated a role for a number of regions including the extended amygdala (including the central nucleus of the amygdala, ventral bed nucleus of the stria terminalis), the shell part of the nucleus accumbens, and the prefrontal cortex in the reinstatement of cocaine self-administration by foot-shock stress, suggesting that these structures would participate in drug seeking behaviour. Similarly, imaging studies have shown that craving for cocaine is associated with metabolic activation of the prefrontal cortex, amygdala, and striatal complex in addicted patients (Childress et al., 1999; Volkow et al., 1999; Kilts, 2001). In addition, recent studies have reported changes in orbitofrontal activity in patients addicted to cocaine. Although theses subjects exhibited a reduction of orbitofrontal cortex and cingulate gyrus compared to healthy subject, they have hyperactivity of the orbitofrontal cortex when they are tested shortly after last cocaine use or during drug-induced craving (Volkow and Fowler, 2000). In fact, this groups postulated that hypermetabolism of orbitofrontal may contribute to compulsive behaviours in patients with obsessive compulsive disorders (Volkow and Fowler, 2000). Based on these different findings Kalivas and Volkow (2005) suggested recently a working model substrate involving in addiction (fig.1.6A) and in the reinstatement (Fig.1.6B.) of drug taking(Volkow, 2005).

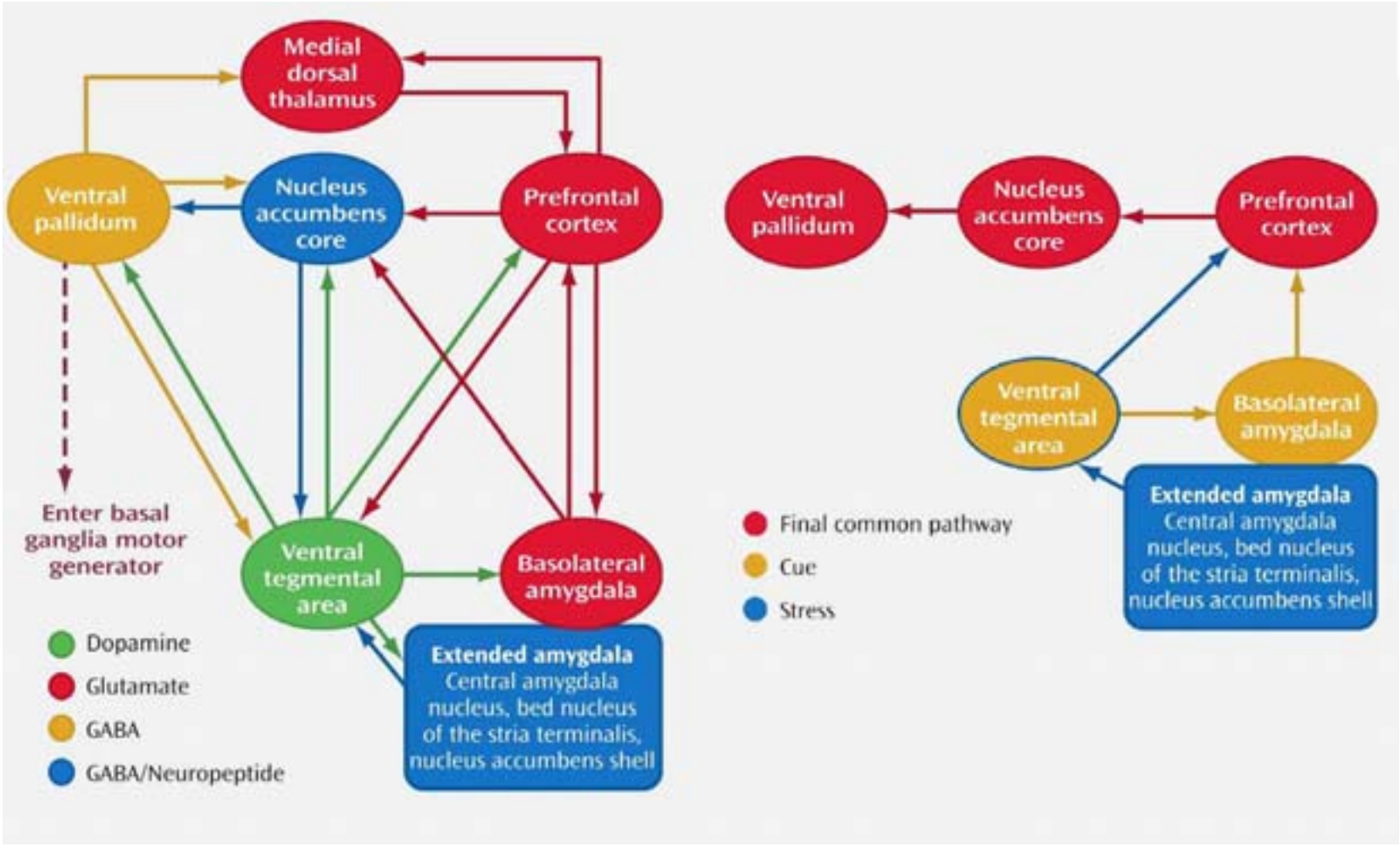

Fig 1.6. Schematic diagram of neural substrates involved in addiction (A) and in reinstatement phenomenon (B).

Fig 1.6. Circuits des substrats neuronaux impliqués dans l'addiction et les phénomènes de rechutes

Although it is undisputed that dopamine plays a key role in addictive behaviours, it appears that other neurotransmitters, including glutamate and GABA, might be involve in this disorders, as it is reported in fig 1.6A. Furthermore, certain neuropeptide, such as cortropin-releasing factor (CRF) seems to become involved in following chronic drug administration. Thus, it has been demonstrated that injection of CRF antagonist in the central nucleus of amygdala would reverse anxiogenic-like effect of ethanol and opiate withdrawal (Koob, 1996). More recently, several groups have investigated the impact of repeated drug administration on several proteins in order to appreciate the long-lasting molecular changes involved in addictives disorders. At this point, one of the most extensively studied mediator of psychostimulant-induced molecular and behavioural changes is the dopamine and adenosine 3', 5'-monophosphate-regulated phosphoprotein (DARPP-32) (Greengard et al., 1999). Addidionally, two different transcription factors have been pointed to be particularly regulate by repeated administration of drugs of abuse : cAMP responsive element binding protein (CREB) and ΔfosB (Nestler and Aghajanian, 1997; Blendy and Maldonado, 1998).

Dopamine and cAMP Regulated Phosphoprotein of 32-kD (DARPP-32) is a converging point of cAMP signaling activated by D1 receptors and the Ca2+/Calmodulin pathway activated by NMDA receptors in the Nucleus Accumbens (Liu and Graybiel, 1996; Greengard et al., 1999). DARPP-32 can be either an inhibitor of protein-phosphatase 1 (PP1) or protein kinase A, whether it is phosphorylated on Threonine 34 (Thr34-DARPP-32) or Threonine-75 (Thr75-DARPP-32), respectively. Acute cocaine exposure increases the levels of Thr34-DARPP-32 in NAc, through a PKA-dependent mechanism (Nishi et al., 1999b), while chronic exposure favors the phosphorylation of DARPP-32 on Thr-75 (Bibb et al., 1999). Loss of DARPP-32 in mice decreases the sensitivity to stimulant and rewarding effects

of cocaine, supporting the role of DARPP-32 activation in addictive disorders (Hiroi et al., 1999; Zachariou et al., 2002).

CREB is a member of the bZIP superfamily of transcription factors, including CREM (cAMP response element modulator) and ATF-1 (activating transcription factor 1). CREB activation via phosphorylation at Ser-133 is required to elicit transcription. Furthermore several signal transduction cascades, including the cAMP pathway via protein kinase A, intracellular Ca^{2+} via Ca^{2+} calmoduline-dependant kinases (CaMK) the Ras/extracellular signal regulated kinase (ERK) protein kinase pathway. Initially, it has been demonstrated that repeated, but not acute, administration of morphine upregulated CREB expression in the locus coeruleus (Guitart et al., 1992). In line with these data, mice containing targeted mutation of the α and Δ isoform of the CREB exhibited attenuated physical symptoms of morphine withdrawal (Maldonado et al., 1996), confirming the role of CREB in the modulation of physical opiate dependence. In addition to its role in locus coeruleus, accumbal CREB have been shown to be implicated in addictive disorders. Indeed, chronic cocaine increases CREB phosphorylation in the NAc (Terwilliger et al., 1991; Kano et al., 1995); whereas, viral-mediated overexpression of CREB in this region decreases the reinforcing properties of cocaine (Carlezon et al., 1998). Moreover, overexpression of a mutant form of CREB increased the reinforcing properties of cocaine (Carlezon et al., 1998). Similar effects are seen in mice containing targeted mutation of the α and Δ isoform of the CREB, which are more responsive to the reinforcing effects of cocaine compared to wild-type littermates (Walters and Blendy, 2001). Taken together, these data suggest that CREB may contribute to reinforcing but also dysphoric effects of drugs. More recently, Kreibich and Blendy demonstrated that CREBαΔ mutant mice exhibited a deficit in the reinstatement of cocaine-induced place preference triggered by stress but not by cocaine administration (Kreibich and

Blendy, 2004) suggesting that CREB might be involved in relapse or drug seeking behaviours.

ΔfosB is a truncated splice variant of the full-length FOSB, a member of the fos family of transcriptional factors, including c-fos, fosB, fos-related antigens 1 and 2 (Fra-1 and Fra-2). Fos family members heterodimerize with Jun family transcription factors to form an activator protein (AP-1) complex, that could induce or repress transcription, depending on the specific AP-1 binding site. Although acute exposure to drugs of abuse, such as cocaine, have been shown to elicited c-fos induction in the nucleus accumbens and dorsal striatum (Graybiel et al., 1990; Young et al., 1991), these protein decline rapidly toward basal levels within 8-12h of drug exposure. Conversely, it appears that although ΔfosB is modestly expressed acutely, it exhibits a particularly high stability and demonstrates in vivo half-life of weeks (Chen et al., 1997). Thus, repeated administration of several drugs of abuse, including cocaine, amphetamine, opiates, ethanol, nicotine and phencyclidine have been shown to induce an accumulation ΔfosB in the nucleus accumbens and dorsal striatum (Hope et al., 1994; Nye et al., 1995; Pich et al., 1997; Kelz and Nestler, 2000). In order to confirm the role of ΔfosB in addictive behaviour, several groups have investigated subsequently the effect of genetic manipulation of ΔfosB in several animal models of addiction. Thus, mice expressing specifically ΔfosB in the nucleus accumbens and dorsal striatum exhibited sensitized behavioural response to cocaine (Kelz et al., 1999). Similarly, these mice exhibit a hypersensitivity to rewarding properties of cocaine and morphine place preference and self-administration paradigms (Kelz et al., 1999; Kelz and Nestler, 2000; Colby et al., 2003). Finally, these mice work harder to self-administer cocaine in a progressive ratio self-administration assay, suggesting that ΔfosB may sensitize animals to the incentive motivational properties of cocaine and thereby lead to a propensity for relapse after drug withdrawal (Colby et al., 2003). As expected, mice expressing the dominant negative

antagonist of antagonist of ΔfosB, Δc-Jun, in the nucleus accumbens and dorsal striatum exhibit an attenuate sensitivity to cocaine in place-conditioning paradigm (Peakman et al., 2003). Thus, these data indicate that the accumulation of ΔfosB might contribute to long-lasting change induced by repeated administration of drugs of abuse and in their long term reinforcing properties.

Because both CREB and ΔFosB are transcription factors, we can speculate that their downstream effects could participate to the neuroplasticity triggered by repeated administration of drugs of abuse. For example, it has been shown that mice over-expressing ΔFosB exhibit upregulation of the Cdk5, in the remodeling of neuronal processes (Benavides and Bibb, 2004). CREB has been shown to modulate dynorphin, an opiod peptide expressed in a subset of accumbal neurons (Cole et al., 1995). Dynorphin has been shown to be particularly involved in the phenomenon of dysphoria during the withdrawal from cocaine (Shippenberg and Rea, 1997). Nevertheless, further studies are require to confirm an eventual role of these transcriptional factor in regulation of gene primordial to neuroplasticity such as BDNF or receptors involved in long term potentiation or depression.

1.3.3.3 Clinical studies implicating $GABA_B$ in addiction

Emerging clinical data indicate that decreased GABAergic function may represent a major etiological step in the development and maintenance of the addictive state suggesting that manipulations that target the GABA system may be useful as treatments for addiction. For example, it has been demonstrated that the alcoholic withdrawal state induce is associated with reduced plasma and cerebrospinal fluid GABA (Coffman and Petty, 1985; Adinoff et al., 1995). In addition recent studies, using brain imaging technology, reported decrease in of GABA concentration in dorsal prefrontal cortex and occipital cortex of cocaine abusers (Hetherington et al., 1997; Ke et al., 2000).

Concerning alcoholism, preliminary human trial with baclofen has been somewhat encouraging. In a preliminary study, Addolorato and collaborators investigated the effects of short-term baclofen administration on alcoholic patients (Addolorato et al., 2000). Thus, they demonstrated that baclofen, administrated for 4 weeks, reduced alcohol craving from the first week the drug administration and remained so throughout the entire treatment period. They also reported that most of subjects exhibit less obsessional thinking about alcohol. In line with these results, Flannery and collaborators, using open-label design, demonstrated that baclofen treatment for, 12 weeks, decrease significantly the number of drinks per dinking day and the number of heavy drinking days and in consequence increase abstinent days (Flannery et al., 2004). In addition, they also reported that baclofen treatment significantly decreased anxiety and craving. Interestingly, a single self-case report by physician reported that baclofen reduces the severity of alcohol craving and produces complete abstinence for 9 consecutive months (Ameisen, 2005).

For opiate addiction, a 12-week, double-blind, placebo controlled, parallel-group experiment in recently detoxified opioid-addicted patents demonstrated that baclofen produced significantly higher treatment retention and non-significant but favorable trend to reduce opioid craving and self-reported alcohol and opiate use (Assadi et al., 2003). Nevertheless, inspection of opiate levels in urine failed to demonstrate differences between baclofen-treated and placebo treated patients ref.

Regarding addiction associated with psychostimulant, such as cocaine, a preliminary open-label, 18 week study revealed that baclofen produced a reduction in both cocaine craving and use, which was verified by urinalysis (Ling et al., 1998). However, patients didn’t report an effect of baclofen of cocaine ”highs” states. More recently, Shoptaw and collaborators, using a follow-up 16-week, double-blind, placebo-controlled study in 70 patients, demonstrated that baclofen significantly reduced cocaine use which was verified by

urinalysis (Shoptaw et al., 2003). Surprisingly, they also shown that baclofen is more effective in subjects with a high consumption of cocaine compared to subjects with low consumptions. In order to confirm the potential anti-addictive properties of baclofen, a recent study , designed with 7 non-treatment seeking volunteers with recent histories of cocaine use, demonstrated that baclofen failed to affect behavioural and cardiovascular effects of intranasal administration of cocaine (Lile et al., 2004). Interestingly, a study, using PET (Positron Emission Tomography), showed that cocaine users given baclofen 1-2 hours before watching a video of cocaine cues showed a reduced cocaine craving and less activation of anterior cingulated and amygdala compared to placebo treatment (Brebner et al., 2002). Consequently, it is becoming evident that $GABA_B$ receptor is involved in the phenomenon of relapse and craving.

Taken together, these studies confirmed that $GABA_B$ activation, via treatment with an agonist, can be viewed a plausible and rational pharmacology strategy for addictive disorders and that $GABA_B$ receptor may play a role in addictive disorders. Nevertheless, the majority of these studies used the prototypical $GABA_B$ receptor agonist which has been demonstrated to have a wide range of side-effects including sedation and muscle relaxant properties (Bowery et al., 2002). Although several reports suggested that tolerability might be adequate, a few reports demonstrated that abrupt cessation of baclofen treatment could elicit withdrawal, including hallucinations, fever, delirium, hypotension, bradycardia (Peng et al., 1998). At this point, the recent development of $GABA_B$ receptor positive modulator, see section 1.2.4.3, could represent a more interesting approach to avoid these side-effects.

1.3.3.4 Preclinical studies on the role of $GABA_B$ receptors in addiction

It has been long known that GABA plays an important role in the modulation of dopaminergic reward circuit. Thus, GABAergic neuronal inputs have been shown to regulate neural tone within the ventral tegmental area (Kalivas et al., 1990) and the nucleus accumbens

(Christie et al., 1987), suggesting that $GABA_B$ receptors might be involved in "reward system" and addictive behaviours. Thus, intra-VTA infusion of baclofen has been shown to block the enhancement of nucleus accumbens induced by the opioid agonist DAMGO (Kalivas et al., 1990); confirming that activation of $GABA_B$ receptors on dopaminergic cell counteract meso-accumbens dopaminergic function. More recently, systemic administration of baclofen has been demonstrated to attenuated cocaine-, nicotine, and morphine-induced enhancement of nucleus accumbens dopamine as measured by microdialysis (Fadda et al., 2003), suggesting that baclofen might alleviate reinforcing properties of these drugs of abuse in behavioural paradigm. Thus, in contrast to anxiety and depression, an extensive number of studies have assessed the effects of $GABA_B$ receptor ligands in animal models related to addictive disorders.

As we discussed previously, locomotor activity is critically dependent on activation of dopaminergic neurotransmission. Thus, several investigators have assessed the effects of $GABA_B$ receptor activation on the locomotor stimulation induced by psychostimulants or another drugs of abuse. Using locomotor activity as a potential behavioural index of dopaminergic activity, Cott and collaborators demonstrated that baclofen eliminates the hyperactivity induced by acute administration of alcohol (Cott et al., 1976). Similarly, systemic administration of baclofen has been demonstrated to attenuate hyperactivity induced by apomorphine, a dopamine D1-D2 receptor agonist, and d-amphetamine (Phillis et al., 2001). Although these results can't provide information on the effects of $GABA_B$ receptor activation on the reinforcing properties of drugs, they support the fact that $GABA_B$ receptor activation might attenuate dopaminergic stimulation elicited by drugs of abuses. It is clear that the sedative effects of baclofen may confound interpretation of such data.

In self-administration paradigms, baclofen has been shown to dose dependently reduces cocaine self-administration under a "fixed ratio" reinforcement (Shoaib et al., 1998;

Campbell et al., 1999; Di Ciano and Everitt, 2003), and induced a dose-dependent reduction of progressive ratio breaking point for intravenous cocaine (Roberts et al., 1996). Similarly, baclofen has also been demonstrated to reduce the breaking point maintained by methamphetamine and nicotine (Ranaldi and Poeggel, 2002; Markou et al., 2004), suggesting that when is administrated systemically, baclofen attenuates the reinforcing properties of these drugs. In addition, a few studies have shown that micro injection of baclofen in the ventral tegmental area also inhibits also nicotine and cocaine self-administration (Shoaib et al., 1998) (Corrigall et al., 2000). As expected, the highly selective $GABA_B$ receptor CGP44532 shows similar anti-addictive properties as seen with baclofen in cocaine self-administration paradigm, decreasing the progressive ratio breaking point maintained by cocaine (Roberts and Brebner, 2000). More recently, a study, using $GABA_B$ receptors positive modulator CGP7930 and GS39783, reported that $GABA_B$ receptor positive modulation suppresses the initiation of cocaine self-administration (Smith et al., 2004), as it was previously observed with baclofen (Roberts et al., 1996). Thus, these data suggested that $GABA_B$ activation, via both agonist and positive modulator alters reinforcing properties of drugs in the self-administration paradigm.

As we previously mentioned in section 1.3.3.3, $GABA_B$ receptor might be involved in the phenomenon of relapse. Thus, baclofen dose-dependently reduced cocaine-induced reinstatement triggered by re-exposure to cocaine (Campbell et al., 1999). In lines with these data, $GABA_B$ receptor agonist, CGP44532, have been shown to inhibit cue-triggered relapse to nicotine seeking behaviour (Paterson et al., 2005). Finally, Hotspenpiller demonstrated that systemic baclofen administration cocaine-conditioned locomotion in rats (Hotsenpiller and Wolf, 2003) confirming the potential anti-relapse properties of baclofen.

Regarding the ICSS paradigm, baclofen, CGP44532, and GS39783, the $GABA_B$ receptor positive modulator, have been shown to attenuate reinforcing properties of cocaine in this paradigm (Dobrovitsky et al., 2002; Slattery et al., 2005b). Nevertheless, Slattery and

collaborators reported that baclofen, but not GS39783, administration elevates ICSS reward thresholds in absence to cocaine suggesting anhedonic and/or sedative effect of baclofen at the doses used. Similarly results were obtained with CGP44532 (Macey et al., 2001).

In the place conditioning paradigm, microinfusion of baclofen in the ventral tegmental area blocks the acquisition of the morphine-induced place preference and both the acquisition and the expression of alcohol-induced place preference (Tsuji et al., 1996; Bechtholt and Cunningham, 2005). In addition, systemic administration of baclofen blocks the acquisition of morphine induced-place preference and both acquisition and expression of place preference elicited by d-methamphetamine (Li et al., 2001; Kaplan et al., 2003). Although these data strongly suggest that baclofen could attenuate reinforcing properties of drugs of abuse, it should be noted that the place preference has an important cognitive component. Thus, the effects observed systemically with baclofen could be partially due to its amnesic properties (Swartzwelder et al., 1987).

Finally, several groups assessed the effects of $GABA_B$ receptor activation on the phenomenon of sensitization to drugs of abuse. Indeed, systemic injection of baclofen blocks both the establishment and the expression of behavioural sensitization to amphetamine (Bartoletti et al., 2005). Similarly, Broadbent and Harless reported that systemic injection of baclofen attenuate the establishment of behavioural sensitization to ethanol (Broadbent and Harless, 1999). More recently, two studies demonstrated an efficacy of local injection baclofen on behavioural sensitization to drugs of abuse. Leite-Morris demonstrated that baclofen injection in the ventral tegmental area blocks both establishment and the expression of morphine induced sensitization (Leite-Morris et al., 2004). Secondly, Steketee and Beyer demonstrated that intra-prefrontal cortex injection of baclofen attenuates the establishment of cocaine sensitization in rats, suggesting that $GABA_B$ receptor of both structure might be involved in sensitization phenomenon (Steketee and Beyer, 2005). Taken together, these data

demonstrated that baclofen might also counteract long-lasting mechanism involved in sensitization phenomenon.

To conclude, this general overview of the effect of $GABA_B$ receptor ligands in several model of addiction led to the belief that $GABA_B$ receptor activation represents a potential new therapy for drug addiction. Nevertheless, the anhedonic-like effect of baclofen observed in the ICSS paradigm combined with its sedative properties could limit its widespread utility in the context of behavioural pharmacology. Thus, the encouraging result obtained using $GABA_B$ receptor positive modulators in ICSS and self-administration paradigms support the use of this pharmacological approach for the assessment of the role of $GABA_B$ receptor in addictive behaviours.

1.4 Specific Objectives of the Research

Although $GABA_B$ receptors were first proposed to play a role in psychiatric disorders such as depression and anxiety over 20 years ago (Pilc and Lloyd, 1984), further progress in the field has been largely hampered by the lack of appropriate tools. Therefore, they have been little interest in the role of $GABA_B$ in neuropsychiatric disorders in the decade preceding the initiation of my research for this thesis in 2002. The recent development of new pharmacological and genetic tools offers a novel opportunity to investigate their contributions in psychiatric disorders. Consequently, the aim of this thesis was to examine the role of $GABA_B$ receptors in anxiety, depression and addiction, using these novel tools. We consciously decided to adopt a multidisciplinary approach to dissect the relevance of $GABA_B$ receptors as a therapeutic target for all three of these psychiatric disorders, namely anxiety, depression and addiction. Furthermore, the comorbidity of these three disorders validated this approach (Markou et al., 1998).

In the first part of the present thesis, we evaluate the impact of targeted deletion of either receptor subunit, in order to confirm the conventional view that heterodimerization of $GABA_{B(1)}$ and $GABA_{B(2)}$ is a prerequisite for $GABA_B$ receptor function. To this purpose, we assessed the effect of genetic ablation of either of $GABA_B$ receptors subunit on hypothermic and motor deficit elicited by the prototypical $GABA_B$ receptor baclofen. To investigate the contribution of $GABA_B$ receptors to GHB action, we evaluated also the hypothermic response elicited by GHB in $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice.

Although GABA-mediated neurotransmission has long been known to have a crucial role in anxiety, there are only sporadic studies investigating the specific contribution of $GABA_B$ in this pathology. This is largely because investigators have used baclofen for their investigations, which has a narrow window of efficacy before confounding side-effects are

manifested in anxiety paradigms. The recent development of $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice and $GABA_B$ positive modulator, offer a novel opportunity to investigate the role of $GABA_B$ receptors in anxiety. Thus, we assessed the effect of targeted deletion of either of $GABA_B$ receptor subunit in well validated models of anxiety, namely the light-dark box test, the staircase test and the elevated-zero maze. In order to corroborate the effect obtained in $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice pharmacologically, we assessed also the effect of a highly potent $GABA_B$ receptor antagonist in the light-dark test. Finally, we took the advantage that $GABA_B$ receptor positive modulators are devoid of side effects associated to $GABA_B$ receptor full agonist, to confirm prior findings supporting a anxiolytic like effect of $GABA_B$ receptor stimulation. Thus, we evaluate the effects of GS39783 in several paradigms of anxiety, acutely and chronically.

As mentioned in the introduction, there is growing body of evidence for a putative involvement of $GABA_B$ receptors in depressive disorders. Considering these prior studies, we hypothesized that genetic ablation of $GABA_B$ receptor might result in antidepressant-like phenotype in mice. To this purpose, we assessed the effect of targeted deletion of either $GABA_B$ receptor subunits in well validated model of antidepressant, namely the forced swim test and the tail suspension test. In order to exclude potential false positive result in these test due to hyperactivity, we secondly measured locomotor pattern of $GABA_B$ knock out animals in a new environment. In order to confirm the antidepressant-like phenotype of the $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice pharmacologically, we assessed the effects of both $GABA_B$ antagonist CGP56433A and $GABA_B$ receptor agonist in the FST and also measured their effects on locomotor activity. Finally, we investigated the interaction of $GABA_B$ receptors and serotoninergic system in the context of depression. Thus, we evaluate the effects of $GABA_B$ receptor antagonist on the antidepressant like-effects of SSRIs. Further, we assessed the effects of $GABA_B$ receptors ligands on the behavioural effects of SSRI and the expression

of 5-HT_{1A} receptor mRNA in the hippocampus. Conversely, we also investigated the impact of pharmacological blockade or genetic ablation of serotonin transporter, on $GABA_B$ receptor function using baclofen induced hypothermia as index of $GABA_B$ receptor function.

Finally, we decided to investigate the role of $GABA_B$ receptors on behavioural and molecular alteration relevant to addictive disorders. To this purpose, we assessed the effect of $GABA_B$ receptor, via administration of baclofen or $GABA_B$ positive modulator, on behaviour and molecular changes elicited by single and repeated administration of drugs of abuse. In a first experiment, we investigated the effect of $GABA_B$ positive modulator and baclofen on hyperactivity and c-fos induction elicited by a single administration of cocaine. Secondly, we evaluated the ability of GS39783 to modulate behavioural sensitization to cocaine and its associated molecular adaptation, namely CREB and DARPP-32 activation and ΔFosB accumulation. In effort to recapitulate these effect in more ethological model of addiction, we decided, finally, to assess the effect of $GABA_B$ receptors positive on conditioned place preference triggered by nicotine and its associated ΔFosB accumulation in nucleus accumbens.

CHAPTER 2 :GENERAL MATERIALS AND METHODS.

2.1 Animals.

2.1.1 Mice.

The $GABA_{B(1)}$ and $GABA_{B(2)}$ knockout mice were generated on a BALB/c genetic background as described previously (Schuler et al., 2001; Gassmann et al., 2004). In order to reduce the influence of strain effects all pharmacological studies were carried out in male BALB/c mice which were obtained from Charles River, France (Formally Iffy Credo). In addition, BALB/c mice have been shown to be highly emotional, or anxious, relative to other strains in several anxiety paradigm such as light-dark box or open-field test (Griebel et al., 2000; Kim et al., 2002). Furthermore, this strain has also been shown to be highly sensitive to the effects of chronic SSRI in forced swim test and novelty-induced hypophagia, confirming that the use of this strain is particularly relevant in anxiety and depression research (Dulawa et al., 2004). Nevertheless, this strain has been also demonstrated to be insensitive to psychostimulant properties of drugs of abuse (see section 6.1). Consequently, all experiment related to cocaine were carried out with C57BL/6J mice which were obtained from Charles River, France.

2.1.2 Rats.

Regarding the studies investigating the effect of role of GS39783 on nicotine-induced place preference, we choose male Wistars Rats (Charles River, France) as a robust place preference to nicotine had been previously validated in our laboratories.

2.2 Assessing $GABA_B$ receptor function in vivo.

2.2.1 Baclofen-induced hypothermia.

Baclofen induces marked hypothermia which can be easily monitored using a rectal probe. This drop in temperature gives a robust indicator of the function of $GABA_B$ receptors in vivo (Serrano et al., 1985; Gray et al., 1987; Jacobson and Cryan, 2005). Rectal temperature was measured to the nearest 0.1 °C by an ELLAB instruments (Copenhagen, Denmark) thermometer Model DM 852 by inserting a lubricated thermistor probe model PRA-22002-A (ELLAB, Copenhagen, Denmark) (2.2 mm diameter) 20 mm into the rectum; the mouse was hand held at the base of the tail during this determination and the thermistor probe was left in place for 15 s. Animals are habituated to the thermometer prior to administration of baclofen

2.2.2 Baclofen-induced impairment of motor coordination.

Rotarod test

Baclofen induces motor impairment which can be easily monitored using the rotarod test. This ability of baclofen to induce motor incoordination also gives a robust indicator of the function of $GABA_B$ receptors in vivo (Schuler et al., 2001; Jacobson and Cryan, 2005). The rotarod apparatus consists of 28 mm diameter rod partitioned into five available lanes 58 mm wide to accommodate individual mice. The rotarod apparatus consists of a cylinder subdivided into five available mice positions of each 6 cm in diameter, which is positioned 30 cm above the Table and rotates at a speed of 12 rpm (Dunham and Miya, 1957). The mice were placed singly on the cylinder. On the day before the start of the experiment animals were trained over two separate sessions to stay on the rotarod for 300 seconds. During the test day the length of time each mouse remained on the cylinder (= ‘endurance time’, maximal score 300 sec) was measured.

Fig.2.1. The Rotarod apparatus.

2.3 Assessing anxiety behaviour in mice.

2.3.1 Light-dark box test.

The light-dark box test was carried out essentially as described previously (Holmes et al., 2002; Cryan et al., 2003c). This paradigm is one of the most widely used of exploratory-based approach avoidance conflicts tests in mice. It's based on the innate aversion of mouse to illuminated areas. The apparatus consisted of a clear Plexiglas cage (44 x 21 x 21 cm) separated into two compartments by a partition, which had a small opening (12 x 5 cm) at floor level. The open compartment was open topped made of transparent Plexiglas and brightly illuminated by a 60 W desk lamp overhead (approx 1000 Lux). The smaller compartment was 14 cm long and made from black Plexiglas. It was covered on top also by black Plexiglas. Mice were individually placed in the centre of the brightly lit compartment,

facing away from the partition and allowed to freely explore the apparatus for 10 minutes. The apparatus was cleaned thoroughly between subjects. The number of light-dark transitions, time spent in the light compartment and latency to enter dark were recorded by a trained observer, with transitions being the most reliable indicator of anxiety-like behaviour in the test (Crawley and Davis, 1982; Holmes, 2001).

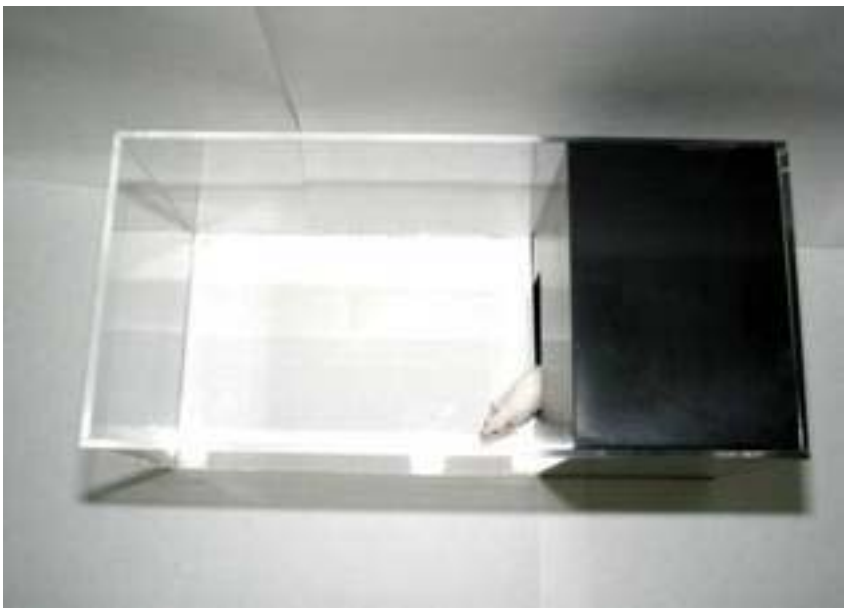

Fig.2.2. The light-dark box.

2.3.2 Staircase test.

Like light-dark box paradigm, staircase test in also exploratory-based approach avoidance conflicts tests. This paradigm exploits the natural tendencies of mice to avoid height. The test was carried out essentially as described earlier (Simiand et al., 1984; Cryan et al., 2003c) and consists of placing an experimentally naïve mouse in an enclosed staircase with five steps made of grey plastic. Each step was 2.5 cm in height 7.5 cm in length and 11 cm in width. The apparatus was 45cm in length with one end 12 cm and the other 25 cm in height. The number of steps climbed and rearings made in a 3-min period was observed. The step-climbing count was increased every time the animal moved from one step to another in

the ascending direction. The apparatus was briefly wiped with a wet paper towel and dried between animals. Animals were moved to the testing room at least one hour prior to testing. The test has been validated using different anxiolytics (Simiand et al., 1984; Pick et al., 1996; Weizman et al., 1999) and has been used to examine anxiety-related phenotypes in genetically modified animals (Cryan et al., 2003c; Salas et al., 2003). The number of steps climbed and the rearing behaviour of the mice are recorded as measures of anxiety-related behaviour.

Fig.2.3. The staircase test.

2.3.3 Elevated-zero maze.

The elevated-zero maze is also a widely used anxiety paradigm. This test use spontaneous tendency of rodents to avoid open areas. In contrast to the elevated-plus maze, it has no central area removing any ambiguity in interpretation of the time spent on the central area of the elevated-plus maze allowing uninterrupting exploration. The apparatus was a 5.5-cm-wide circular track constructed of grey Plexiglas with an inside diameter of 34 cm, a mid-track circumference of approximately 121 cm, and an elevation of 40 cm. It consisted of two

open quadrants with a raised, 2-mm edge and two closed quadrants with walls 11 cm high. Mice were placed in one of the closed quadrants designated as the starting quadrant and were allowed to investigate the zero maze for a period of 5 min. During this time, an observer scored mice on several anxiety-related variables as identified in previous studies (Shepherd et al., 1994; Tarantino et al., 2000). These included times spent in both open and closed quadrants, number of transitions between quadrants, latency to leave the closed quadrant, stretchings (elongated body posture with at least snout over open/closed divide) into open quadrant, rearings, head-dips.

Fig.2.4. Elevated-zero maze.

2.3.4 Stress-induced hyperthermia.

As mentioned in the introduction, the stress-induced hyperthermia is a model of anticipatory anxiety. The test procedure for the modified stress-induced hyperthermia was adopted from Van der Heyden et al. (Van der Heyden et al., 1997) and is based on the original

description of stress-induced hyperthermia by Lecci et al. (Lecci et al., 1990): rectal temperature was measured to the nearest 0.1 °C by an ELLAB instruments thermometer (Copenhagen, Denmark) via a lubricated thermistor probe (2 mm diameter) inserted 20 mm into the rectum while the mouse was hand held near the base of the tail. The probe was left in place until steady readings were obtained (± 15 seconds). Twenty-four hours before testing the animals were individually housed in smaller cages (26 x 21 x 14 cm). Stress-induced hyperthermia was assessed as follows: The core-temperature of each mouse was measured twice. The second measurement (T2) was 15 minutes after the first measurement (T1), which served as the basal value for each condition. The dependent variable, i.e. the stress-induced hyperthermia, was defined as the difference between T2 - T1. T1 was used to evaluate whether the test-compound by itself would have a potential effect on basal body temperature.

2.3.5 Novelty-induced hypophagia.

The novelty-induced hypophagia is anxiety paradigm with a high predictive validity. Indeed, it has been shown to be highly sensitive acute administration of benzodiazepines, but also to chronic administration of SSRI. In this paradigm, the exposure to a novel environment is anxiety-provoking leading to an increase in latency to drink and decrease in food consumption. In the present thesis, the novelty-induced hypophagia was adopted from that described by Dulawa et al. (Dulawa et al., 2004). Mice were singly housed one week prior experiment began. Mice were trained to drink diluted (1 : 3; milk : water) sweetened condensed milk for 30 min. during 3 consecutive days. Milk was presented in 10 ml serological pipettes cut at the bottom and closed with a 0.5 ml ependorff. Pipettes were positioned through wire cage lids. Home cage testing occurred on day 4 when mice were briefly removed from their cages to position pipettes containing the milk, and testing began when mice were returned to their cages. The latency to drink and the volume consumed were recorded every 5 min for 30 min. Home cage testing occurred under normal lighting. Novel

cage testing occurred on day 5, when mice were placed into new clean cages of the same dimensions but without shavings, with pipets containing the milk positioned. In order to enhance the aversiveness of the new environment, novel cage testing occurred under bright lighting (approx. 1300 lux), with white paper placed under cages.

2.4 Assessing antidepressant-like activity in animals.

2.4.1 Forced-swim test.

As mentioned in the introduction, the forced swim test is probably the most extensively used paradigm to screen antidepressant. Here, it was conducted as previously described (Cryan et al., 2001; Cryan et al., 2003c). Briefly, mice were placed individually into plexiglass cylinders (24 cm tall x 21 cm in internal diameter) filled with water (23 – 25˚C) to a depth of 15 cm. All test sessions were recorded by a video camera positioned directly above the cylinders. Videotapes were subsequently scored blind by a trained observer. The behavioural measure scored from videotape was the duration of immobility during the last four minutes of the six minute test period as previously validated (Porsolt et al., 1978). A mouse was judged to be immobile when making only those movements necessary to keep its head above water.

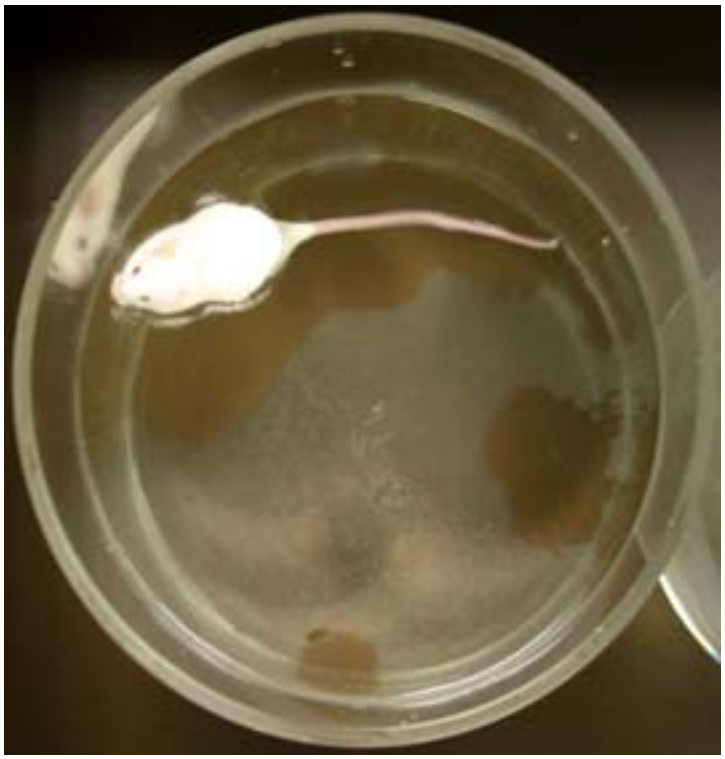

Fig.2.5. The forced swim test.

2.4.2 Tail suspension test.

The second animal of depression used in the present thesis is the tail suspension test. Like the forced swim test paradigm, this paradigm is highly predictive of antidepressive activity and widely used in depression research. The test was carried out essentially as described previously (Steru et al., 1985; Cryan et al., 2003c; Cryan et al., 2005) with the exception that an automated device was used to score immobility (BioSeb, Chaville, France)(Cryan et al., 2005). Mice were individually suspended by the tail to a metal hook (distance from floor = 18 cm) using adhesive tape (distance from tip of tail = 2 cm). Typically, mice demonstrated several escape-oriented behaviours interspersed with temporally increasing bouts of immobility. The computer recorded the number of seconds spent immobile over the entire 6 minute period.

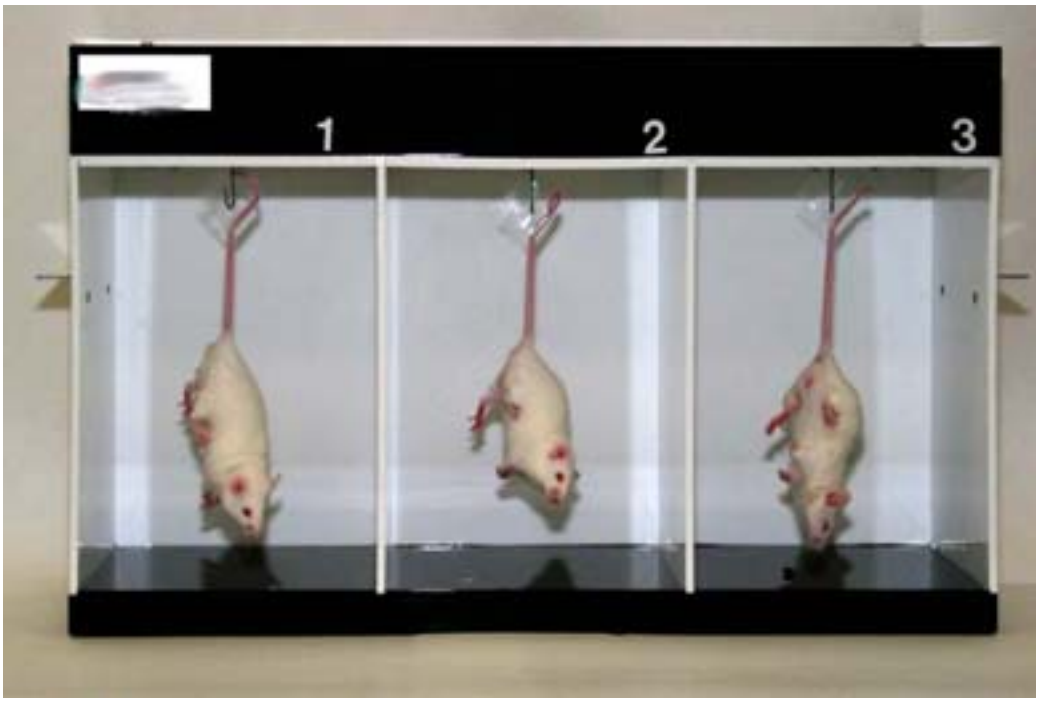

Fig.2.6.The tail suspension test.

2.5 Determination of 5-HT_{1A} receptor mRNA expression in hippocampus.

2.5.1 RNA isolation, DNAse I digest and reverse transcription

Hippocampi were disrupted (2 x 2 minutes at 20Hz) using a Mixer Mill 300 (Qiagen). Total RNA was isolated from disrupted brain tissue using the “absolute RNATM” RT-PCR Miniprep Kit (Stratagene) according to manufacturer’s protocol. The quantity and quality of the RNA was determined by optical density (OD) at 260 nm and 280 nm (NanoDrop®). To remove traces of genomic DNA contamination, a small aliquot of total RNA was digested with RNAse-free DNAse (RNAse-free DNAse Set, Qiagen). The enzyme was inactivated by addition of EDTA and heating up to 65°C. After the DNAse I digest, a spot check of the RNA samples was performed by real-time PCR assays to ensure the absence of genomic DNA. DNAse I-treated total RNA was reverse transcribed into cDNA by random priming using the StrataScriptTM Reverse Transcriptase kit (Stratagene).

2.5.2 Real-time quantitative RT-PCR

5-HT_{1A} primers and probe (see Table 1) were designed with the aid of the Primer Express software (Version 1.0; Applied Biosystems). Probes were labeled with 6-carboxyfluorescein (FAM) at the 3′-end and with 6-carbox-tetramethyl-rhodamine (TAMRA) at the 5′-end. For both the amplification of 5-HT_{1A} and the internal control 18S cDNA, the standard amplification program was used (1 cycle of 50°C for 2 min, 1 cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min). Direct detection of the PCR product was enabled by the 5′→3′ exonuclease activity of Taq polymerase, thereby terminating the close proximity of the reporter dye to the quencher dye by reporter fluorochrome cleavage leading to an increase of reporter dye fluorescence equivalent to the increase of amplified PCR product. Real-time RT PCR assays were performed using the qPCRTM Mstermix (Eurogentec) on an ABI Prism® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). 5-HT_{1A} primers and probe were used at concentrations of 300 nM and 175 nM, respectively. Real-time RT PCR assays for the internal control gene 18S rRNA were performed by using the “18S Genomic Endogenous Control Kit” (Eurogentec).

Relative levels of target transcripts are reported after normalization to 18S rRNA. The normalized raw data were analyzed with the so-called “comparative C_T method” as described in detail in Livak and Schmittgen, 2001(Livak and Schmittgen, 2001).

2.6 Locomotor activity and animal models relevant to addiction.

2.6.1.Locomotor activity.

Animals were placed in automated locomotor activity cages (31 cm length, 19 cm width, 16 cm height; TSE, Bad Homburg, Germany) and the distance traveled was measured

by the number of horizontal beam-breaks as previously described (Spooren et al., 2000). Data were collected using personal computer in 5-minute intervals.

Fig.2.7.Locomotor activity chamber.

2.6.2 Behavioural sensitization to cocaine.

The mice were habituated to the test environment for three days and basal locomotor activity was measured. After an intraperitoneal injection of saline the mice were placed in the test cages (as above) for 30 min and locomotor activity was recorded. From days 4-10, mice were injected with cocaine (20 mg/kg/d, i.p.) or saline and locomotor activity was recorded. To assess the effects of drugs on the acquisition of behavioural sensitization to cocaine, tested drugs or its associated vehicle was applied 30 minutes before each cocaine injection. This period of acquisition of sensitization was followed by 14 days without drug treatment. In order to investigate the effect of GS39783 on the expression of cocaine sensitization, we designed a challenge trial (see e.g. (Kalivas and Stewart, 1991). On day 23 (challenge day), mice were administrated a dose of 10 mg/kg i.p. cocaine. In order to assess the effect of a

drug on the expression of sensitization, drugs or its associated vehicle was applied 30 min prior to the cocaine injection.

2.6.3 Place conditioning paradigm.

The apparatus was as described previously (Chaperon and Thiebot, 1996). Briefly, rats were trained and tested in black wooden open fields (76×76×50 cm) located in a room dimly lit room (red-light). The floor of each open field was covered with removable quadrants made from one of two textures, wire mesh or rough plexiglas. These textures were chosen on the basis of previous studies indicating that naive rats exhibited no unconditioned preference for either of them.

The general procedure consisted of two phases: conditioning and testing. Conditioning consists of an alternation of four drug-paired conditioning sessions with four vehicle-paired sessions. During drug-paired sessions, drugs were administered prior to exposure to the open-field covered with one floor texture. During the vehicle-paired session, rats received vehicle injection a 30 min exposure to the other floor texture. For half of rats, the first conditioning session was a vehicle-paired session, and for the other half a drugs-paired session. The day following the last conditioning session, rats were placed for a single 20 min testing session in the open field whose floor was made up of two quadrants of the vehicle-paired texture and two quadrants of the dug-paired texture. The quadrants of the same texture were positioned diagonally opposite to each other floor texture. During the test session, time spent on each texture was automatically recorded by means of the video system and analyzed by appropriate in-house developed software (SuperG Software, Hans C. Neijt; Novartis Pharma, Basel, Switzerland).

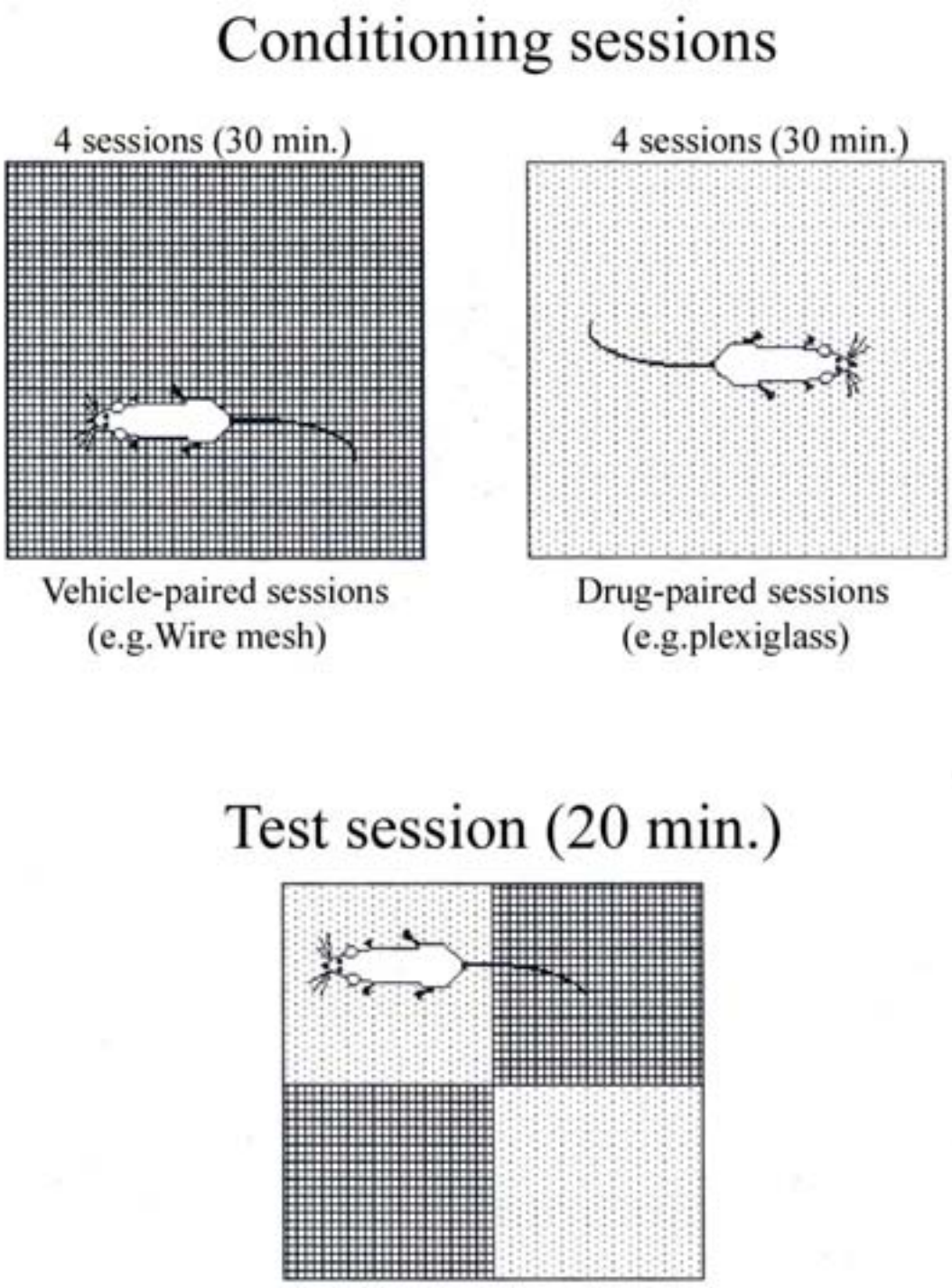

Fig.2.8. Place conditioning paradigm.

2.7. Determination of specific molecular markers affected by administration of drugs of abuse.

2.7.1 Tissue Preparation

Brains were quickly removed, chilled in ice-cold phosphate-buffered saline (PBS) and nucleus accumbens, dorsal striatum and hippocampus were dissected on ice-chilled glass plate and flash-frozen in dry ice.

2.7.2 Immunoblotting

Individual tissue samples for western blot analysis were homogenized with a glass-glass homogenizer in ice-cold buffer containing (in mM) Hepes (pH 7.9) 20, NaCl 400, $MgCl_2$ 5, EDTA 0.5, EGTA 0.1, including complete protease (Roche) and phosphatase inhibitors (Sigma). Homogenates were kept 20 min on ice and centrifuged 15 min at 20000 g, 4°C. Pellets containing particulate fractions enriched in organelles and nuclei were mixed with 2X Laemmli sample buffer (125 mM Tris pH 6.8, 4% sodium dodecyl sulfate, 0.005% (w/v) bromophenol Blue, 200 mM dithiothreitol, 20% glycerol), heated to 90°C for 5 min and loaded on 10% SDS acrylamide gels (Bio-Rad). After electrophoretic transfer (Biorad blotting device), nitrocellulose membranes were incubated 1 hour at room temperature in PBS containing 0.1% Tween 20 and 5% fat-free powdered milk (PBST/milk). After three washes in PBST the membranes were incubated overnight at 4°C with primary antibodies in PBST/milk, and washed again three times in PBST. Incubation with horseradish-peroxydase-conjugated secondary antibody was for 1-2 hours at room temperature. Peroxydase activity was detected using Supersignal West Pico substrate (Pierce) and Kodak MR-1 X-ray films (Amersham Biosciences). Afterwards, bound antibodies were removed (restore buffer, Pierce) and the membranes incubated with an anti-actin antibody to check for loading. X-ray films were scanned and analyzed with NIH ImageJ software (http://rsb.info.nih.gov/ij/, v1.31) according to manufacturer's indications. Intensity values are presented as the ratio of the

optical density of the band of interest and of its actin control. Individual samples were not pooled and care was taken not to overexpose X-ray films in order to ensure linearity of signals. All experiments were performed in duplicate or triplicate.

2.7.3 Reagents

Primary antibodies used were rabbit anti- Fos (sc-253, Santa Cruz Biotechnology, 1:1000), rabbit anti-FosB (sc-48, Santa Cruz Biotechnologies, 1:500), mouse anti-phosphoCREB (clone 1B6, Cell Signaling, 1:1000), rabbit anti-DARPP-32 (2302, Cell Signaling, 1:2000), rabbit anti-CREB (sc-58, Santa Cruz Biotechnology, 1:1000), rabbit anti-actin (A2066, Sigma, 1:5000). Secondary antibodies were HRP-linked goat anti-mouse IgG (1:2000, Bio-Rad) or goat anti-rabbit IgG (7074, Cell Signaling, 1:1000 to 1:5000). All other reagents are from Sigma.

2.8. Drugs

Desipramine, chlordiazepoxide, diazepam, cocaine and (-)-nicotine were obtained from Sigma (St. Louis, MO). Fluoxetine, L-baclofen, GS39783 (N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine), CGP56433A (3-{1(*S*)-[3-(cyclohexylmethyl)hydroxyphosphinyl)2(*S*)hydroxypropylamino]nethyl} benzoic acid) and CGP44532 (((S)-3-amino-2-hydroxy-propyl)-methyl-phosphinic acid) were synthesized in-house. In mice, all drugs were made up fresh prior to use and administered orally in a suspension of 0.5% methylcellulose at a concentration of 10 ml/kg expected cocaine which was administrated intra-peritoneally and dissolved in NaCl 0.9%. Regarding rat studies, GS39783 were dissolved in a suspension of 0.5% methylcellulose at a concentration of 5 ml/kg and administrated orally. (−)Nicotine bitartrate free base dissolved in NaCl 0.9%, and the pH was adjusted between 7.3 and 7.5 with a few drops of 0.1 M NaOH; and administrated at a concentration of 5ml/kg sub-cutaneously.

2.9. Statistics

For all behavioural studies, all data were analyzed using the appropriate within-subject, and mixed-design ANOVAS or Student's *t*-test (in the case of comparisons between just two groups of animals) followed by where appropriate by Fishers's posthoc tests, using the SYSTAT software package. The level of significance was set at $P < 0.05$. Data from Section 5.5.3 were analyzed using t-test. Indeed, each group was compared to their proper controls groups. Specifically, chronic handled animals were compared to naïve animals and animals treated with $GABA_B$ ligands and antidepressants were compared individually to chronic handled animals. Finally, all molecular results obtained in chapter 6 were analyzed using the appropriate within-subject, and mixed-design ANOVAS or Student's *t*-test (in the case of comparisons between just two groups of animals) followed by *post hoc* Newmann-Keuls test where appropriate.

CHAPTER 3: IMPACT OF TARGETED DELETION OF $GABA_B$ RECEPTOR SUBUNITS ON $GABA_B$ RECEPTOR FUNCTION: IN VIVO STUDIES.

3.1 Introduction.

In vivo assays receptor function using selective agonists using physiological or behavioural readouts of have been long employed to probe alterations in receptors following specific manipulations (e.g. (Goodwin et al., 1985; Cryan et al., 1999)). A key necessity for such research strategy is to have a robust and rapidly obtainable measure that is able to capture in a temporal and dose dependent manner the actions of the agonist. This is certainly been the case for $GABA_B$ receptors where the selective tool baclofen to probe $GABA_B$ receptor function in both animals and humans.

Among other effects in vivo, stimulation of $GABA_B$ receptors, is known to produce a marked hypothermia which parallels behavioural changes, including impairment of motor coordination (Brogden et al., 1974; Gray et al., 1987; Schuler et al., 2001; Cryan et al., 2004). This effect is considered to be a central origin, since intracerebroventricular injection of the prototypic $GABA_B$ receptor agonist baclofen lowers body temperature. The specific site at which $GABA_B$ receptor agonists act is unknown, but it probably resides within the hypothalamic thermoregulation center (Yakimova et al., 1996). For these reasons, it has been well accepted that baclofen-induced hypothermia might be considered as a good in vivo index of $GABA_B$ receptor function, and in particular the function of hypothalamic $GABA_B$ receptors.

Thus, several investigators have assessed the effects of genetic or pharmacological treatment on this parameter. $GABA_B$ receptor antagonists have been shown to block the

hypothermic response to baclofen (Jackson and Nutt, 1991; Humeniuk et al., 1995). More recently, two different groups used this phenomenon in order to confirm the loss $GABA_B$ receptor function in mice lacking $GABA_{B(1)}$ receptor subunit (Schuler et al., 2001; Queva et al., 2003). In their studies, both of them observed that targeted deletion of $GABA_{B(1)}$ receptor subunit totally blocked hypothermic response associated to systematic administration of baclofen, suggesting that $GABA_B$ receptor function require $GABA_{B(1)}$ subunit.

Another widely used in vivo assay for $GABA_B$ receptor activation is motor incordination and sedation which can be measured using a rotarod or open field apparatus Baclofen has long been known to produce motor discoordination in a range of species such as mice (Gray et al., 1987; Schuler et al., 2001; Cryan et al., 2004; Gassmann et al., 2004; Jacobson and Cryan, 2005), rats (Kasture et al., 1996) and rabbits (Frosini et al., 2004). Clearly, it is these muscle relaxant effects that have made baclofen the drug of choice for treatment of spasticity in man (Bowery et al., 2002).

When assessing $GABA_B$ receptor function it is important to look at multiple readouts as they may be controlled by different populations of $GABA_B$ receptors. Indeed, recently Jacobson and Cryan (Jacobson and Cryan, 2005) have shown that certain mice strains had a marked hypothermic response to baclofen in the absence of motor impairing effects and vice-versa.

Accordingly, two different groups corroborated this hypothesis, using in vitro techniques, including electrophysiology and functional approaches. Indeed, these researchers observed that $GABA_{B(1)}^{-/-}$ lack pre or postsynaptic $GABA_B$ responses (Prosser et al., 2001; Schuler et al., 2001). Altogether these data support the idea that heterodimerization of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunits is a prerequisite for $GABA_B$ function (Marshall et al., 1999) excluding that $GABA_{B(2)}$ receptor subunit might assemble functional receptors by itself or in association with a protein other that $GABA_{B(1)}$.

Nevertheless, several findings lead to speculate that $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit might have separate role. Firstly, $GABA_{B(1)}$ exhibits a more widespread cellular distribution than $GABA_{B(2)}$ (Kaupmann et al., 1998a). In addition, despite the fact that $GABA_{B(1)}$ subunit require $GABA_{B(2)}$ for its delivery to plasma membrane (Margeta-Mitrovic et al., 2000), $GABA_{B(1)}$ was originally cloned by surface expression in mammalian cells (Kaupmann et al., 1997). Finally, $GABA_{B(1)}$ receptor subunit occasionally yields electrophysiological responses when transfected alone into heterologous cells (Kaupmann et al., 1997). Thus, it is conceivable that the $GABA_{B(1)}$ receptor subunit is functional either alone or in combination with unknown protein.

Like baclofen, γ-Hydroxybutyrate (GHB) have been shown to induced hypothermia in rodents (Snead, 1990) and in humans (Chin et al., 1998) and also induce sedation and motor incordination (Lobina et al., 2005). Although it has been proposed to function as neurotransmitter (Cash, 1994), its receptor interaction are a matter of much debate. As mentioned in the general introduction, two different theories coexist at this time. The first suggest that the action of GHB is mainly mediated by specific GHB receptors, but not by $GABA_B$ receptors. Indeed, it has been shown that GHB binding differ from the $GABA_B$ receptors binding (Snead, 1994). In addition, the putative GHB antagonist NCS-382 has been shown to have no affinity to $GABA_B$ receptors (Maitre et al., 1990). Conversely, the second theory supports the idea that GHB response might be $GABA_B$ receptor dependent. To clarify whether $GABA_B$ can participate in GHB response, we sought to assess the effect of targeted inactivation of $GABA_B$ receptors on hypothermia and rotarod performance induced by GHB.

Thus, the aims of the present chapter were three-fold. In order to confirm that genetic inactivation of $GABA_{B(1)}$ receptor subunit abolish $GABA_B$ receptor function, we analyzed both hypothermic and motor effects of baclofen in $GABA_{B(1)}^{-/-}$ mice. Secondly, using the recently generated $GABA_{B(2)}^{-/-}$ mice, we investigated the effect of targeted on the deletion

$GABA_{B(2)}$ on the physiological and behavioural effects of baclofen. Finally, we examined the effect of genetic ablation of $GABA_{B(1)}$ or $GABA_{B(2)}$ on the well characterized hypothermic response to GHB, in clarify which receptors can be involved in physiological response induced by GHB.

3.2 Experimental Design.

$GABA_{B(1)}$ and $GABA_{B(2)}$ knockout mice were generated on BALB/c genetic background as described previously (Schuler et al., 2001; Gassmann et al., 2004). Mice were used at an age of 10-17 weeks. Regarding baclofen experiments, animals were placed on the rotarod cylinder immediately before and 1, 2 and 4 hours following R-baclofen (12.5 mg/kg, p.o.) administration. Temperatures were taken immediately after completion of the rotarod trial. The dose of baclofen was selected from previous studies (Schuler et al., 2001). Rectal temperature was measured 60 minutes and immediately before; 30 minutes, 60 minutes, 2 hours and 4 hours after GHB administration.

3.3 Effects of targeted deletion of $GABA_{B(1)}$ subunit on $GABA_B$ receptor function.

3.3.1 Results.

3.3.1.1 Effects of targeted deletion of $GABA_{B(1)}$ receptor subunit on baclofen induced hypothermia.

A two-way repeated measures ANOVA revealed a significant effect of baclofen treatment[$F(1,35) = 78.85$, $P < 0.001$] , genotype [$F(1,35) = 9.603$, $P = 0.004$] ,a significant genotype X baclofen treatment interaction [$F(1,35) = 45.21$, $P < 0.001$]. As reported previously, $GABA_{B(1)}^{-/-}$ mice have slightly lower body temperature than their wildtype counterparts (Queva et al., 2003), however in these studies it failed to reach the level of statistical significance ($P > 0.05$). Baclofen induced a dramatic decrease of body temperature during with maximal effect two hours following administration in $GABA_{B(1)}^{+/+}$ mice.

However, $GABA_{B(1)}^{-/-}$ mice had a totally blunted response to baclofen-induced hypothermia (See Fig. 3.1.).

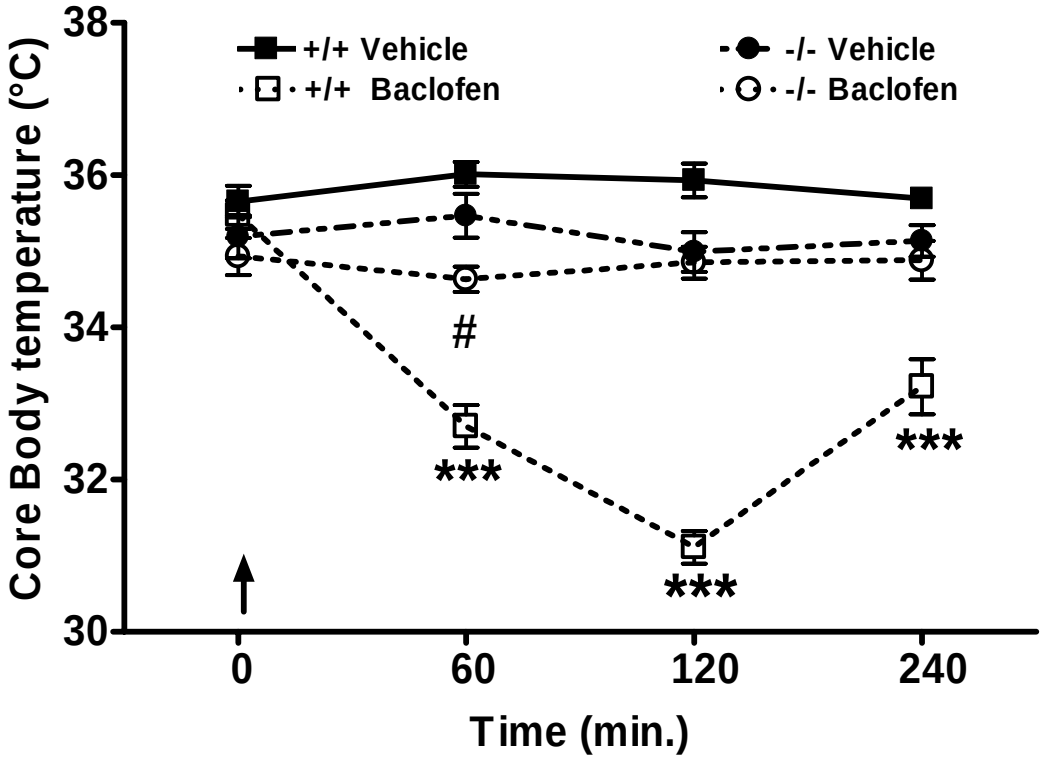

Fig.3.1.Lack of baclofen-induced hypothermia in $GABA_{B(1)}^{-/-}$ mice.
$GABA_{B(1)}^{+/+}$ mice treated with 12,5 mg/kg (p.o.) of baclofen (n = 10) exhibited a decrease of core body temperature compare to $GABA_{B(1)}^{+/+}$ mice treated with vehicle (n = 10). $GABA_{B(1)}^{-/-}$ mice treated with baclofen (n = 10) exhibited no reduction of body temperature compare to $GABA_{B(1)}^{-/-}$ mice treated with vehicle (n = 9). All data points represent mean ± SEM values. The arrow denotes the time of compound application. ***Groups that differed significantly from to $GABA_{B(1)}^{+/+}$ mice treated with vehicle ($p<0.001$). # groups that differed significantly to $GABA_{B(1)}^{-/-}$ mice treated with vehicle.

3.3.1.2 Effects of targeted deletion of $GABA_{B(1)}$ receptor subunit on baclofen-induced motor coordination impairment.

On examination of rotarod test ANOVA revealed a significant effect of baclofen treatment [$F(1,35) = 842.64$, $P < 0.001$] , genotype [$F(1,35) = 721.77$, $P < 0.001$] and a [$F(1,35) = 758.05$, $P < 0.001$]. No baseline difference in rotarod performance was observed, however $GABA_{B(1)}^{-/-}$ mice were devoid of the motor impairing effects of baclofen which was

clearly observed in $GABA_{B(1)}^{+/+}$ mice (see Fig.3.2). Thus, these results confirm that the absence of $GABA_B$ receptor function in mice lacking $GABA_{B(1)}$ receptor subunit.

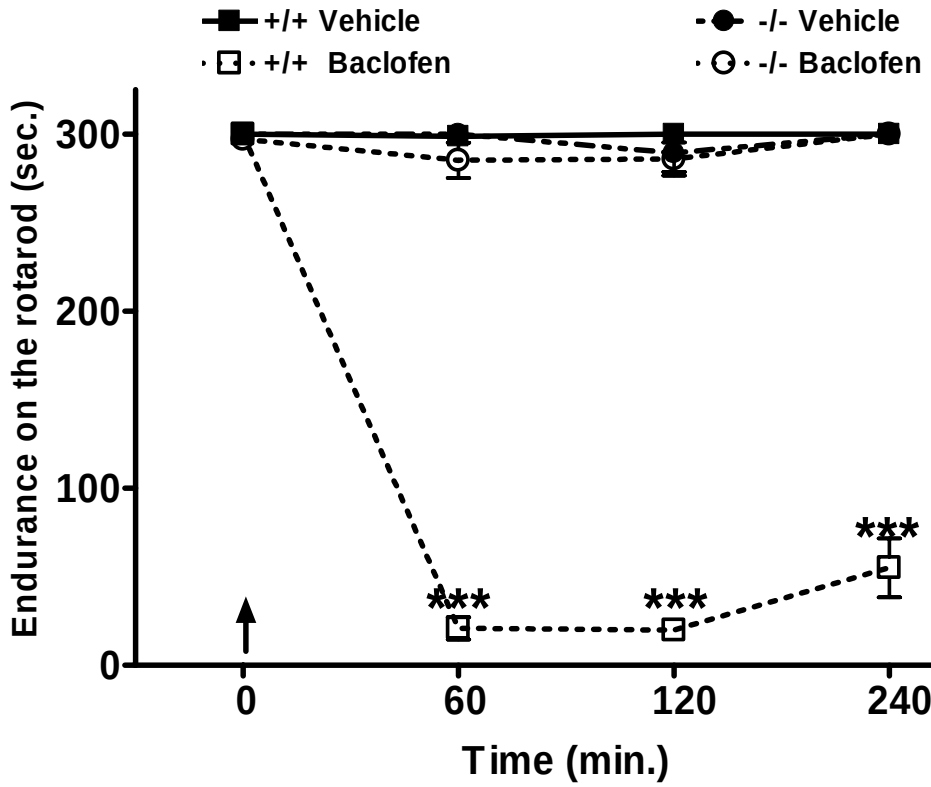

Fig.3.2.Baclofen fails to induced motor impairment in $GABA_{B(1)}^{-/-}$ mice.
$GABA_{B(1)}^{+/+}$ mice treated with 12,5 mg/kg (p.o.) of Baclofen (n = 10) exhibited a motor impairment compare to $GABA_{B(1)}^{+/+}$ mice treated with vehicle (n = 10). $GABA_{B(1)}^{-/-}$ mice treated with baclofen (n = 10) exhibited no change in the endurance on the rotarod compared to $GABA_{B(1)}^{-/-}$ mice treated with vehicle (n = 9). All data points represent mean ± SEM values. The arrow denotes the time of compound application. ***Groups that differed significantly from to $GABA_{B(1)}^{+/+}$ mice treated with vehicle ($p<0.001$).

3.3.1.3 Effects of targeted deletion of $GABA_{B(1)}$ receptor subunit on GHB-induced hypothermia.

ANOVA (repeated measures) revealed that there was a significant difference in temperature responses to GHB between both genotypes [$F(1,15) = 15.05$, $P < 0.001$] and a genotype X time interaction [$F(5,75) = 69.40$, $P < 0.001$]. γ-Hydroxybutyrate induced a marked (6 °C) hypothermia in wild-type animals. However, there was no significant effect of GHB on temperature in $GABA_{B(1)}^{-/-}$ mice over the 3-h recording period after GHB application

(Fig.3.3). Posthoc analysis revealed that there was a slightly, but significantly, lower basal temperature in GABA$_{B(1)}^{-/-}$ mice compared with wild-type mice at both time points prior to GHB administration (Fig3.3).

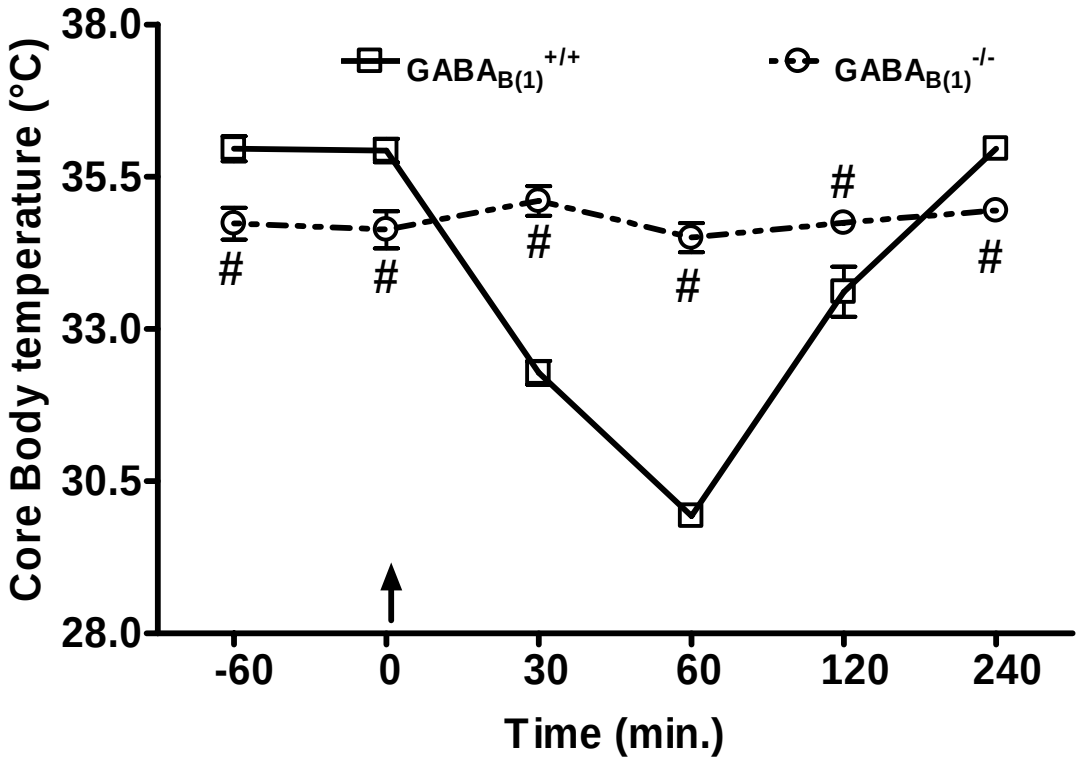

Fig.3.3. Core body temperature after γ-hydroxybutyrate (GHB) application in wild-type and GABA$_{B(1)}^{-/-}$ mice.
Body temperature after application of 1 g/kg GHB (p.o.) to wild-type ,white squares, (n = 10) and GABA$_{B(1)}^{-/-}$ mice, white dots (n = 7). The arrow denotes the time of compound application. # Groups that differed significantly from genotype control ($P < 0.05$, Fisher's posthoc tests). All data points represent mean ± SEM values.

3.4 Effects of targeted deletion of GABA$_{B(2)}$ subunit on GABA$_B$ function.

3.4.1 Results.

3.4.1.1 Effects of targeted deletion of GABA$_{B(2)}$ receptor subunit on baclofen induced hypothermia.

Somewhat surprisingly, we observed that $GABA_{B(2)}^{-/-}$ mice respond similarly to administration of baclofen. Indeed, a two-way repeated measures ANOVA revealed a significant effect of baclofen treatment [$F(1,32) = 79.973$, $P < 0.001$] , genotype [$F(1,32) = 9.187$, $P = 0.003$] ,a significant genotype X baclofen treatment interaction [$F(1,32) = 95.21$, $P < 0.001$.]. Intriguingly, basal temperature of $GABA_{B(2)}^{-/-}$ also was slightly lower than that of wild-type animals. Although baclofen, in wild-type animals, elicits a marked hypothermia which reached its nadir at 120 min after administration, it seems to be without effect in $GABA_{B(2)}^{-/-}$ mice. (see Fig 3.4)

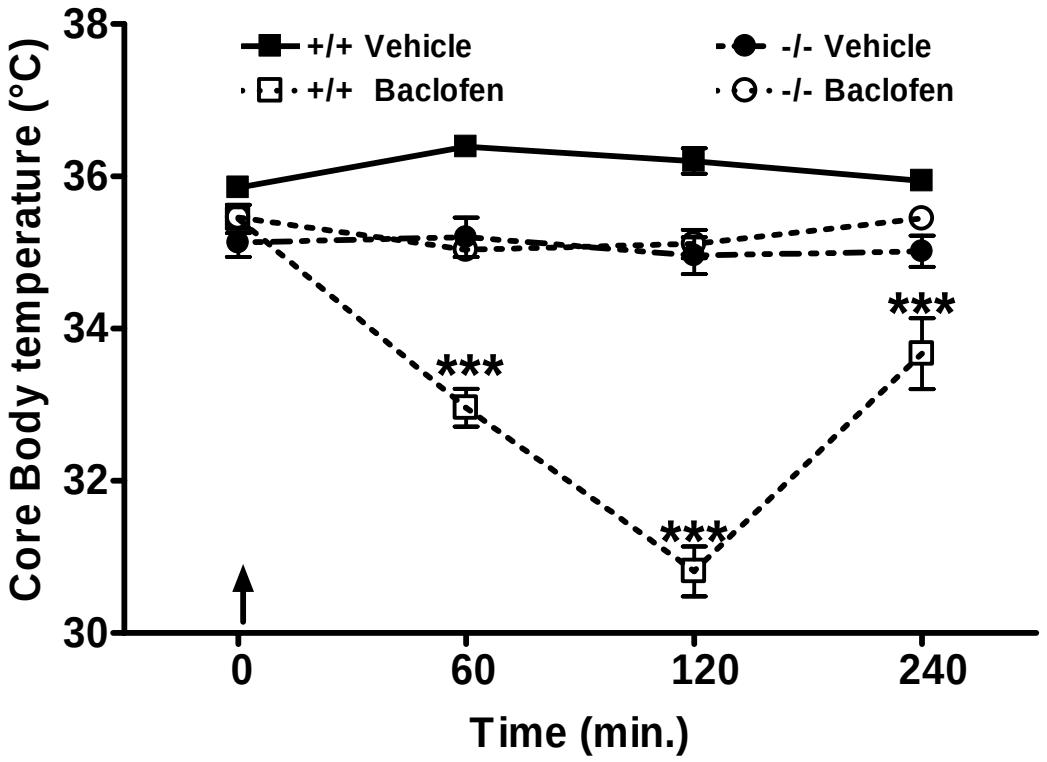

Fig.3.4.Lack of baclofen-induced hypothermia in $GABA_{B(2)}^{-/-}$ mice.
$GABA_{B(2)}^{+/+}$ mice treated with 12,5 mg/kg (p.o.) of Baclofen (n = 10) exhibited a decrease of core body temperature compare to $GABA_{B(2)}^{+/+}$ mice treated with vehicle (n = 10). $GABA_{B(2)}^{-/-}$ mice treated with baclofen (n = 9) exhibited no reduction of body temperature compare to $GABA_{B(2)}^{-/-}$ mice treated with vehicle (n = 7). All data points represent mean ± SEM values. The arrow denotes the time of compound application. ***Groups that differed significantly from to $GABA_{B(2)}^{+/+}$ mice treated with vehicle ($p<0.001$).

3.4.1.2 Effects of targeted deletion of $GABA_{B(2)}$ receptor subunit on baclofen-induced motor coordination impairment.

Regarding the impact of targeted deletion of $GABA_{B(2)}$ subunit on motor impairment induced by baclofen , analysis of variance (repeated measures) revealed a significant effect of genotype [$F(1,32) = 786.013$, $P < 0.001$], and effect baclofen treatment $F(1,32) = 786.195$, $P < 0.001$] and a genotype X treatment interaction [$F(1,32) = 786.195$, $P < 0.001$]. Post-hoc analysis indicated that baclofen produced a marked hypothermia in wildtype animals (Fig.3.5.). Conversely, this response appears to be totally abolished in $GABA_{B(2)}^{-/-}$ mice. Altogether, these data , combined with the experiment on core temperature, suggested that $GABA_{B(2)}$ receptor subunit seems to be essential to $GABA_B$ receptor responses in vivo.

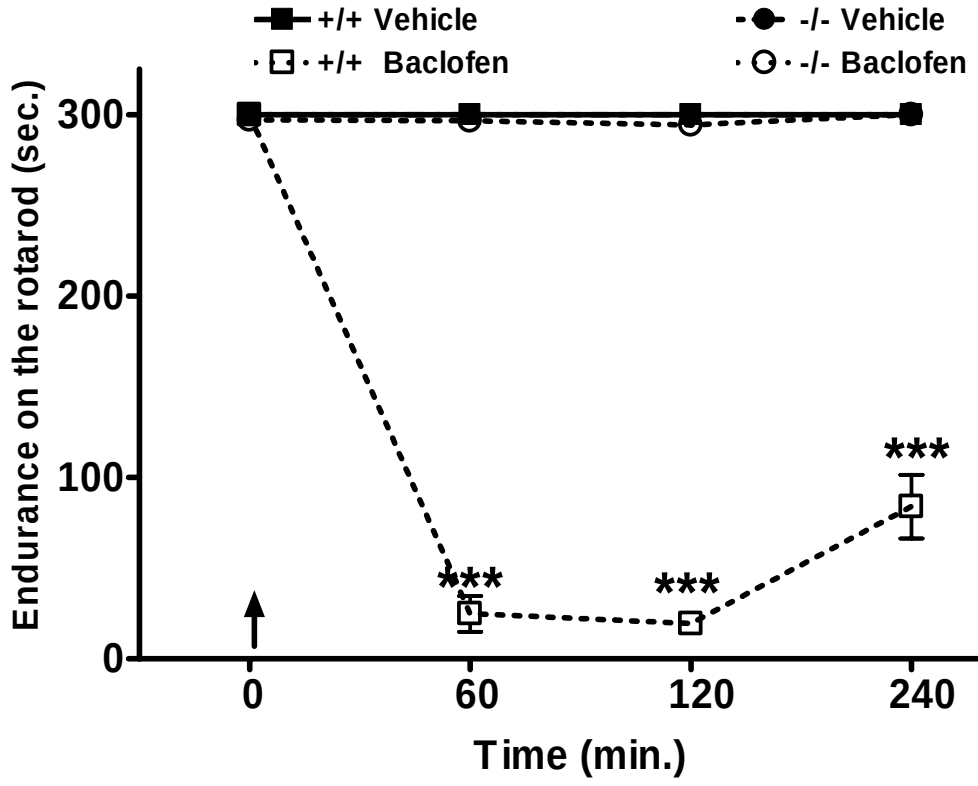

Fig.3.5. Lack of baclofen-induced motor impairment in $GABA_{B(2)}^{-/-}$ mice.
$GABA_{B(2)}^{+/+}$ mice treated with 12.5 mg/kg (p.o.) of Baclofen (n = 10) exhibited a motor impairment compare to $GABA_{B(2)}^{+/+}$ mice treated with vehicle (n = 10). $GABA_{B(1)}^{-/-}$ mice treated with baclofen (n = 9) exhibited no change in the endurance on the rotarod compared to $GABA_{B(2)}^{-/-}$ mice treated with vehicle (n = 7). All data points represent mean ± SEM values. The arrow denotes the time of compound application. ***Groups that differed significantly from to $GABA_{B(2)}^{+/+}$ mice treated with vehicle ($p<0.001$).

3.4.1.3 Effects of targeted deletion of $GABA_{B(2)}$ receptor subunit on GHB-induced hypothermia.

A one-way repeated measures ANOVA revealed no significant effect of genotype [$F(1,14) = 3.695$, $P = 0.075$] but a significant effect of time [$F(5,70) = 14.396$, $P < 0.001$] and a significant genotype X time interaction [$F(5,70) = 14.223$, $P < 0.001$]. Post-hoc analysis indicated that $GABA_{B(2)}^{-/-}$ exhibit significantly lower basal core temperature. Furthermore, GHB, at 1 g/kg, induced a strong hypothermia in wild-type mice. As we observed in $GABA_{B(1)}^{-/-}$ mice, GHB, at the dose use, failed to induced hypothermia in $GABA_{B(2)}^{-/-}$ mice.

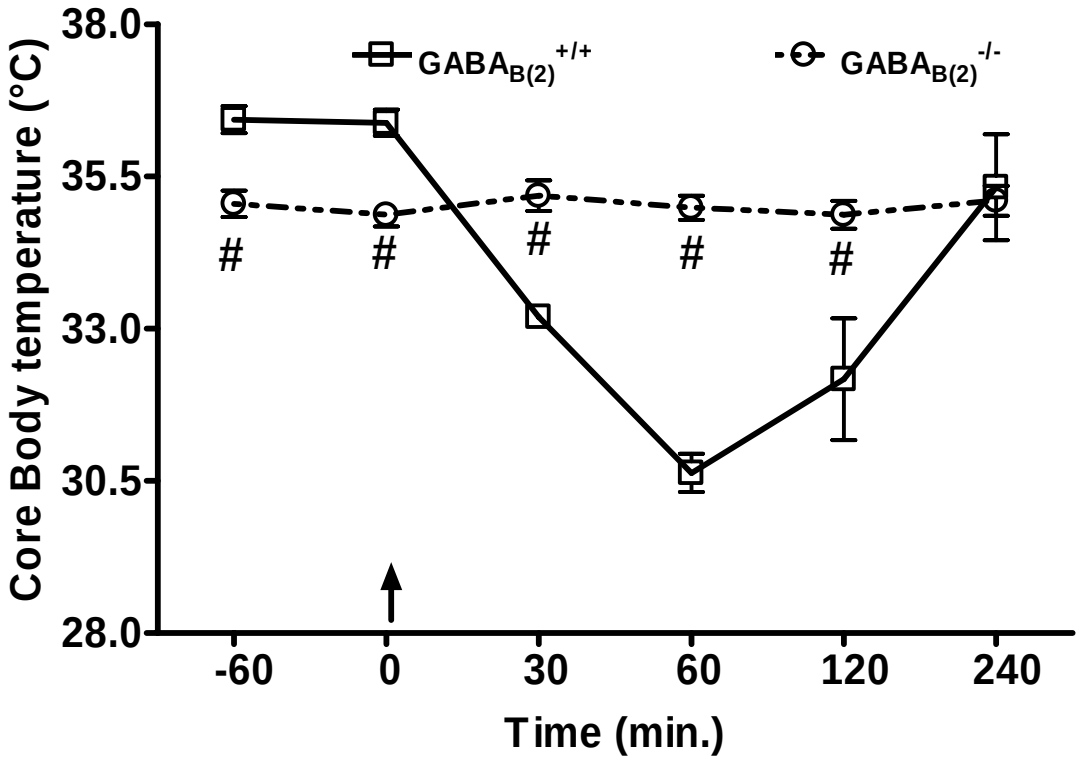

Fig.3.6. Core body temperature after γ-hydroxybutyrate (GHB) application in wild-type and $GABA_{B(2)}^{-/-}$ mice.
Body temperature after application of 1 g/kg GHB (p.o.) to wild-type, white square, (n = 10) and $GABA_{B(2)}^{-/-}$ mice, white dots, (n = 6). The arrow denotes the time of compound application. #Groups that differed significantly from wild type genotype ($P < 0.05$, Fisher's posthoc tests). All data points represent mean ± SEM values.

3.5 Discussion.

The present set of data demonstrates that both $GABA_B$ receptor subunits are required to elicit the hypothermic and motor response induced by baclofen. Indeed, in agreement with a previous study, we shown that targeted deletion of $GABA_{B(1)}$ receptor subunit abolished totally motor impairment , measured by endurance on the rotarod, and hypothermic effect of baclofen. Secondly, using recently generated $GABA_{B(2)}^{-/-}$ mice, we observed that that genetic ablation of $GABA_{B(2)}$ receptor subunit blunted also these responses, confirming that heterodimerization of $GABA_{B(1)}$ and $GABA_{B(2)}$ subunit is a prerequisite for $GABA_B$ function. Finally, the hypothermic response triggered by GHB appeared to be totally blocked in both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice, suggesting that physiological response to GHB seems to be mediated by $GABA_B$ receptors.

In agreement with the study of Schuler and colleagues (2001), baclofen neither affected temperature regulation neither motor coordination in $GABA_{B(1)}^{-/-}$ mice, which confirms the notion that $GABA_{B(1)}$ receptor subunit is necessary for these effects of baclofen and that $GABA_{B(2)}$ receptor subunit can be alone(Schuler et al., 2001). Accordingly, it has been shown also that $GABA_{B(1)}^{-/-}$ exhibits a lack of $GABA_B$ bindings sites, disruption of $GABA_B$ receptor function (characterized by GABA- or baclofen [^{35}S]GTPγS binding), and absence of $GABA_B$ auto-or heteroreceptors and postsynaptic $GABA_B$ receptor function (Prosser et al., 2001; Schuler et al., 2001). In addition, pharmacological studies have demonstrated that $GABA_B$ receptor antagonists are also able to block hypothermic response elicited by baclofen (Humeniuk et al., 1995). Given that both pharmacological blockade and genetic inactivation of $GABA_B$ receptors abolished hypothermic response, it is safe to conclude that baclofen-induced hypothermia and its associated motor response can be viewed as a powerful and simplistic translational in vivo read-out of $GABA_B$ receptor function. Nevertheless, the characterization of the biological and, more specially, specific $GABA_B$ receptors involved in these responses could reinforce its interest. Thus, the discovery that

hypothermic response to 8-OH-DPAT is mainly caused by 5-HT_{1A} autoreceptor stimulation in mice (Goodwin et al., 1985) provided a huge tool in the context of psychiatric researched. Indeed, this technique is the most used to assessed desensitization of somatodendritic 5-HT_{1A} receptor characterizing antidepressant-like response.

Regarding the $GABA_{B(2)}^{-/-}$ mice, we demonstrated that targeted deletion of $GABA_{B(2)}$ receptor subunit is sufficient to abolish hypothermic response and motor impairment induced by baclofen. These results are analogous to the results obtained in $GABA_{B(1)}^{-/-}$ mice and suggest that these responses relate to $GABA_{B(1,2)}$ heterodimer activation. In addition, parallel studies, obtained in collaboration with the group of Bernhard Bettler, Martin Gassmann, and colleagues at the University of Basel, confirmed the lack of other baclofen-induced responses in $GABA_{B(2)}^{-/-}$ mice (Gassmann et al., 2004). Thus, using saturation binding experiment, they demonstrated a decrease of number of $GABA_B$ receptor bindings sites in $GABA_{B(2)}^{-/-}$ mice. In addition, they observed $GABA_{B(2)}^{-/-}$ mice exhibits an absence of functional $GABA_B$ receptor, using GTPγ[^{35}S] binding. In vivo, they also demonstrated that baclofen failed to induce delta waves in EEG. Altogether, these data corroborated the absence of effect of $GABA_B$ receptor agonist in absence of $GABA_{B(2)}$ receptor subunit. Although they demonstrated also a loss of presynaptic $GABA_B$ functions in $GABA_{B(2)}^{-/-}$ mice, electrophysiological experiments demonstrated that these mice exhibit atypical post-synaptic response to baclofen in hippocampus. Indeed, baclofen elicits an inward current , in $GABA_{B(2)}^{-/-}$ mice, instead of the typical outward current observed in wild-type mice, suggesting neurons that lack naturally $GABA_{B(2)}$ receptor subunit might have the potential to express functional $GABA_B$ receptor. Although behavioural and physiological effects of baclofen, such as hypothermia, motor impairment or EEG delta waves induction seems to require heteromeric $GABA_{B(1,2)}$ receptor, it is therefore plausible that the potential functional $GABA_B$ receptor, observed in $GABA_{B(2)}^{-/-}$ mice, could participate to another neurobiological function.

Intriguingly, we also observed a basal hypothermia in both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$, suggesting that $GABA_B$ receptors might also participate in thermal homeostasis. Although the hypothermic effects of $GABA_B$ receptor agonists have been known for a long time (e.g. (Gray et al., 1987)) antagonist experiments have to our knowledge not suggested that there would be any endogenous $GABA_B$ergic tone in this system. Thus, we can hypothesize that basal hypothermia observed in both knock-out lines might be a consequence of compensatory changes.

Concerning the involvement of $GABA_B$ receptors in the GHB-related responses, we demonstrated that hypothermic elicited by GHB is totally abolished in both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice. Moreover, parallels studies achieved in collaboration with the group of Bernhard Bettler, demonstrated that targeted deletion of $GABA_{B(1)}$ receptor subunit blocked also pharmacological response to GHB, such as hypolocomotion, increase in striatal dopamine synthesis and EEG delta-waves induction. In lines with these data several authors demonstrate that many effects of GHB are antagonized by $GABA_B$ receptor. Indeed, $GABA_B$ antagonist have been shown to block the sedative properties of GHB (Carai et al., 2001), its effects of dopamine release in striatum (Waldmeier, 1991). Together, these studies demonstrated that these pharmacological effects of GHB are directly mediated by the activation of the functional heteromeric $GABA_B$ receptors. Nevertheless, autoradiography and ligands-binding assays revealed the presence of specific high-affinity binding sites for GHB in the brain of $GABA_{B(1)}^{-/-}$, suggesting $GABA_B$ receptor do not contribute to GHB-binding sites. However, the functional relevance of these GHB binding sites is still unclear.

To conclude, in this chapter, we have demonstrated that hypothermic and motor response to baclofen or GHB require the presence of both $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit receptor, confirming the hypothesis that functional $GABA_B$ receptors assemble from two subunit $GABA_{B(1)}$ and $GABA_{B(2)}$. However, the atypical electrophysiological response,

observed in $GABA_{B(2)}^{-/-}$ mice, led two think that the $GABA_{B(1)}$ and $GABA_{B(2)}$ subunits might have a differential role in the modulation of neural activity and perhaps in emotional behaviours. Further these studies allowed to concluded that $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice would be very useful tools to assess the role of $GABA_B$ receptor in animal model of psychiatric disorders.

CHAPTER 4: THE ROLE OF GABA$_B$ RECEPTORS IN ANXIETY.

4.1 Introduction.

As mentioned in the introduction, GABA$_B$ receptors seem to play a role in the modulation of anxious behaviour. More precisely, GABA$_B$ activation appears to induce anxiolytic like effect in animal models of anxiety (see (Cryan and Kaupmann, 2005). Nevertheless, previous data investigating GABA$_B$ mechanisms in anxiety are limited and rather variable; this is largely because investigators relied on using the prototypical full GABA$_B$ receptor agonist baclofen for such analysis. Indeed, baclofen has a narrow efficacy window before confounding side-effects are manifested in anxiety paradigms (Dalvi and Rodgers, 1996). Thus, the sedative properties of GABA$_B$ receptor agonists, such as baclofen, limit their used in exploratory-based approach-avoidance conflicts tests (Cryan and Holmes, 2005). Similarly, the memory impairment associated to GABA$_B$ receptor agonist or its analgesic properties (Bowery et al., 2002) may also represent a major problem in animal model of conflict paradigms, such as Vogel test (Vogel et al., 1971), Geller-Seifter conflict (Geller et al., 1962). Consequently, the anxiolytic properties of baclofen observed in these two paradigms (Ketelaars et al., 1988; Shephard et al., 1992) seem to be questionable and could be considered as false positive. Regardless these considerations, sporadic clinical studies report that baclofen reversed the anxiety associated with alcohol withdrawal (Addolorato et al., 2002b) and post traumatic stress (Drake et al., 2003), supporting the involvement of GABA$_B$ receptor in emotional behaviours.

Two recent developments have added innovative new tools to the armamentarium of researchers. Firstly, mice that lack the GABA$_{B(1)}$ subunit or GABA$_{B(2)}$ (Prosser et al., 2001; Schuler et al., 2001; Queva et al., 2003; Gassmann et al., 2004) have been generated. As

mentioned in previous chapter, these mice exhibit an abolished response to baclofen in vivo. Furthermore, $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ share atypical phenotype including spontaneous seizures, hyperalgesia or cognitive impairment (Schuler et al., 2001; Gassmann et al., 2004). Secondly, with positive allosteric modulators, novel pharmacological tools for $GABA_B$ receptors have been characterized (Urwyler, et al., 2001; Urwyler, et al., 2003). These molecules enhance the action of GABA at the GABAB receptor and have little or no intrinsic agonistic efficacy on their own (Urwyler et al., 2001; Urwyler et al., 2003). Application of $GABA_B$ receptor positive modulators in the presence of an agonist shifts the concentration-response curve to the left, as the modulators increase the potency of GABA. In addition, the maximal efficacy of GABA is increased. Allosteric positive modulation of metabotropic receptors is a recently identified phenomena, providing novel means for the pharmacological manipulation of G-protein-coupled receptors acting at a site apart from the orthosteric binding region of the receptor protein (Soudijn et al., 2002). Such properties suggest that allosteric modulators may offer a number of potential pharmacological improvements over the use of conventional agonists as has been demonstrated for modulators acting at ligand-gated ion channels (Costa, 1989). In the case of $GABA_A$ receptors, such modulation has been therapeutically utilized with the benzodiazepines, which amplify the action of the endogenous neurotransmitter GABA. Therefore, we hypothesized that $GABA_B$ receptor positive modulators will be superior drugs, devoid of the side effect profile associated with full agonists such as baclofen.

Thus, these novel tools, including mice lacking $GABA_B$ receptors and positive modulators, provide a new opportunity to explore the role of $GABA_B$ receptor in behavioural paradigms relevant to anxiety. Thus, we investigated the behavioural effect of genetic inactivation of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit in several anxiety paradigms, such as light-dark box or staircase test. We attempt also to corroborate the effect of targeted

deletion of $GABA_B$ receptor on anxiety with antagonist. Finally, we also assessed the effect of $GABA_B$ receptor positive modulator in several animal model of anxiety.

4.2 Experimental Design.

The $GABA_{B(1)}$ and $GABA_{B(2)}$ knockout mice were generated on a BALB/c background as described previously (Schuler et al., 2001; Gassmann et al., 2004). Age and sex matched mice were used at an age of 3 to 8 months. Both male and female animals were used in all experiments in approximately equal amounts. There was no effect of gender on behaviours observed.

In order to minimize the influence of strain effects all pharmacological studies were carried out in male BALB/c mice (23-26 g) which were obtained from Iffa Credo, France excepted for the elevated zero maze experiment where both BALB/c and OF-1 (30-40g) mice were used. In a number of initial studies heterozygous mice ($GABA_B^{+/-}$) were also used, no gene dosage effect was found in any of the behaviours analyzed with heterozygotes behaving similarly to wildtypes. Regarding pharmacological studies, all drugs were administrated orally 1 hour prior behavioural testing.

Concerning the novelty-induced hypophagia experiment, the drugs was administrated 1 hour prior the test in the new environment. All drugs were made up fresh prior to use and administered in a suspension of 0.5% methylcellulose at a concentration of 10 ml/kg.

In the case of chronic studies, animals were injected in the afternoon (2-4 pm) for 21 days and tested on the morning following last injection. They were again injected immediately after the initial test and for the consecutive day, locomotor activity testing was carried out approx 24 hours following this last injection. Doses for chronic studies were selected from the dose-response studies of acute administration of the compounds (data presented in these studies).

4.3 Impact of targeted $GABA_{B(1)}$ receptor subunit on anxiety related behaviours.

4.3.1 Light-dark box.

Upon being placed in the light side of the apparatus, freezing behaviours was observed in 30% of the $GABAB_{(1)}^{-/-}$ mice but none of the wildtype. As shown in Fig.4.1, $GABA_{B(1)}^{-/-}$ mice displayed marked increases in anxiety-related behaviours in the light-dark box paradigm compared with wildtype ($GABA_{B(1)}^{+/+}$) or heterozygous ($GABA_{B(1)}^{-/+}$) mice. ANOVA revealed a significant effect of genotype on the time spent in light compartments [$F(2,45) = 11.02$, $P = 0.001$] and on the number of transitions [$F(2,45) = 4.39$, $P = 0.018$]. Further, there was a genotype influence on the latency to enter in the dark compartment [$F(2,45) = 4.86$, $P = 0.012$.]. Post-hoc analysis revealed that $GABA_{B(1)}^{-/-}$ mice exhibited a decrease of the latency to enter into the dark compartment compared to wildtype and heterozygous mice. $GABA_{B(1)}^{-/-}$ mice showed a significant decrease in the time spent in light compartment compared to heterozygote or wildtype mice (Fig.4.1B) and exhibited significantly fewer light-dark transitions (Fig.4.1A). This latter parameter being the most reliable indicator of anxiety in the light-dark box test. Heterozygote mice behaved in the same manner as wildtype mice in all parameters in this test. Altogether, these effects are indicative of an increased anxiety in $GABA_{B(1)}^{-/-}$ mice. In order to confirm the reliability of the phenotype a second cohort of animals were tested in the light-dark box. These $GABA_{B(1)}^{-/-}$ mice had both qualitatively and quantitatively the same (anxious) phenotype (data not shown.).

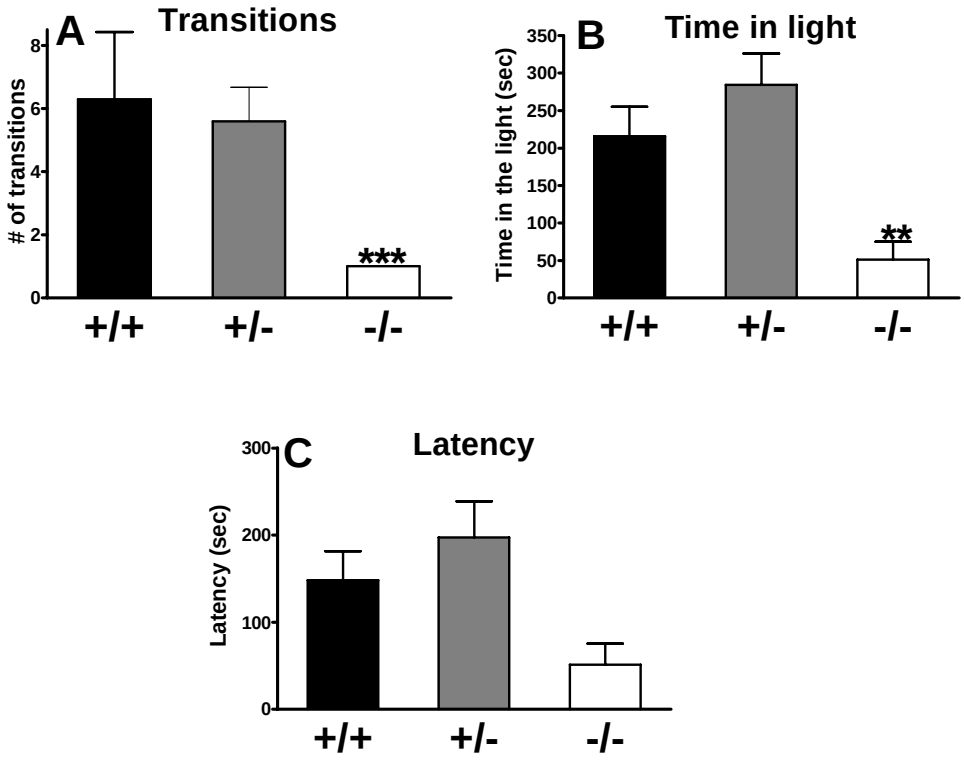

Fig.4.1. Increased anxiety in GABA$_{B(1)}$ deficient mice in light-dark box.
(A) GABA$_{B(1)}^{-/-}$ mice had a marked decrease of transitions between light and dark compartments compared with heterzygote or wildtype mice. (B) GABA$_{B(1)}^{-/-}$ mice spent less time in light compartment in comparison to heterozygous or wildtype mice. (C) GABA$_{B(1)}^{-/-}$ mice (n = 16) exhibited a decrease in latency to enter in the dark compartment, compared to heterozygous (n = 16), but not compared to wildtype mice (n = 16). All bars represent mean values, with vertical lines indicating one SEM. *,**,***groups that differed significantly to wildtype mice (P < 0.05, P < 0.01 and P < 0.001 respectively).

4.3.2 Response to classical benzodiazepines in the light-dark box.

Regarding the latency to enter in the dark compartment, two-way ANOVA revealed an effect of genotype [F(1,58) = 9.645, P = 0.03], but not effect of treatment [F(2,58) = 0.802, P = 0.453] and no genotype x treatment interaction [F(2,58) = 1.360 , P = 0.265]. These data suggest that GABA$_{B(1)}^{-/-}$ mice are more anxious that their wildtype counterparts, but that benzodiazepines failed to significantly affect this parameter in both GABA$_{B(1)}^{-/-}$ mice and wildtype. Concerning the time spent in the light compartment, two-way ANOVA failed to reveal any significant effect of genotype [F(1,58) = 1.867, P = 0.177], but an effect of

treatment [F(2,58) = 3.390, P = 0.041] and no genotype x treatment interaction [F(2,58) = 0.188, P = 0.829]. On examination of the number of transitions, Analysis of Variance (ANOVA) revealed an effect of genotype [F(1,58) = 6.623, P = 0.013], treatment [F(2,58) = 3.283, P = 0.045], but no genotype x treatment interaction [F(2,58) = 0.829, P = 0.442]. Post-hoc analysis indicated that diazepam or chlordiazepoxide increased significantly the number of transitions in wildtype mice, but this effect was markedly blunted in $GABA_{B(1)}^{-/-}$ mice (Fig 4.2A). Nevertheless, taking together, these data corroborated that $GABA_{B(1)}^{-/-}$ mice are more anxious than their wildtype counterpart and demonstrated that targeted deletion of $GABA_{B(1)}$ receptor subunit attenuated the response to benzodiazepines in the light-dark box. Intriguingly, it has to be noted that the anxious phenotype of $GABA_{B(1)}^{-/-}$ mice is less robust than in experiment of section 4.3.1. These discrepancies might be due to that many $GABA_{B(1)}^{-/-}$ mice froze upon being placed in the apparatus (30% in vehicle treated animals) in the present study.

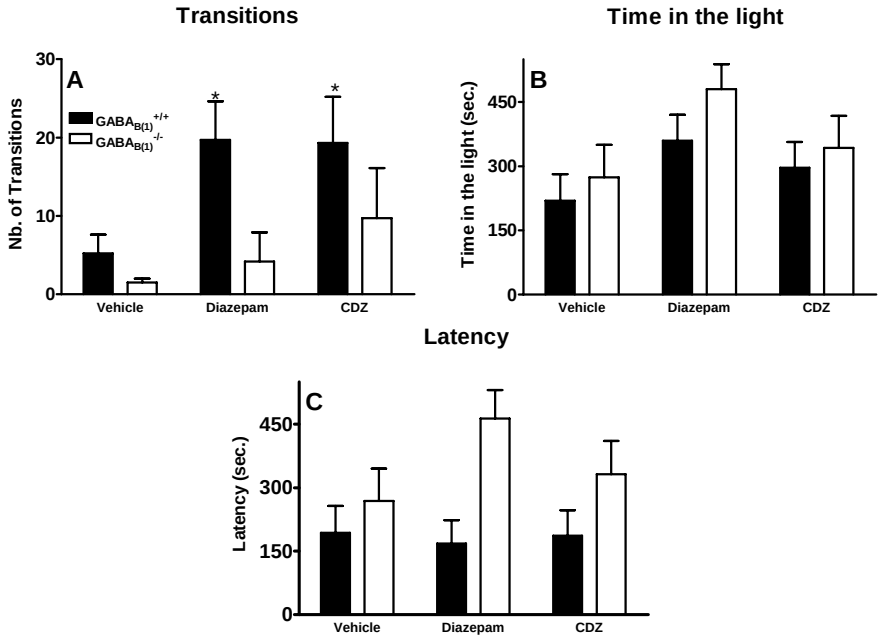

Fig.4.2. Blunted anxiolytic effect of benzodiazepines in GABA$_{B(1)}^{-/-}$ mice.
A) Effects of acute administration of diazepam (7.5 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) on the number of transitions in GABA$_{B(1)}^{+/+}$ and GABA$_{B(1)}^{-/-}$ mice: acute administration of diazepam (7.5 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) increased the number of transitions in GABA$_{B(1)}^{+/+}$ mice. n = 10-11 per treatment group. These two treatments didn't affect the number of transitions in GABA$_{B(1)}^{-/-}$ mice. n = 10-11 per treatment group. B) Effects of acute administration of diazepam (7.5 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) On the time spent in light compartment in GABA$_{B(1)}^{+/+}$ and GABA$_{B(1)}^{-/-}$ mice. (C) Effects of acute administration of diazepam (7.5 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) on the latency to enter in dark compartment in GABA$_{B(1)}^{+/+}$ and GABA$_{B(1)}^{-/-}$mice. All bars represent mean. Values, with vertical lines indicating 1 SEM.* groups that differed significantly to vehicle treated GABA$_{B(1)}^{+/+}$ mice treated with vehicle($P < 0.05$).

4.3.3 Staircase test.

In the staircase test, another paradigm for assessing anxiety-related behaviours, GABA$_{B(1)}^{-/-}$ mice had lower number of rearings than wildtype and heterozygote mice [$F(2,45) = 23.15$, $P = 0.001$] (Fig.4.3B). In addition, the number of steps climbed by GABA$_{B(1)}^{-/-}$ mice was decreased compared to wildtype and heterozygote mice [$F(2,45) = 52.61$, $P = 0.001$] (Fig.4.3A).This lack of exploration in the test was associated with a substantial amount of freezing behaviour.

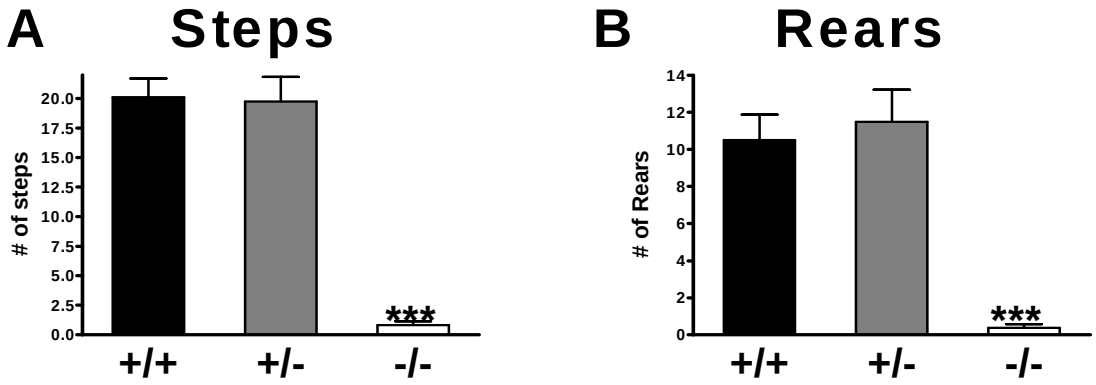

Fig.4.3. Increased anxiety in GABA$_{B(1)}^{-/-}$ mice in the staircase test:
(A) GABA$_{B(1)}^{-/-}$ mice ($n = 16$) exhibited a decrease in the steps climbed compared to heterozygous (n = 16) and wildtype (n = 16) mice. (B) GABA$_{B(1)}^{-/-}$ mice had significantly less rearing events to heterozygous or wildtype mice. All bars represent mean values, with vertical lines indicating one SEM. *** groups that differed significantly to wildtype mice ($P < 0.001$).

4.3.4 Stress-induced hyperthermia.

Unfortunately, ANOVA revealed no significant effect of genotype on the magnitude of the SIH response [$F(2,46) = 2.657$, $p < 0.081$; Fig.4.4]. However, analysis of variances (repeated measures) indicated a significant effect of genotype on body temperatures [$F(2,46) = 7.960$, $p < 0.001$], effect of stress on the body temperature [$F(1,46) = 91.210$, $p < 0.001$] but no interaction genotype X stress [$F(2,46) = 2.657$, $p < 0.081$]. Thus, the stress induced by the measurement of basal rectal temperature increased significantly the temperature measured 15 min. after. However, post-hoc analysis revealed a significant decrease of temperature during the second measurement in GABA$_{B(1)}^{-/-}$ compared to wild-type animals. Altogether, these data might suggest that targeted deletion of GABA$_{B(1)}$ subunit produced anxiolytic effect in mice, however this phenomenon would be mainly due to dysfunction of thermoregulation processes in these mice.

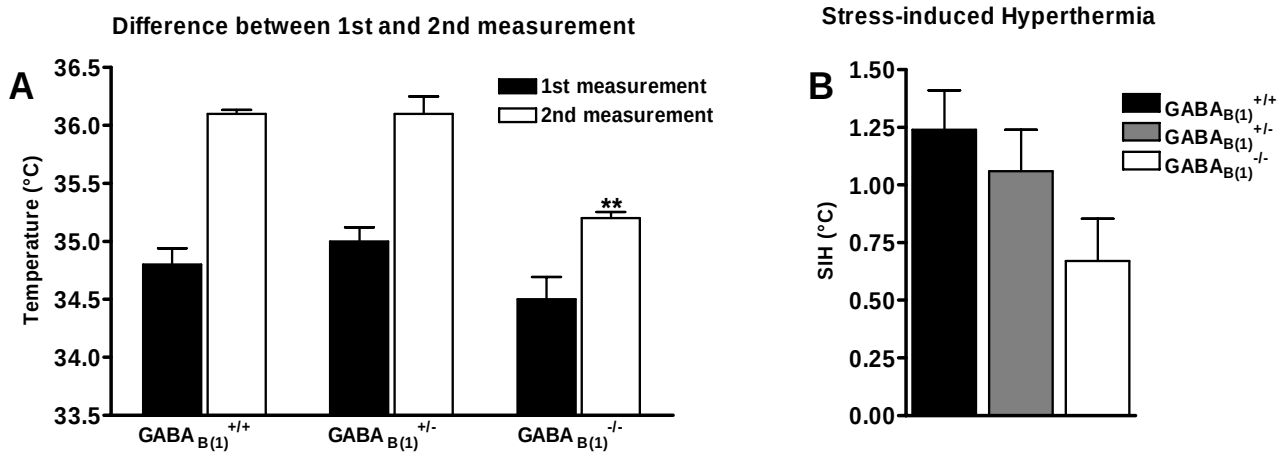

Fig.4.4. Effect of targeted deletion of GABA$_{B(1)}$ receptor subunit in stress-induced hyperthermia paradigm:
(A) Effects of targeted deletion of GABA$_{B(1)}$ receptor subunit on the basal temperature (first temperature) and body temperature (second measurement)15 min after stress compared with baseline temperature (B) The stress induced hyperthermia in GABA$_{B(1)}^{-/-}$ (n= 16) , GABA$_{B(1)}^{-/+}$ (n= 16) and GABA$_{B(1)}^{+/+}$ (n= 17)All bars represent mean values, with vertical lines indicating one SEM. ** groups that differed significantly to wildtype mice ($P < 0.01$).

4.3.5 Elevated-zero maze.

No functional data were obtained from examining the behavioural response of GABA$_{B(1)}^{-/-}$ mice in the elevated zero maze due to the fact that all of the GABA$_{B(1)}^{-/-}$ mice actively jumped off the maze. Reasons for this increased flight response are likely to reflect an increase in anxiety/panic like behaviour as opposed to lack of motor co-ordination as evidenced by absence of motor deficits in rotarod tests (Schuler et al., 2001)(Schuler et al., 2001; Mombereau and Cryan unpublished observations). Further, similar flight reactions from an unstable elevated maze has been recently characterized as a novel model of panic/anxiety in rodents (King, 1999a; 1999b; Jones et al., 2002a; Jones et al., 2002b). Additionally such an ethological response has also been demonstrated in the wild house mouse *(Mus musculus)* in the elevated plus maze (Holmes et al., 2000).

4.4 Impact of targeted $GABA_{B(2)}$ receptor subunit on anxiety related disorders.

4.4.1 Light-dark box.

Given that the most robust effect of targeted deletion of $GABA_{B(1)}$ receptor subunit was observed in the light dark box, we investigate the effect of genetic ablation of $GABA_{B(2)}$ receptor subunit in this test. Somewhat surprisingly, $GABA_{B(2)}^{-/-}$ mice displayed a similar anxious phenotype to that of $GABA_{B(1)}^{-/-}$ mice in the light-dark box. $GABA_{B(2)}^{-/-}$ mice spent less time in the light compartment [t-test, $P = 0.0028$], and they had significantly lower number of transitions compare to wild-type mice [t-test, $P < 0.001$]; which is indicative of increased anxiety in this paradigm. In addition, the latency to move from the light to the dark area of the arena was decreased (Fig.4.5C). These data suggest that deletion of either subunit of the functional $GABA_B$ heterodimer results in an enhancement of anxiety-behaviours in the light-dark box paradigm.

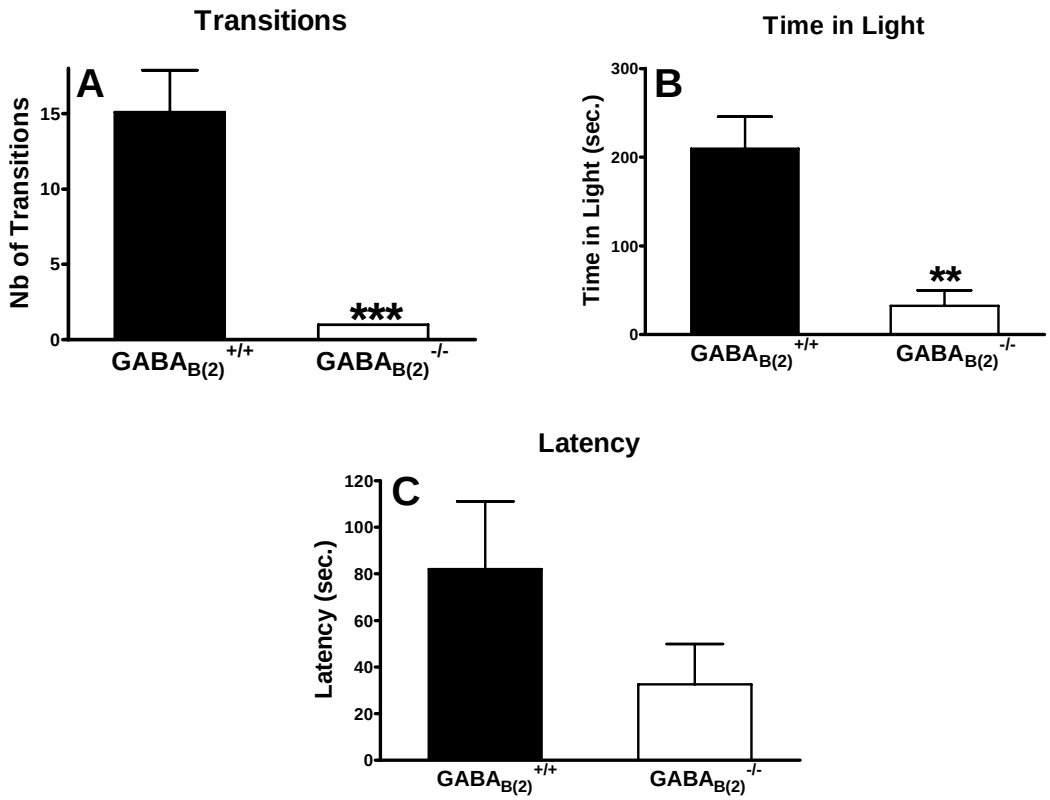

Fig.4.5. Increased anxiety in GABA$_{B(2)}$ receptor subunit deficient mice in light-dark box.
(A) GABA$_{B(2)}^{-/-}$ mice (*n* = 6) had a marked decrease of transitions between light and dark compartments compared with their respective counterpart wildtype mice (*n* = 10). (B) GABA$_{B(2)}^{-/-}$ mice spent less time in light compartment in comparison to wildtype mice. (C) GABA$_{B(2)}^{-/-}$ mice didn't exhibit a decrease in latency to enter in the dark compartment. All bars represent mean values, with vertical lines indicating one SEM.*,**,***groups that differed significantly to wildtype mice (P < 0.05, P < 0.01 and P < 0.001 respectively).

4.5 Pharmacological studies.

4.5.1 Effects of GABA$_B$ receptor positive modulator on anxiety-related behaviours

4.5.1.1 Light-Dark box.

As shown in Fig.4.6, ANOVA indicated an effect of drug treatment on the number of transitions between dark and light compartments [F(4,45) = 10.06, P = 0.001]. Post-hoc analysis revealed that GS39783 (0.3-30 mg/kg, p.o.) and the benzodiazepine chlordiazepoxide (10 mg/kg, p.o.) increased the number of transitions. Treatment with GS39783 or

chlordiazepoxide one hour prior to testing failed to influence the latency to enter the dark chamber but increased the time spent in the light compartment [F(4,45) = 9.30, P = 0.001]. Post-hoc analysis indicated a significant effect of both chlordiazepoxide and GS39783 (only at the highest dose tested (30 mg/kg). These effects are not due to any confounding effect of GS39783 on locomotor activity as acute administration of GS39783 is devoid of any effects on locomotor activity (Cryan et al., 2004). It is of interest that the basal levels of anxiety the light-dark test in Fig.4.6. are considerably different between those in Fig.4.1. The reason for this may lie in the fact that these mice are purchased from Iffa Credo and those in Fig.4.1. are wildtype BALB/c mice which were housed with their more anxious littermates.

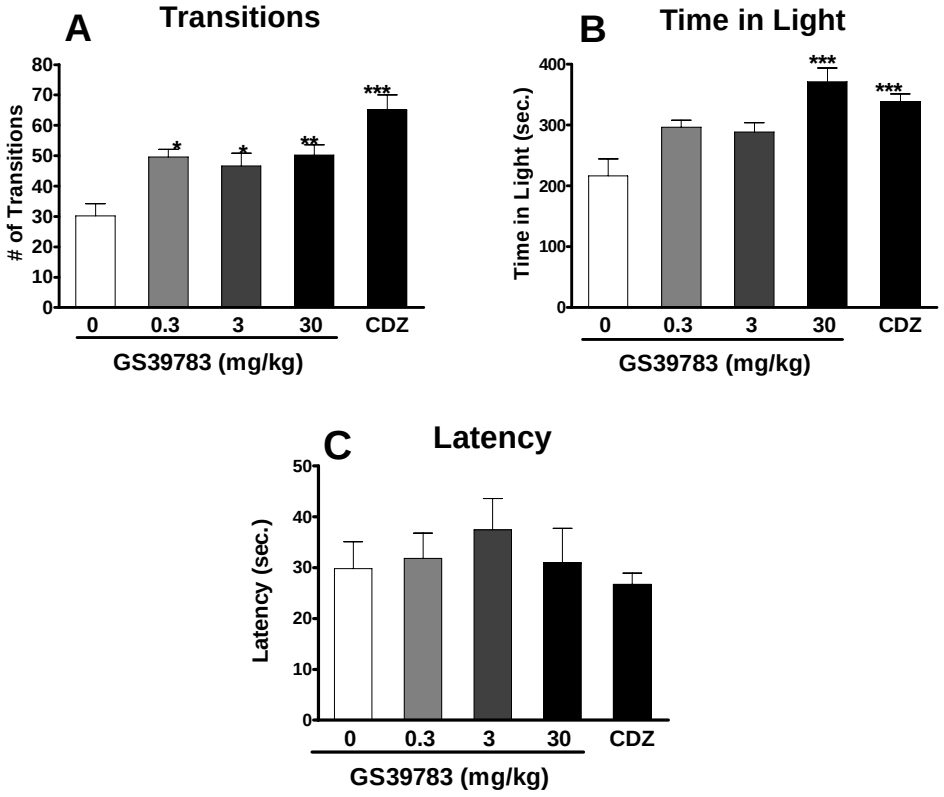

Fig.4.6. Anxiolytic effects of acute treatment with the GABA$_B$ receptor positive modulator GS39783 in the light-dark test.

Effects of acute GABA$_B$ positive modulator treatment (doses: 0, 0.3, 3 or 30 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) on (A) the number of transitions between light and dark compartments during the test, (B) the time spent in light compartment and (C) the latency to enter into the dark compartment. n = 10 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *,**,***groups that differed significantly to vehicle treated mice(P < 0.05, P < 0.01 and P< 0.001, respectively).

4.5.1.2 Chronic effects.

In an attempt to assess the effects of chronic administration of the positive modulator on anxiety-like behaviour, we tested GS39783, in addition to CGP56433A (a selective $GABA_B$ receptor antagonist) and the antidepressants fluoxetine and desipramine in the light-dark box (20 to 24 hours following last treatment). ANOVA revealed an effect of chronic drug treatment on the time spent in the light side of the arena [$F(4,55) = 2.573$, $P = 0.04$] and the number of transitions between the light and the dark sides [$F(4,55) = 2.637$, $P = 0.04$], but had no effect on latency to enter into the dark compartment (Fig.4.7A). Post-hoc analysis revealed that GS39783 was the only compound tested to significantly modify the number of transitions (Fig.4.7A) and the time spent in the light side of the arena (data not shown). Taken together, these results indicate a potential anxiolytic effect of acute and chronic GS39783 treatment. As shown in Fig.4.7B, these effects are not due to any confounding effect of GS39783 on locomotor activity as chronic administration of GS39783 did not affect locomotor activity [$F(4,53) = 0.9289$, $P = 0.4543$]. It is of interest that the basal levels of anxiety in the light-dark test in Fig.4.7 are considerably different between those in Fig. 4.6. The reason for this may lie in the fact that although all mice are purchased from Iffa Credo, those in Fig.4.7. have been handled and injected daily for 21 days such stress has been shown to influence anxiety-like behaviours.

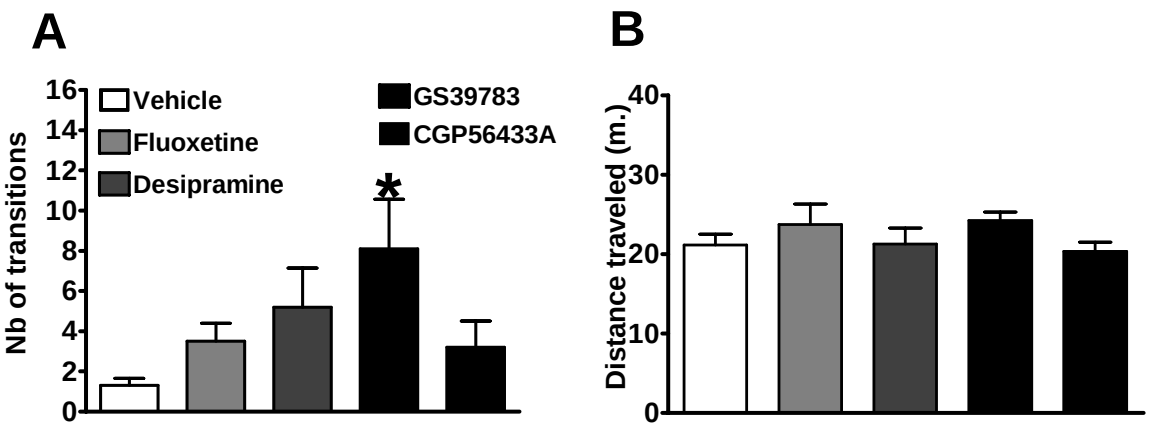

Fig.4.7. Chronic treatment with the GABA$_B$ receptor positive modulator reveals anxiolytic effects in the light-dark box test:
Chronic treatment (21 days) with GABA$_B$ the positive modulator GS 398783 (10 mg/kg, p.o., once daily), significantly increased (A) the number of transitions between light and dark compartments during the test, whereas fluoxetine (10 mg/kg, p.o., once daily), desipramine (15 mg/kg, p.o., once daily) and the GABA$_B$ receptor antagonist (3 mg/kg, p.o., once daily) was without effect. N = 12per treatment group. All bars represent mean values, with vertical lines indicating one SEM. * groups that differed significantly to vehicle treated mice($P < 0.05$). (B) Locomotor activity in a novel environment following chronic (23 days) administration of the GABA$_B$ receptor positive modulator (10 mg/kg, p.o.), fluoxetine (10 mg/kg, p.o.), desipramine (15 mg/kg, p.o.) and GABA$_B$ receptor antagonist (3 mg/kg, p.o.) during the 30 minutes of test. Testing was carried out for thirty minutes 24 hours following last dose in the same animals previously tested in light-dark box. None of the treatments altered locomotor activity, indicating that the effects of GS39783 in the light-dark box are not due to any secondary stimulant effect. n = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM.

4.5.1.3 Staircase test.

In the staircase, ANOVA indicated a significant effect of treatment on the number of steps climbed [$F(4,55) = 10.362$, $P < 0.0001$] but not on the number of rearings [$F(4,55) = 0.745$, $P = 0.566$]. Unfortunately, only the classical benzodiazepine, chlordiazepoxide, increased the number of step climbed and GS39783 appears to be without effect on the number of step climbed and rearings (Fig.4.8). Although GABA$_B$ positive modulator, GS39783, was anxiolytic in light/dark box, it failed to affect behaviour in the staircase test.

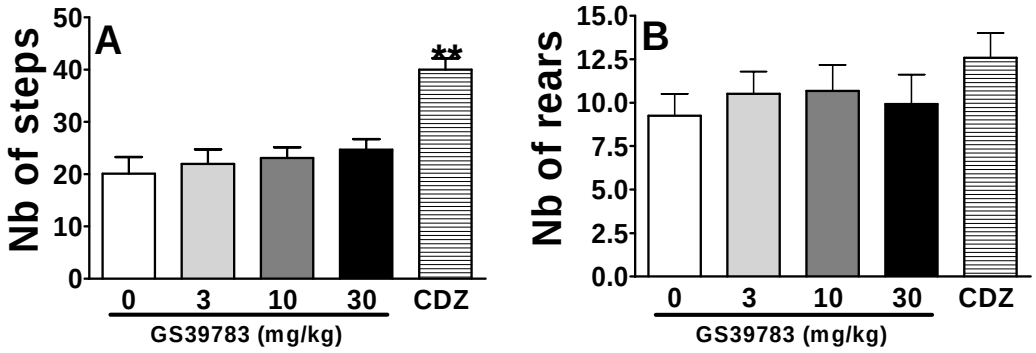

Fig.4.8. The effects of GABA$_B$ receptor positive modulator, GS39783, and chlordiazepoxide in staircase test.
(A) the effects of GS39783, CDZ, on number of steps climbed, 60 min following injection. Values represent mean and S.E.M. (n = 12 animals). **, p ≤0.001 versus control group (0) (Fisher's post hoc test after ANOVA). (B) the effects of GS39783, CDZ, on number of rears, 60 min following injection. Values represent mean and S.E.M. (n = 12 animals).

4.5.1.4. Elevated-zero test.

BALB/c

To further confirm the anxiolytic effects of GS39783 we tested it in comparison with chlordiazepoxide in the elevated zero maze in BALB/c mice, the background strain on to which $GABA_{B(1)}^{-/-}$ mice were generated. ANOVA revealed that drug treatment decreased the latency to enter the open sides of the maze [F(4,55) = 3.192, P = 0.020], the number of stretched-attend postures [F(4,55) = 13.16, P < 0.0001] and increased the time spent in the open side of maze [F(4,55) = 3.932, P = 0.007], increased the number of head dips [F(4,55) = 6.995, P < 0.00001], number of rearing [F(4,55) = 8.233, P < 0.0001], and the number of line crossings [F(4,55) = 33.76, P < 0.0001]. Post-hoc analysis revealed that chlordiazepoxide (10 mg/kg p.o.) significantly affected all parameters tested whereas GS39783 treatment reduced the latency to enter the open side at the highest dose tested (30 mg/kg, p.o.; P <0.05) (Fig.4.9A) and at doses of 3 to 30 mg/kg, reduced the number of stretch-attend postures (Fig.4.9D) only. There was a trend toward GS 39783 increasing the time in the open parts of arena which failed to reach the level of significance (Fig.4.9B). GS 39783 failed to affect the number of head dips, number of rearings and the number of line crossings at any dose. Taken

together, these data further suggest an anxiolytic effect of GS39783, although the magnitude of the effects in this test are much less robust compared with that induced by benzodiazepine anxiolytics.

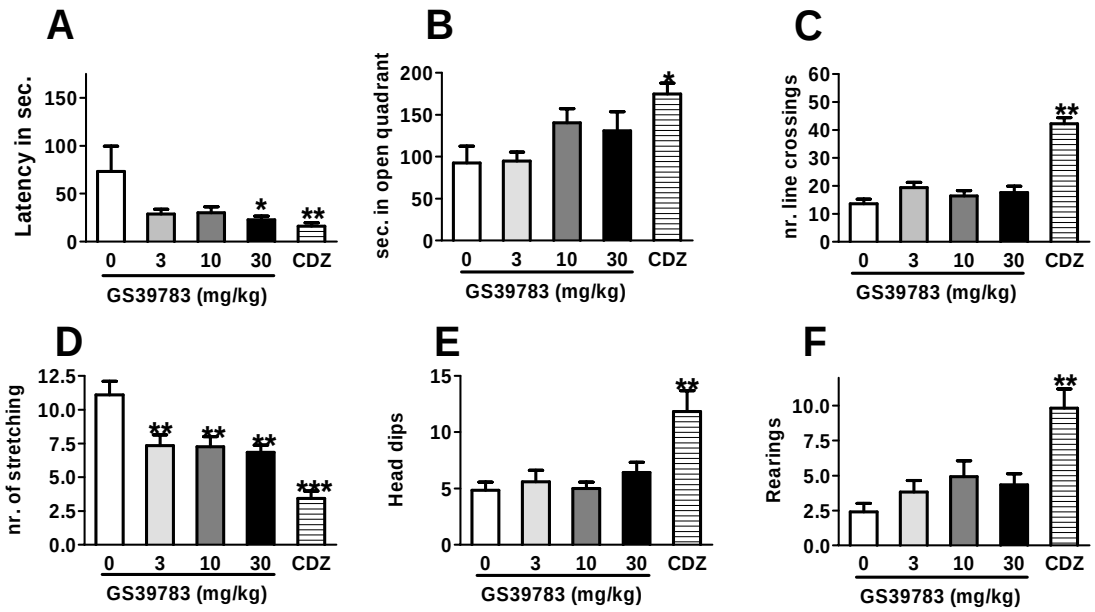

Fig.4.9. Effects of acute treatment with GS39783 in the elevated zero-maze test for anxiety behaviour in BALB/c mice.
Both the effects of acute GABA$_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) affected (A) the latency to enter in open side of maze and (D) the number of stretched attend postures. However, only chlordiazepoxide significantly increased the time spent in the open quadrants of the maze (B), increase head-dips (E), rearings (F) and the number of line crossed during the 5 min. of test (C). n = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *,**,***groups that differed significantly to vehicle treated mice($P < 0.05$, $P < 0.01$ and $P< 0.001$, respectively).

OF-1

In OF-1 mice, ANOVA revealed that drug treatment decreased the latency to enter the open sides of the maze [$F(5,63) = 2.82$; $p=0.023$], the number of stretched-attend postures [$F(5,63) = 13.89$; $p<0.001$] and increased the time spent in the open side of maze [$F(5,63) = 10.36$; $p<0.001$], increased the number of head dips [$F(5,63) = 5.622$; $p<0.01$], number of rearing [$F(5,63) = 4.162$; $p=0.002$], and the number of line crossings $F(5,63) = 19.79$; $p<0.001$].In line with result obtained in BALB/c, posthoc analysis revealed that chlordiazepoxide (10 mg/kg p.o.) significantly affected all parameters tested. Unlike

BALB/c, GS39783 treatment failed to reduced the latency to enter the open side at the highest dose tested (Fig.4.10A), however GS39783 increased significantly the number of rearing at the highest dose used, and significantly decreased the number of stretched attend posture at 10 and 30 mg/kg (Fig.4.10D). In this strain, GS39783 failed to affect the time spent in open quadrants, the number of head dips and the number of line crossings at any dose (Fig.4.10B,C,E). Taken together, these data confirm the anxiolytic-like properties of GS39783 in elevated-zero maze paradigm.

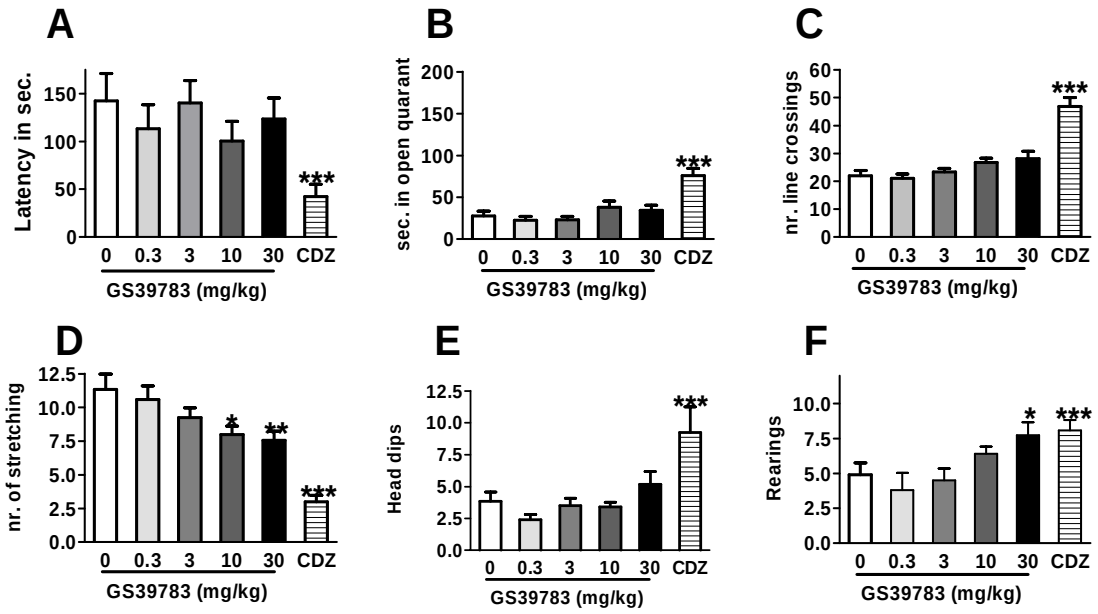

Fig.4.10. Effects of acute treatment with GS39783 in the elevated zero-maze test for anxiety behaviour in OF-1 mice.
Both the effects of acute GABA$_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) affected (D) the number of stretched attend postures and (F) the number of rearings. However, only chlordiazepoxide significantly decreased the latency to enter in open quadrant (A) and increased the time spent in the open quadrants of the maze (B), increase head-dips (E) and the number of line crossed during the 5 min. of test (C). n = 10-12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *,** groups that differed significantly to vehicle treated mice($P < 0.05$, $P < 0.01$ and $P< 0.001$, respectively).

4.5.1.5 Novelty-induced hypophagia

As mentioned in the introduction, the novelty induced-hypothermia is currently considered as an interesting model of anxiety in animals, mainly because it is sensitive to chronic antidepressant (Dulawa, 2005). Analysis of variances (repeated measures) indicated a

significant effect of treatment [$F(3,32) = 3.269$; $p=0.034$], of environment (Home vs New) [$F(1,32) = 231.68$; $p<0.001$] and treatment X environment interaction [$F(3,32) = 3.391$; $p = 0.03$] on the latency to drink. Post-hoc analysis revealed that latency to drink is significantly higher in the new environment and that mice treated with chlordiazepoxide exhibit a lower latency to drink in the new environment than vehicle treated animals (Fig.4.11A). In contrast, GS39783 appears to be without effect on this parameter. Concerning the volume intake during the first 5 minutes of test, Analysis of variances (repeated measures) indicated no significant effect of treatment [$F(3,32) = 0.624$; $p=0.605$] and treatment X environment interaction [$F(3,32) = 1.998$; $p = 0.134$], but an effect of environment (Home vs New) [$F(1,32) = 262.586$; $p<0.001$]. Post-hoc analysis revealed that both GS39783, at 30 mg/kg, and chlordiazepoxide increase the volume intake during the first 5 minutes in the new environment (Fig.4.11B). Regarding the total volume intake during the test, analysis of variances (repeated measures) indicated no significant effect of treatment [$F(3,32) = 2.109$; $p=0.119$], but an effect of environment (Home vs New) [$F(1,32) = 24.395$; $p<0.001$] and treatment X environment interaction [$F(3,32) = 4.379$; $p = 0.134$]. Post-hoc analysis revealed that chlordiazepoxide increases the total volume intake (Fig.4.11C).

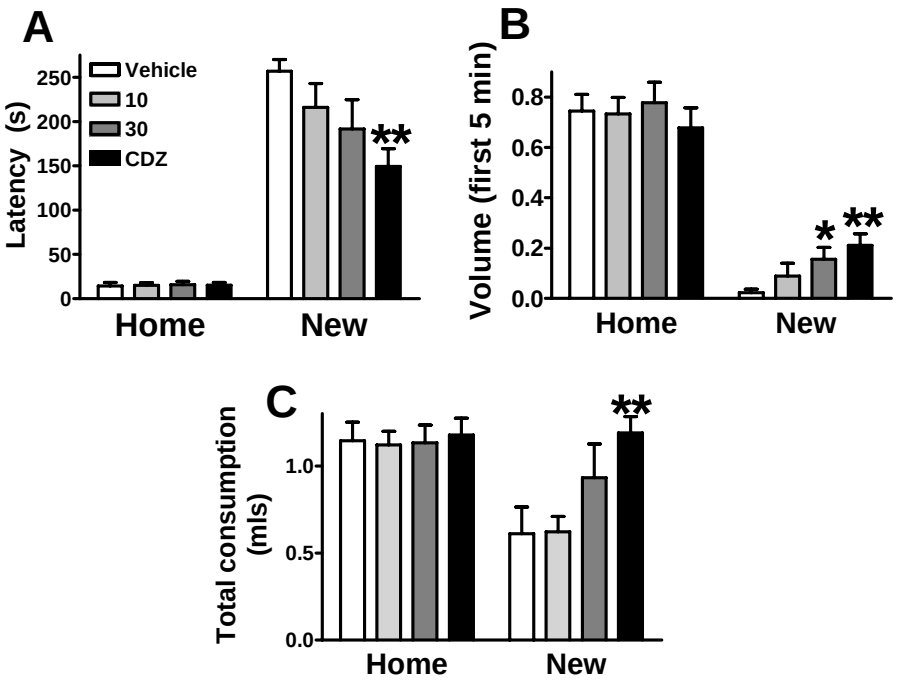

Fig.4.11. Effects of acute treatment with GS39783 novelty-induced hypophagia. A. Effects of acute GABA$_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) on latency to consume in the home vs. a novel cage (B), Effects of acute GABA$_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) on the volume intake during the first 5 minutes in the home vs. a novel cage (C) Effects of acute GABA$_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) on the total volume intake during the 30 minutes of test. n = 9 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *,** groups that differed significantly to vehicle treated mice($P < 0.05$ and $P < 0.$, respectively).

4.5.2 .Effects of GABA$_B$ receptor antagonist on anxiety-related behaviour.

4.5.2.1 Acute effect.

Given the anxiolytics properties of GABA$_B$ receptor positive modulator in the several paradigms, we also assessed the effect of GABA$_B$ receptor antagonist in the light-dark box. Surprisingly, we observed an anxiolytic like effect of GABA$_B$ receptor antagonist whereas anxiogenic effect would be more expected. Thus, ANOVA revealed a significant effect of treatment on the time spent in light compartment [$F(5,54) = 2.692$; $p = 0.0304$], on the number of transitions between both compartments [$F(5,54) = 154.9$; $p < 0.0001$] but not on

the latency to enter in the dark compartment [F(5,54) = 0.245; p = 0.940]. Post-hoc analysis indicated that both Chlordiazepoxide and CGP564333A, at 10 and 30 mg/kg increased the time spent in the lit compartment (Fig.4.12A). In addition, Chlordiazepoxide increased also significantly the number of transitions and there was a trend toward GS 39783 increasing this parameter which failed to reach the level of significance (Fig.4.12B).

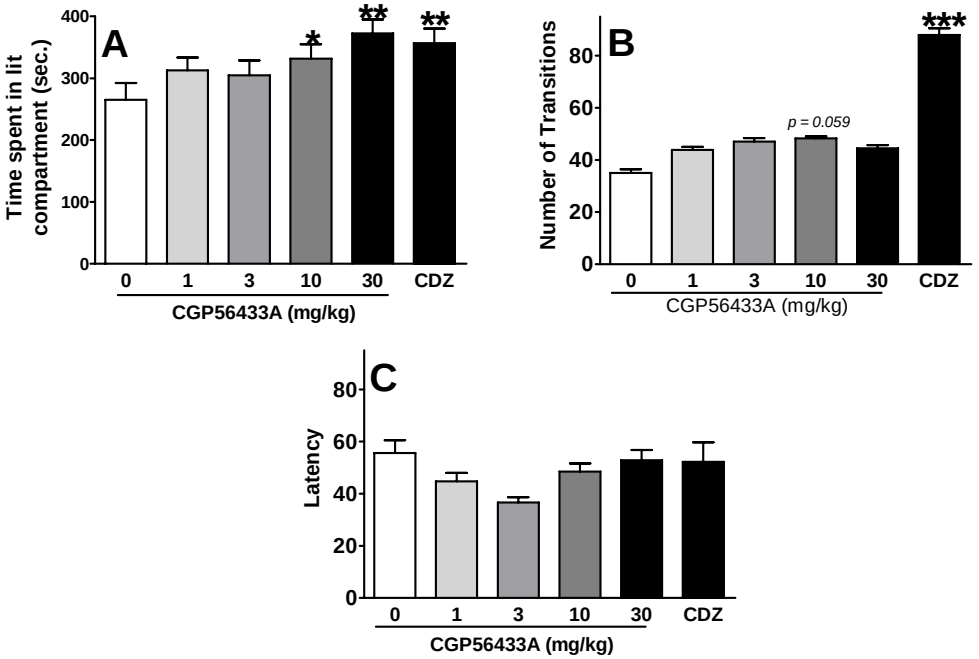

Fig.4.12. Anxiolytic effects of acute treatment with the GABA$_B$ receptor antagonist CGP56433A in the light-dark test.
Effects of acute GABA$_B$ receptor antagonist receptor (doses: 0, 1 , 3, 10 or 30 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) on (A) the number of transitions between light and dark compartments during the test, (B) the time spent in light compartment and (C) the latency to enter into the dark compartment. n = 10 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *, **,***groups that differed significantly to vehicle treated mice(P < 0.05 P < 0.01 and P< 0.001, respectively).

4.6 Discussion.

In this chapter, we sought to combine pharmacological and genetic approaches to obtain converging information on the function of GABA$_B$ receptors in behavioural processes. Using this dual approach, we demonstrate that through differential pharmacologically manipulation of GABA$_B$ receptors one can modify behaviours relevant to anxiety related

behaviours. Thus, deletion of $GABA_{B(1)}$ receptor subunit results in a more anxious phenotype in mice and a decreased sensitivity to classical benzodiazepines in the light-dark box. Similarly, we observed that genetic ablation of $GABA_{B(2)}$ receptor subunits produced analogous anxious phenotype than observed in $GABA_{B(1)}^{-/-}$ mice. Congruent with these data, we demonstrated that activation of $GABA_B$ receptors, via $GABA_B$ receptor positive modulator, results in anxiolysis. Interestingly, treatment with a $GABA_B$ receptor antagonist also resulted in a mild anxiolytic-like effect with no anxiogenic-like effects observed in the light-dark box. Given the complex overt behavioural phenotype of $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice, which includes a high propensity for spontaneous epileptic seizures, hyperalgesia and amnesia (Schuler et al., 2001; Gassmann et al., 2004), it was important to combine both genetic and pharmacological approaches. Together, these studies clearly demonstrate that $GABA_B$ receptors play a role in the modulation of behaviours relevant to anxiety related behaviours (Holmes, 2001).

Using the light-dark box, one of the most widely used tests for assessing anxiety-related behaviour in rodents, we clearly show that $GABA_{B(1)}^{-/-}$ mice are more anxious than their wildtype counterparts (Fig.4.1). Complimentary data were also found in the staircase anxiety test, where $GABA_{B(1)}^{-/-}$ mice had a substantial increase in freezing behaviour and failed to explore the elevated platform compared to wildtype animals (Fig.4.3.). It should be noted that this increase in anxiety related behaviours is robust and not masked by the already high anxiety of the parental strain. In a variety of paradigms it has been shown that BALB/c mice exhibit increased anxiety-related behaviours compared to other inbred strains of mice (Belzung and Griebel, 2001). The use of mice on this background strain was essential for the generation of $GABA_B$ related knockout animals, as mice on other strains died very prematurely (Prosser et al., 2001; Queva et al., 2003). Interestingly, unlike genetic deletion chronic pharmacological antagonism of $GABA_B$ receptors with CGP56433A failed to alter anxiety related behaviour in the light-dark-box (Fig.4.7.). This indicates that loss of $GABA_B$

receptor during development may be critical for the increased anxiety phenotype to be unveiled, indeed using conditional knockout technology, such an assertion has recently been ascertained for the 5-HT_{1A} receptor (Gross et al., 2002). It is unlikely that the increased anxiety-like behaviour is due to motor failure in the animals. Although $GABA_{B(1)}^{-/-}$ have less activity in locomotor chambers their activity increases over time as the habituate to the environment (See chapter 5).

Concerning the putative anxiolytic effect of targeted deletion of $GABA_{B(1)}$ receptor subunit in stress-induced hyperthermia paradigm, we cannot exclude a putative dysfunction of thermoregulation in these mice. Indeed, as we shown in the previous chapter, $GABA_B$ receptor plays a key role in the regulation of body temperature. Although $GABA_B$ receptor antagonist has no effect on basal temperature, suggesting that there would be any endogenous $GABA_B$ ergic tone under basal condition, we can hypothesize that $GABA_B$ receptor might be recruited under certain circumstances such as stress.

We also demonstrated that that selective ablation of $GABA_{B(1)}$ receptor subunit attenuated the response to benzodiazepines in the light-dark box test (4.2.). This suggests a functional relationship between both $GABA_B$ and $GABA_A$ receptors in vivo. Thus, we can speculate that targeted deletion of $GABA_{B(1)}$ receptor subunit might induced an alteration in benzodiazepine binding and/or $GABA_A$ subunit expression as is the case with the 5-HT_{1A} receptor knockout (Sibille et al., 2000). Indeed, an attenuated response to benzodiazepines in 5-HT_{1A} receptor knockout mice has been shown to be correlate with a downregulation of α_1 and α_2 $GABA_A$ subunits and decrease in benzodiazepine binding in the amygdala. Conversely, Queva and collaborators (2003), in a recent study, demonstrated that $GABA_{B(1)}^{-/-}$ mice are more, but not less, sensitive to muscimol, using hypothermia as physiological readout of GABA function. However, we can presume that genetic ablation of $GABA_{B(1)}$ receptor subunit might differentially affect GABAergic function involved in thermoregulation

and in anxiety-related behaviour. Although these result confirm that $GABA_B$/$GABA_A$ interaction might be involved in the hyposensitivity to benzodiazepines observed in $GABA_{B(1)}^{-/-}$ mice, we cannot exclude that this attenuated efficacy of chlordiazepoxide and diazepam is due to an interaction between loss of $GABA_B$ receptor function and the background strain employed (Lepicard et al., 2000).

In line with the result obtained in $GABA_{B(1)}^{-/-}$ mice, we demonstrated that genetic inactivation of the $GABA_{B(2)}$ receptor subunit produces the same behavioural effects as that following $GABA_{B(1)}$ receptor subunit deletion. Indeed, the $GABAB_{(2)}^{-/-}$ mice exhibited a decreased time spent in the light compartment and a decrease in the number of transitions between the light and dark compartment, analogous to the behaviour observed in $GABAB_{(1)}^{-/-}$ mice. However, it should be noted that although we demonstrated that genetic inactivation of $GABA_{B(1)}$ or $GABA_{B(2)}$ subunit produces an anxiogenic effect, pharmacological studies fail to demonstrate anxiogenic profile of $GABA_B$ antagonists (Fig.4.12.). In addition, the behavioural similarities between $GABA_{B(2)}^{-/-}$ and $GABA_{B(1)}^{-/-}$ mice observed in the present study, agree with the current $GABA_B$ receptor models implying that genetic inactivation of one of the two subunit blocks receptor function (Kaupmann et al., 1998a). Thus, these data confirmed the results obtained in the previous chapter suggesting that $GABA_B$ function require the assemble of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit. Nonetheless, several studies demonstrated that $GABA_{B(1)}$ subunit may be functional either alone or in combination with an unknown protein (Bettler et al., 2004). Moreover, a differential expression of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor protein in subpopulations of striatum has been demonstrated (Ng and Yung, 2001). In addition, the recent description of a residual electrophysiological $GABA_B$ receptor response in hippocampal slices from $GABA_{B(2)}^{-/-}$ mice suggests that $GABA_{B(1)}$ receptor subunits may be able to form functional receptors on their own (Gassmann et al., 2004). Therefore, although our data do not provide further evidence for this hypothesis, distinctive roles of individual $GABA_B$ receptor subunits in the regulation of

behaviour cannot be formally excluded. However, heterodimerization seems to be a prerequisite for the contribution of $GABA_B$ in the neurophysiology of anxiety.

Given that $GABA_{B(1)}^{-/-}$ mice have elevated anxiety-like behaviour, we hypothesized that by activating $GABA_B$ receptors we would be able to decrease anxiety in normal animals placed in an aversive environment. As expected, following acute administration of the recently identified $GABA_B$ receptor positive modulator GS37983 (Cryan et al., 2004), animals displayed reduced anxiety in the majority of behavioural paradigms used. More specifically, we demonstrated that GS39783 decreased the avoidance to potentially dangerous area, such as lit compartment in the light-dark box (Cryan and Holmes, 2005). Indeed, GS39783 decreased the time spent in the lit compartment in the light-dark box. Furthermore, we also observed that $GABA_B$ positive modulation increase exploratory behaviour in the elevated zero maze, increasing the number of rearing, and in the light-dark box, increasing the number of transitions between both compartments. Therefore, both BALB/c and OF-1 mice treated with GS39783 exhibits less total stretch attends posture in elevated-zero maze, suggesting that $GABA_B$ positive modulator might decrease risk assessment in this paradigm (Dawson and Tricklebank, 1995). In addition, we also demonstrated that GS39783, (at 30 mg/kg only), attenuated the phenomenon of hyponeophagia, confirming the putative anxiolytic-like properties of $GABA_B$ receptor positive modulator (Dulawa and Hen, 2005). Although we failed to demonstrated any effect of GS39783 in the staircase paradigm, a recent study demonstrated anxiolytic properties of GS39783 in stress-induced hyperthermia suggesting that $GABA_B$ positive modulator could attenuate anticipatory anxiety measured in stress-induced hyperthermia paradigm (Cryan et al., 2003c). Further, the anxiolytic effects of GS39783 were also observed following chronic treatment (Fig.4.7). Thus, the present chapter reports that GS39783 is effective in several models of anxiety and on several forms/aspects of anxiety. Being a positive modulator GS37983 is potentially advantageous over full $GABA_B$ receptor agonists which potentially engenders it more amenable for use *in vivo*, the major side

effects associated with full agonists include sedation, muscle relaxation, hypothermia cognitive impairing effects (Cryan et al., 2004). Therefore, given the clinical effectiveness of baclofen in the treatment of panic disorder and post-traumatic stress disorder (Breslow et al., 1989; Drake et al., 2003), it might be also essential to assess the effect of $GABA_B$ receptor positive modulator in animal model of these disorders. Indeed, paradigms such as lactate-induced panic-like behaviour (Shekhar et al., 2001) or predatory-stress induced memory impairment (El Hage et al., 2005) may be valuable behavioural tools to confirm the putative therapeutic properties of $GABA_B$ receptor positive modulator.

Although positive modulation and genetic ablation of $GABA_B$ receptors appear to modulate anxiety in opposite manners, we also found that pharmacological blockade of $GABA_B$ receptor, via acute administration of CGP56433A, induced moderated anxiolytic effect in the light-dark box paradigm. These data corroborate the results obtained with another highly potent $GABA_B$ receptor antagonist in the elevated plus maze. Indeed, intracerebroventricular injection of CGP35348 has been shown to increased percent of open arm entries (Zarrindast et al., 2001). Although these results need to be confirmed in other behavioural paradigms, we can presume that GS39783 and CGP56433A recruit differentially pre-or post-synaptic $GABA_B$ receptor, leading to the same behavioural effect. On the other hand, these effects could also be due to also agonistic or partial agonistic efficacy of CGP56433A. Indeed, Urwyler and collaborators demonstrated that CGP35348 and 2-OH-saclofen, which did not stimulate GTP(γ)^{35}S binding at all on their own, might become partial $GABA_B$ receptor agonists in the presence of the allosteric modulators (Urwyler et al., 2005).

In summary the present set of data is clearly indicative of potential anxiolytic activity of $GABA_B$ receptor positive modulator and conversely genetic ablation of one of the two $GABA_B$ receptor subunits induced anxious phenotype in mice. Nevertheless, the mechanisms responsible for the influence of $GABA_B$ receptors on anxiety behaviour are not well

understood. Therefore, future studies should focus on behavioural and electrophysiological responses of $GABA_B$ receptor activation in key brain regions that are associated with anxiety. Currently, several neural substrates might be hypothesized to be involved in this phenomenon, such as the amygdala or raphé nuclei. Taking together, these data reconfirm the concept that $GABA_B$ receptors are relevant therapeutic target for anxiety disorders.

CHAPTER 5: THE ROLE OF $GABA_B$ RECEPTORS IN DEPRESSIVE DISORDERS.

5.1 Introduction.

In addition to a putative role of $GABA_B$ receptor in the modulation of anxiety related behaviours, preclinical and clinical data have also implicate $GABA_B$ receptor in the pathophysiology of mood disorders (see section 1.3.2). Indeed, depressed patients exhibit a blunted growth hormone response to baclofen (Marchesi et al., 1991; O'Flynn and Dinan, 1993). Furthermore, learned helplessness has been shown to decrease $GABA_B$ receptor expression in the frontal cortex and that this decrease was reversed following antidepressant administration (Martin et al., 1989). In line with these results, Gray and Green demonstrated an increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks (Gray and Green, 1987). More recently, several authors have pointed that $GABA_B$ receptor antagonist might have antidepressant-like properties (Cryan and Kaupmann, 2005). Indeed, $GABA_B$ receptor blockade have been shown to elicit antidepressant-like effects in several depression paradigms , including learned helplessness (Nakagawa et al., 1996a), forced swim test (Nakagawa et al., 1996c; Slattery et al., 2005a) or chronic mild stress (Bittiger et al., 1996). Thus, the recent generation of $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice provide a novel opportunity to further explore the role of $GABA_B$ receptors in depression-related disorders.

The release of monoamines has been shown to be modulated via $GABA_B$ receptors (Bowery et al., 1980). In particular, emerging evidence suggests a strong interaction between $GABA_B$ receptors and serotoninergic system. $GABA_B$ receptors are densely localized on, and intricately interact with serotonergic neurons in the dorsal raphé nucleus (Abellan et al., 2000;

Burman et al., 2003; Serrats et al., 2003). Moreover, $GABA_B$ receptor activation is known to inhibit 5-HT cell firing in the raphé nucleus (Innis, 1988). In addition, microdialysis studies have shown that $GABA_B$ receptor activation in raphé nucleus decreases serotonin release (Tao et al., 1996). Mice lacking the 5-HT transporter have been shown to exhibit desensitization of $5\text{-}HT_{1A}$ autoreceptors and of $GABA_B$ receptors in the dorsal raphé nucleus (Mannoury la Cour et al., 2004) providing further evidence supporting an interaction between the serotonergic system and $GABA_B$ receptors. More recently, $GABA_B$ receptor antagonists have an antidepressant-like profile in the forced-swim test similar to that of serotonergic antidepressants (Slattery et al., 2005a) which is blocked via serotonin depletion. Taken together these, data suggest that antidepressant-like effect of $GABA_B$ receptor antagonist might be mediated via serotonin-$GABA_B$ receptor interaction.

The hypothalamic-pituitary-adrenal (HPA) axis is a key regulator of the stress reaction. Dysregulation of this axis is though to play a central role in the pathophysiology of depressive disorders(de Kloet et al., 2005). In addition, it is clear , from both preclinical and clinical studies that exposure to stressors could participate in the ontogenesis of affective illness, such as depressive syndrome, via alteration of the negative feedback of HPA axis (Holsboer et al., 1980). Furthermore, the serotonin (5-HT) system and the HPA axis have complex inter-relationships (Porter et al., 2004). In particular, the $5\text{-}HT_{1A}$ receptor is very susceptible to modulation by stress and HPA-axis activation and is well known to play an important role in the pathophysiology of mood disorders (Cryan and Leonard, 2000; Leitch et al., 2003). Indeed, it is well accepted that adrenal steroids, such as corticosterone, exerts a tonic inhibitory action on $5\text{-}HT_{1A}$ receptor expression in the hippocampus. In particular adernalectomy have been shown to up-regulated hippocampal $5\text{-}HT_{1A}$ gene expression (Kuroda et al., 1994; Meijer and de Kloet, 1998). Conversely, several studies have demonstrated that long-term corticosterone treatment can counteract adrenalectomy-induced $5\text{-}HT_{1A}$ receptor

upregulation (Chalmers et al., 1994). More recently, Lopez and co-workers demonstrated, in a series of elegant studies, a down-regulation of hippocampal $5\text{-}HT_{1A}$ receptors in both chronically stressed rats and major depressed patients (Lopez et al., 1998). Additionally, they demonstrated also that chronic administration of desipramine, a tricyclic antidepressant, prevent the effect of chronic stress on hippocampal $5\text{-}HT_{1A}$ receptors mRNA, suggesting that these receptors might be involved in antidepressant response. Supporting this hypothesis, recent studies demonstrated an up-regulation of $5\text{-}HT_{1A}$ receptor mRNA in the hippocampus of mice exhibiting antidepressant-like phenotype (Fabre et al., 2000; Mitsukawa et al., 2005).

In the present studies, we investigated the impact of both genetic inactivation and pharmacological blockade of $GABA_B$ receptors on behaviour in two animal models of antidepressant action, the forced swim test and the tail suspension test. In order to investigate the interaction between $GABA_B$ receptor and serotonergic system, we also assessed the effects of a $GABA_B$ receptor antagonist on the anti-immobility effect of an SSRI. Using baclofen-induced hypothermia, we assessed the impact of pharmacological blockade and genetic inactivation of serotonin transporter on $GABA_B$ receptor function. Finally, we investigated the effects of routine daily handling and oral injection on hippocampal $5\text{-}HT_{1A}$ receptor mRNA in the BALB/c mouse strain. It has been hypothesized that the BALB/c strain may be a relevant strain to model trait, pathological anxiety (Belzung and Griebel, 2001) and are particularly sensitive to the effects of chronic antidepressant treatments (Dulawa et al., 2004). Further, we investigated the effects of antidepressants from two different classes to alter any potential changes. Similarly, we examined whether $GABA_B$ receptors play any role in stress-induced modulation of $5\text{-}HT_{1A}$ receptors and investigated the effects of $GABA_B$ receptor agonist, novel $GABA_B$ receptor positive modulator GS39783 and the $GABA_B$ receptor antagonist CGP56433A on $5\text{-}HT_{1A}$ mRNA expression.

5.2 Experimental Design.

The $GABA_{B(1)}$ and $GABA_{B(2)}$ knockout mice were generated on a BALB/c background as described previously (Schuler et al., 2001; Gassmann et al., 2004). Age and sex matched mice were used at an age of 3 to 8 months. Both male and female animals were used in all experiments in approximately equal amounts. There was no effect of gender on behaviours observed. In order to minimize the influence of strain effects, all pharmacological studies were carried out in male BALB/c mice (23-26 g) which were obtained from Charles River (formally Iffa Credo), France. In a number of initial studies heterozygous mice ($GABA_B^{+/-}$) were also used, no gene dosage effect was found in any of the behaviours analyzed with heterozygote behaving similarly to knockouts.

Regarding pharmacological studies, all drugs were administrated orally 1 hour prior behavioural testing. All drugs were made up fresh prior to use and administered in a suspension of 0.5% methylcellulose at a concentration of 10 ml/kg. In the case of chronic studies, animals were injected in the afternoon (2-4 pm) for 21 days and tested on the morning following last injection. They were again injected immediately after the initial test and for the consecutive day, locomotor activity testing was carried out approx 24 hours following this last injection. Doses for chronic studies were selected from the dose-response studies of acute administration of the compounds (data presented in these studies).

Baclofen-induced hypothermia experiments were carried out using male BALB/c mice (23-26 g) which were obtained from Iffa Credo France. Regarding acute study, (9 mg/kg; p.o.) L-baclofen and fluoxetine (20 mg/kg; p.o.) were injected simultaneously and rectal temperature was measured 60 minutes and immediately before; 60 minutes and 2 hours after L-baclofen administration. Doses were selected from previous studies showing robust effects at these doses.

In the case of chronic studies, animals were injected with fluoxetine (20 mg/kg; p.o.)in the afternoon (2-4 pm) for 21 days and baclofen-induced hypothermia were performed 24, 48 and 72 hours after the last injection of fluoxetine. Rectal temperature was measured immediately before; 60 minutes and 2 hours after L-baclofen administration (9 mg/kg; p.o.).Finally, 5-$HTT^{-/-}$ and 5-$HTT^{+/+}$ (18-24 g) were obtained from heterozygous mutants of C57BL/6. Rectal temperature was measured 60 minutes and immediately before; 30 minutes, 60 minutes, 2 hours and 4 hours after (±)baclofen administration (12 mg/kg; p.o) (dose corrected in order to have robust effect on hypothermia)

Concerning the experiments assessing the impact of $GABA_B$ receptor ligands and antidepressants on downregulation of 5-HT_{1A} receptor induced by chronic handling, mice were given daily injections (o.d; p.o.) for 21 days. During this period animals they were given active drug treatment for the last 1, 7, or 21 days of this treatment regimen with one of these six treatments (N=12): vehicle, desipramine (15 mg/kg); fluoxetine (20 mg/kg); the prototypical GABAB agonist L-baclofen, (5 mg/kg), GS39783, a novel $GABA_B$ receptor positive modulator (10 mg/kg) and CGP56433A, a $GABA_B$ receptor antagonist (3 mg/kg). This treatment regimen was adopted to ensure equal amount of daily handing and injection stress in all groups. Handling was minimal and was just the amount needed to restrain the animal for injection and to weigh it daily. In addition, twelve mice were untreated and unhandled but kept in the same environment for the duration of the experiment; these constituted the control group (unstressed group). Hippocampi were collected one hour after the last injection and frozen at -80°C and 5-HT_{1A} receptor mRNA were analyzed via RT-PCR (see section 2.5)

5.3 Impact of targeted deletion of GABA$_{B(1)}$ or GABA$_{B(2)}$ receptor subunits on depression related disorders.

5.3.1 Effects of targeted deletion of GABA$_{B(1)}$ receptor subunit in models of antidepressant activity.

5.3.1.1 Forced swim test.

The FST is the most widely used tool for assessing depression and antidepressant-related phenotypes in genetically altered mice (Porsolt, 2000; Cryan et al., 2002a; Cryan and Mombereau, 2004), hence we examined the effects of mice with a targeted deletion of the GABA$_{B(1)}$ receptor subunit on behaviour in this test. As shown in Fig. 7A, there was a significant effect of genotype on immobility time in the FST [*t*-test, P = 0.012]. GABA$_{B(1)}^{-/-}$ mice had a significantly lower immobility time as compared to wildtype control mice. The magnitude of reduced immobility of the GABA$_{B(1)}^{-/-}$ mice in this test is similar to that which we and others have reported for a variety of antidepressants, including selective monoamine reuptake or oxidase inhibitors (Porsolt et al., 1978; Cryan et al., 2001; Lucki et al., 2001). It is noteworthy that there was no observable occurrence of seizures or altered motor patterns in animals subsequent to being submerged in the water.

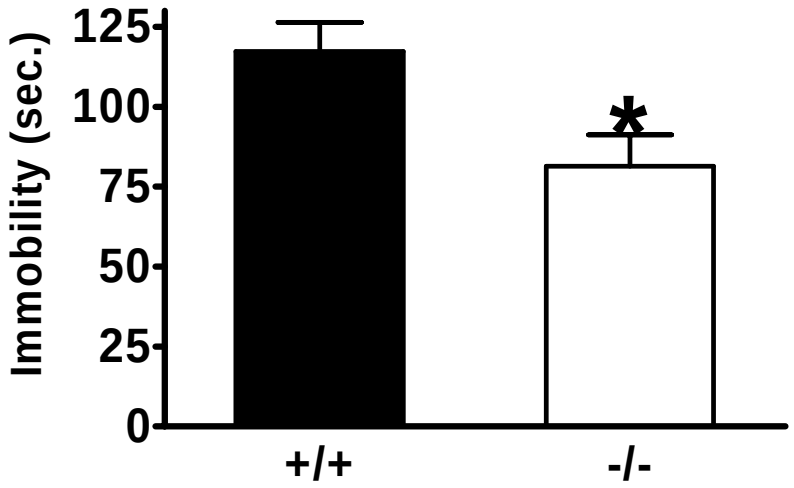

Fig.5.1. Effect of targeted deletion of GABA$_{B(1)}$ receptor subunit in the forced swim test.
GABA$_{B(1)}^{-/-}$ mice (n = 16) had a much lower immobility score than wildtype (n = 16) in the mouse forced swim test, which indicates an antidepressant-like effect. All bars represent mean values, with vertical lines indicating one SEM. *Groups that differed significantly from to wildtype mice (P <0.05).

5.3.1.2 Tail suspension test.

We also tested the animals in the tail suspension test, another well validated model for assessing depression related behaviour in mice (Steru et al., 1985). Further confirming accumulating evidence, that both tests rely on different neurochemical substrates to mediate their behavioural effects (see (Cryan et al., 2005), deletion of GABA$_{B(1)}$ receptor subunit failed to affect the immobility score in this test (*t*-test, P = 0.710) (Fig.7B). There was no observable occurrence of seizures or altered motor patterns in animals subsequent to being suspended by the tail. Further, no tail climbing was observed as has been reported with other background strains of mice (Mayorga and Lucki, 2001)..

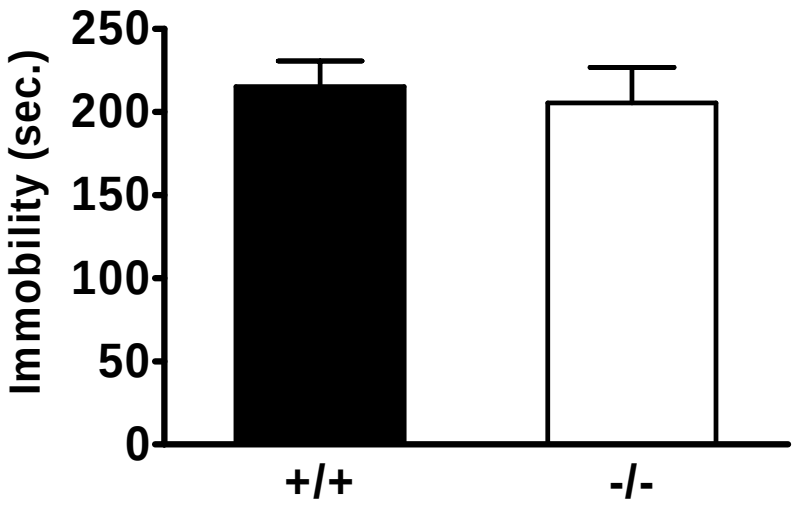

Fig.5.2. Effect of targeted deletion of $GABA_{B(1)}$ receptor subunit on tail suspension test.
$GABA_{B(1)}^{-/-}$ mice (n = 15) exhibited no difference in immobility compared to wildtype mice (n = 16) in the mouse tail suspension test. All bars represent mean values, with vertical lines indicating one SEM.

5.3.1.3 Locomotor activity.

In order to address the issue of whether the behavioural effects of $GABA_{B(1)}^{-/-}$ mice seen in the FST are related to potential hyperactivity, we analyzed the locomotor pattern of both $GABA_{B(1)}^{-/-}$ and $GABA_{B(1)}^{+/+}$ mice. In a novel environment the locomotor activity of the same mice that had previously undergone the FST was recorded over a period of 30 minutes. Repeated measures ANOVA revealed a clear impact of the targeted deletion of $GABA_{B(1)}$ receptor subunit on locomotor activity [$F(1,29) = 9.9$, $P = 0.001$]. As shown in Fig. 8A, $GABA_{B(1)}^{-/-}$ mice exhibited a lower horizontal activity compared to wildtype mice during the first 20 minutes of the trial. This reduction of locomotor activity during the first minutes of trial, could translate into a deficit in habituation to a novel environment in $GABA_{B(1)}^{-/-}$ mice and/or to an increased freezing behaviour, conforming the anxious phenotype observed in anxiety paradigms.

Correlations were also made between activity in the FST and the first 10 minutes in the novel locomotor activity chambers. Similar correlations were made with data obtained in

tail suspension test. As shown in Fig. 8B, there was no correlation between locomotor activity (distance travelled) and immobility in forced swim test in wildtype mice [R = 0.349, P = n.s.] as well as in $GABA_{B(1)}^{-/-}$ mice [R = 0.008, P = n.s.]. These results suggest an absence of a stimulant effect as a result of $GABA_{B(1)}$ deletion. Additionally, no correlation was observed between immobility in tail suspension test and locomotor activity in a novel environment (data not shown).

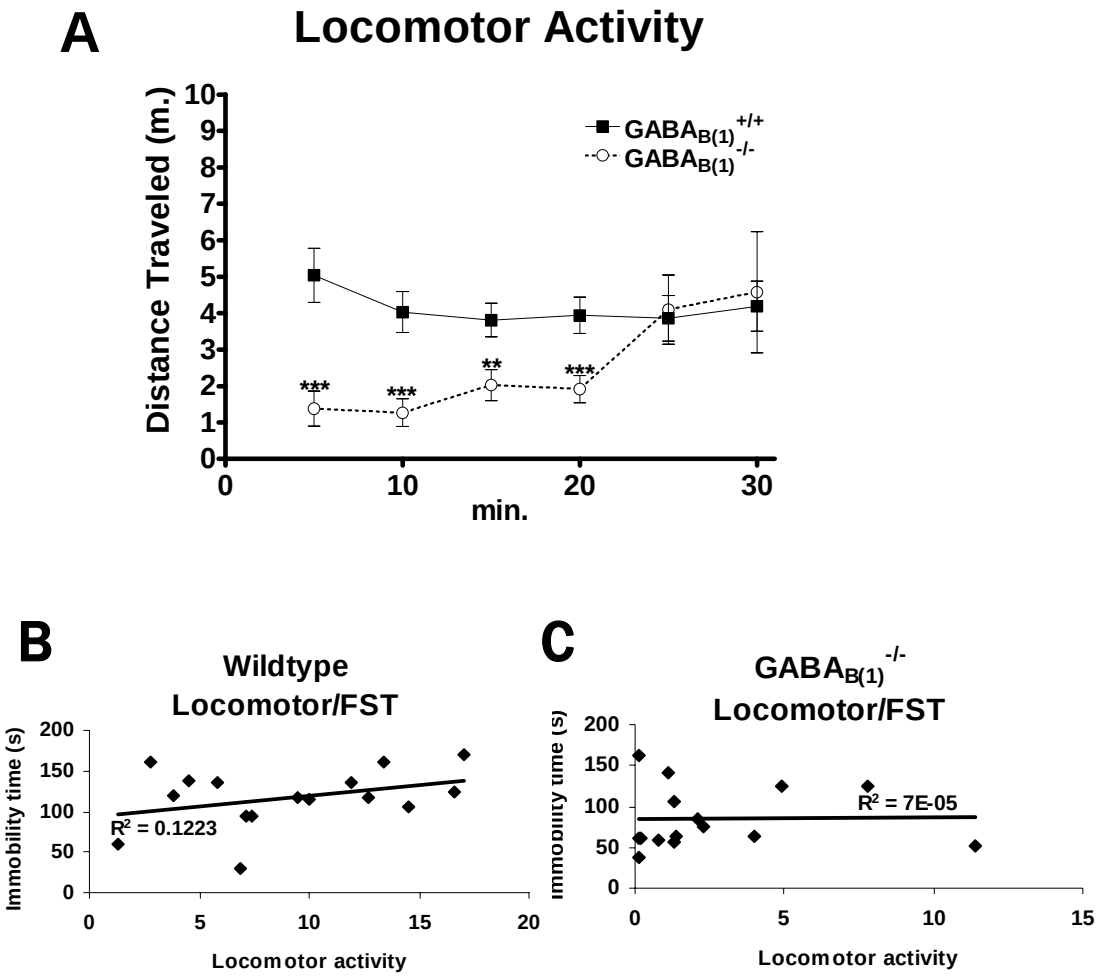

Fig.5.3. Effect of $GABA_{B(1)}$ deletion on locomotor activity in mice pre-tested with FST: Deficits in habituation and lack of correlation with FST.
(A) $GABA_{B(1)}^{-/-}$ mice (n = 15) had a much lower locomotor activity score than wildtype mice(n = 16) during the first 20 minutes of the 30-minute trial. There was no consistent correlations between immobility score in the FST and locomotor activity score in (Panel B) wildtype mice and (Panel C) $GABA_{B(1)}^{-/-}$ mice. All bars represent mean values, with vertical lines indicating one SEM. **, ***Groups that differed significantly from to wildtype mice ($p < 0.01$ and $p < 0.001$ respectively).

5.3.3 Effects of targeted deletion of $GABA_{B(2)}$ receptor subunit in models of antidepressant activity.

5.3.3.1 Forced swim test.

In the previous chapter, we provide evidence that $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice behave similarly in paradigm related to anxiety disorders. In effort to assess whether these similarities generalize across other behavioural domains we assessed the effects of targeted deletion of $GABA_{B(2)}$ receptor subunit in the well characterized animal model of antidepressant activity, the FST As we expected, $GABA_{B(2)}^{-/-}$ mice spent less time in immobility than their littermate wild-type [*t-test*, $P < 0.0001$], indicating an antidepressant-like effect. Combined with the antidepressant-like phenotype observed in $GABA_{B(1)}^{-/-}$ mice, these data suggest that disruption of $GABA_B$ receptor function *via* genetic ablation of one of the two $GABA_B$ subunits, induced antidepressant-like effect in the forced swim test.

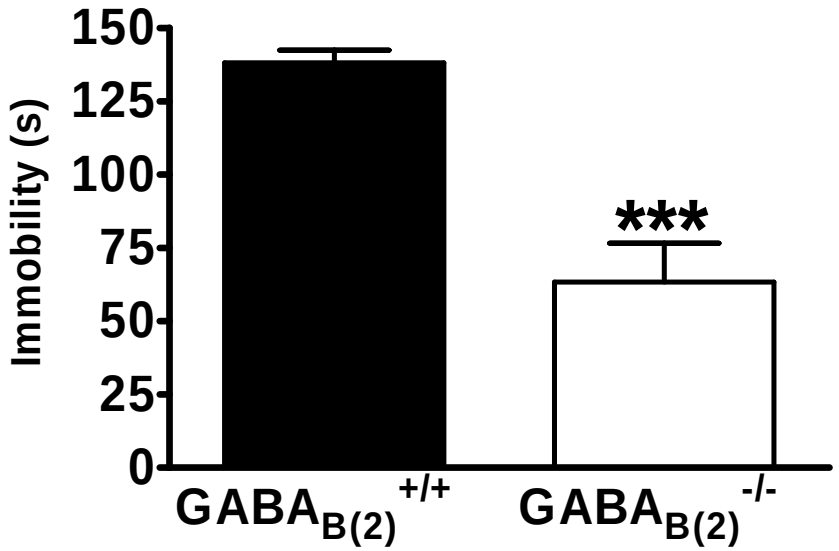

Fig. 5.4. Targeted deletion of $GABA_{B(2)}$ receptor subunit induced antidepressant-like behaviour.
$GABA_{B(2)}^{-/-}$ mice (n=11) had a much lower immobility score than wildtype (n = 12 in the mouse forced swim test, which indicates an antidepressant-like effect. All bars represent mean values, with vertical lines indicating one SEM. ***groups that differed significantly to wildtype mice ($P < 0.001$).

5.4 Pharmacological studies.

5.4.1 Effects of GABA$_B$ receptors antagonist in models of antidepressant activity.

5.4.1.1 Forced swim test.

To test whether the antidepressant-like effect due to genetic inactivation of GABA$_B$ receptor could be recapitulated following pharmacological antagonism, we tested the highly selective and potent GABA$_B$ receptor antagonist CGP56433A in the FST. As shown in Fig. 9A, acute administration of CGP56433A affected immobility time in the FST [$F(4,53) = 4.56$, $P = 0.003$]. Post-hoc analysis revealed that CGP56433A (10 and 30 mg/kg) produced a significant decrease in immobility, supporting that genetic inactivation or pharmacological blockade of GABA$_B$ receptor result in the same antidepressant-like effect in the forced swim test. As shown in fig. 5.5, CGP56433A failed to significantly influence locomotor activity in habituated mice [$F(4,55) = 0.8533$, $P = 0.49$]. These data exclude any potential stimulant effect of CGP56433A contributing to behaviour in the FST.

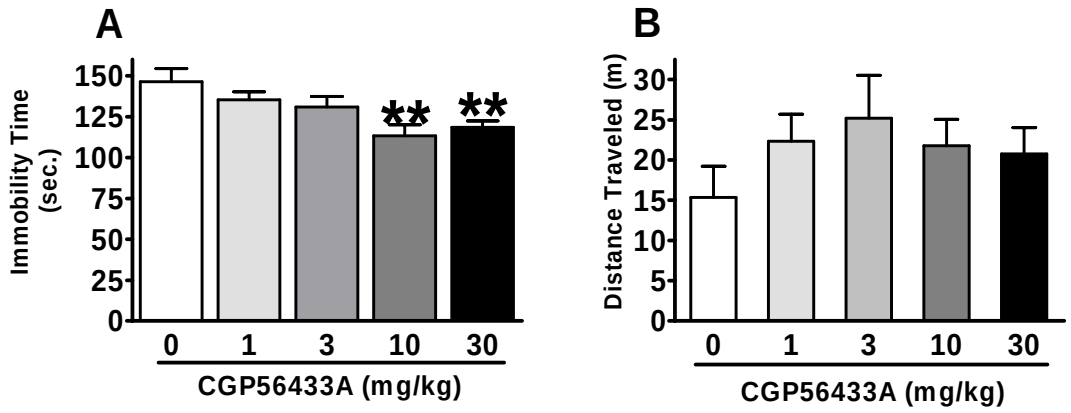

Fig.5.5. Acute treatment with CGP56433A reduces immobility in FST but not TST.
(A) Effect of CGP56433A treatment (doses: 1, 3, 10 and 30 mg/kg, p.o.) on immobility time in forced swim test. *n* = 10-12 per treatment group. (B) Effect of CGP56433A treatment (doses:1, 3, 10 and 30 mg/kg, p.o.) on locomotor activity (60 minutes) in mice that were habituated (for 60 minutes) to the novel environment. *n* = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. **groups that differed significantly to vehicle treated mice (P< 0.01).

5.4.1.2 Tail suspension test.

Targeted deletion of $GABA_{B(1)}$ receptor decrease the time spent in immobility in the FST but not in the TST. In attempt to assess whether this behavioural profile can be replicated the following pharmacological antagonism, we also assessed the effect of $GABA_B$ receptor antagonist in TST. Interestingly, ANOVA revealed no effect of treatment on time spent in immobility in the TST [F(2,27)=0.24, *P*=0.791]. Combined with results observed in FST, these data confirmed a growing body of data which suggest that the FST and the TST don't recruit the same neurochemical substrate (see Cryan et al, 2005).

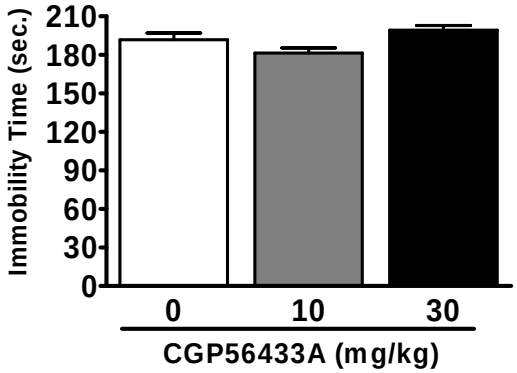

Fig.5.6. Acute treatment with CGP56433A failed to reduce immobility in TST.
Effect of CGP56433A treatment (doses:0, 10 and 30 mg/kg, p.o.) on immobility time in tail suspension test. *n* = 10 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. **groups that differed significantly to vehicle treated mice (P< 0.01).

5.4.1.3 Chronic studies

Although acute administration of antidepressants produces effects in the FST, depressive patients must be treated with antidepressants chronically for several weeks to produce full therapeutic effects (Mann, 2005). Thus, we assessed the effect of chronic treatment (21 days) with sub-effective dose of CGP56433A , 3 mg/kg, in addition to GS39783 (a GABA$_B$ receptor positive modulator) and desipramine in the forced swim test (20 to 24 hours following last treatment). As shown fig.5.7 , animals administrated chronically (21 days) with both CGP56433A (3 mg/kg, p.o., once daily) and desipramine (10 mg/kg, p.o., once daily) reduced immobility times in the FST whereas GS39783 was without any effect [F(3,44) = 7.966, P =0.001]. Taken together, these data further suggest an antidepressant effect of CGP56433, although the magnitude of the effects after chronic treatment are less robust compared with that induced by the antidepressant desipramine.

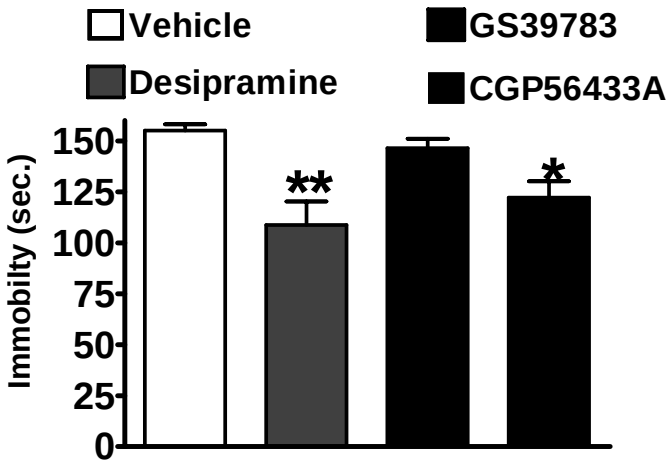

Fig. 5.7. Chronic treatment with CGP56433A and desipramine reduces immobility in the FST.
Effects of chronic treatment (21 days) with the GABA$_B$ antagonist CGP56433A(3 mg/kg, p.o.), desipramine (15 mg/kg, p.o.) and the GABA$_B$ positive modulator GS39783 (10 mg/kg, p.o.) on immobility time in the FST. n = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *,**groups that differed significantly to vehicle treated mice($P < 0.05$ and $P< 0.01$, respectively).

5.4.2 Effects of GABA$_B$ receptors agonist in models of antidepressant activity.

Given the antidepressant-like properties of GABA$_B$ receptor antagonist, we assessed the effect of selective and potent GABA$_B$ receptor agonist, CGP44532, in the forced swim test. Analysis of variance indicates a significant effect of treatment [$F(4,53) = 9.039$, $P =0.001$]. Post-hoc analysis indicated that CGP44532 , at 0.3 and 1 mg/kg, increase the time spent in immobility in the forced swim test paradigm ($P < 0.05$ and $P < 0.001$, respectively). In order to exclude a potential sedative effect of CGP44532, we assessed the effects of this ligand on locomotor activity. Although ANOVA revealed an absence of effect of treatment on distance travelled during the 60 minutes of test [$F(4,55) = 2.414$, $P =0.0597$], there was a trend toward CGP44532 decreasing the distance travelled which failed to reach the level of significance. Although GABA$_B$ receptor activation appears to induce depressant effect in the forced swim test, we cannot exclude that the sedative properties of GABA$_B$ receptor agonist confounds this effect.

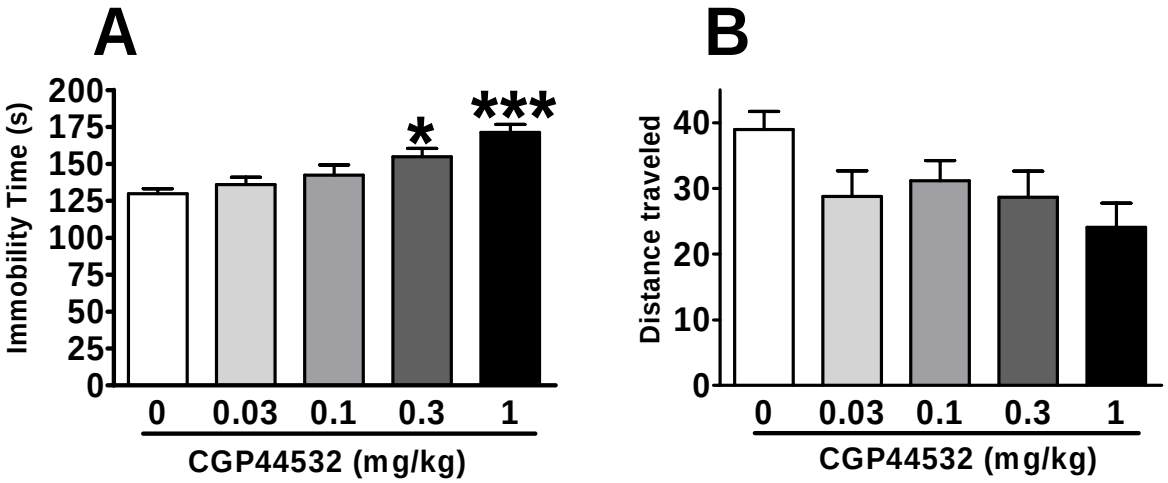

Fig.5.8. Acute treatment with GABA$_B$ receptor agonist increases immobility in FST.

(A) Effect of CGP44532 treatment (doses: 0.03, 0.1, 0.3 and 1 mg/kg, p.o.) on immobility time in forced swim test. *n* = 10-12 per treatment group. (B) Effect of CGP44532 treatment (doses:1, 3, 10 and 30 mg/kg, p.o.) on locomotor activity (60 minutes) in mice that were habituated (for 60 minutes) to the novel environment. *n* = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *, ***groups that differed significantly to vehicle treated mice (P < 0.05 and P< 0.001, respectively.).

5.5 Assessing interactions between GABA$_B$ receptors and serotoninergic system.

5.5.1 Effects of GABA$_B$ receptor antagonist on anti-immobility properties of SSRI in the forced swim test.

Emerging evidence suggest that GABA$_B$ receptor antagonists may recruit serotoninergic mechanisms to elicit their behavioural effects (Slattery et al., 2005a). In order to investigate the interactions between GABA$_B$ and serotoninergic system, we examined the combinations of different doses of GABA$_B$ receptor antagonist (0, 3 and 10 mg/kg) with fluoxetine treatments (0, 3, 10 and 30 mg/kg) in the forced swim test. Two-way ANOVA revealed an significant effect of treatment with CGP56433A [F(2,132) = 8.470, P =0.001], of fluoxetine [F(3,132) = 3.068, P =0.05], nevertheless there is no fluoxetine X CGP56433 interactions [F(3,132) = 0.919, P =0.483]. Although post-hoc tests revealed an antidepressant like effect of fluoxetine alone at 10 and 30 mg/kg and CGP56433A at 10 mg/kg, GABA$_B$

receptor antagonist failed to potentiate anti-immobility effect of fluoxetine in the forced swim test.

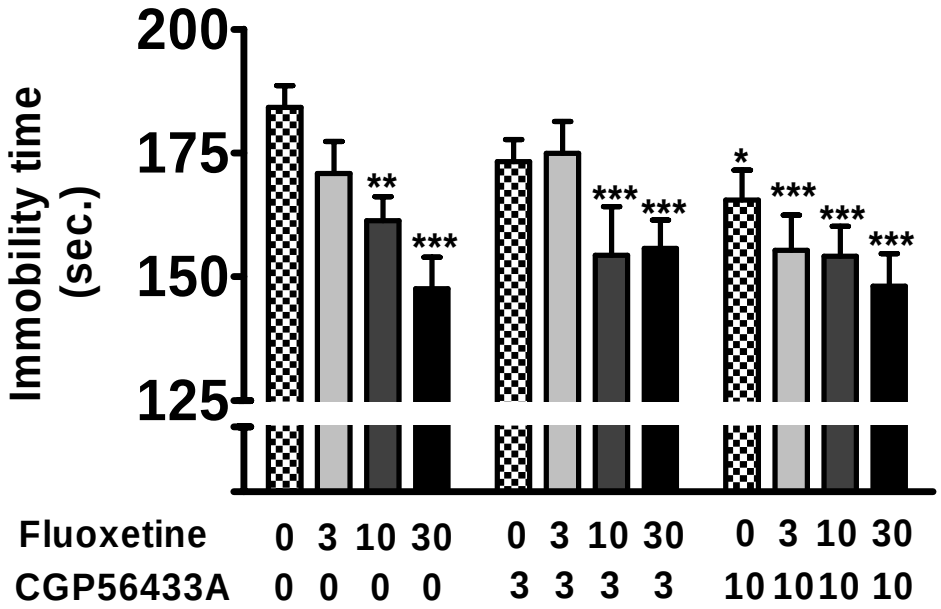

Fig. 5.9. Effects of GABA$_B$ receptor antagonist on anti-immobility properties of SSRI in the forced swim test.
(A) Effect of CGP56433A treatment (doses: 0, 3, 10 mg/kg, p.o.) combined with Fluoxetine treatment (doses: 0, 3, 10 and 30 mg/kg, p.o.)immobility time in forced swim test. *n* = 12 per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *, **, ***groups that differed significantly to vehicle treated mice (P < 0.05 and P< 0.001, respectively.).

5.5.2 Differential hypothermic response to baclofen following pharmacological or genetic blockade of serotonin transporter.

5.5.2.1 Impact of acute fluoxetine treatment on baclofen-induced hypothermia.

As mentioned in the third chapter, baclofen-induced hypothermia can be considered as a good in vivo index of GABA$_B$ receptor function. Consequently, it provides a powerful tool in order to investigate the impact of serotonergic treatment on GABA$_B$ receptors function. Thus, we assessed the effect of acute treatment with fluoxetine on the hypothermic response induced by baclofen. The ANOVA (repeated measures) indicate a significant effect of treatment with baclofen [$F(1,36) = 223.224$, $P < 0.001$], an fluoxetine X baclofen interaction

[F1,32) = 13.802, P =0.001] but no effect of treatment with fluoxetine [F(1,36) = 01.245, P =00.272]. Therefore, the two-way repeated measures ANOVA revealed also an effect of time [F3,108) = 172.66, P <0.001], time X baclofen interaction [F3,108) = 13.802, P =0.001] but no time X fluoxetine interaction [F3,108) = 1.423, P <0.241] and time X fluoxetine X baclofen interaction [F3,108) = 1.655, P <0.183]. Acute administration of fluoxetine (in vehicle treated animals) failed to affect temperature. In contrast, baclofen induced a marked decrease in body temperature in mice treated with fluoxetine and with vehicle. Finally, acute administration of fluoxetine attenuated the hypothermic response induced by baclofen one hour but not two hours after administration of baclofen. Thus, we can conclude that acute blockade of serotonin transporter failed to affect GABA$_B$ receptor function.

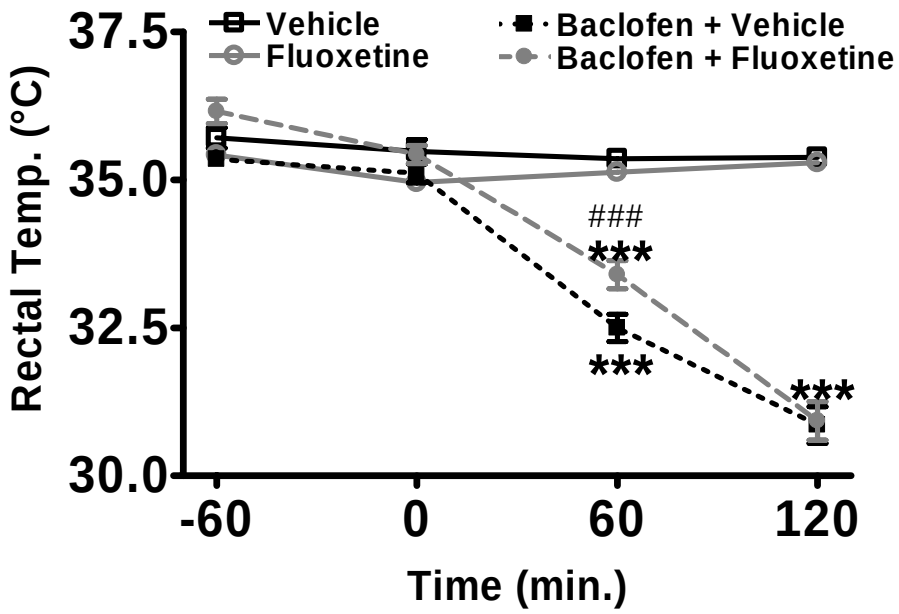

Fig.5.10. Effect of acute fluoxetine treatment on baclofen-induced hypothermia. Acute administration of fluoxetine (20 mg/kg; p.o.) failed to affect body temperature compared to mice treated with vehicle. Baclofen (9 mg/kg, p.o.) induced a strong hypothermia in group treated with fluoxetine and in the group treated with vehicle. N = 12 per treatment group All data points represent mean ± SEM values.. ***Groups that differed significantly from to vehicle treated animals (p<0.001).. ### groups that differed significantly to animals control animals treated with baclofen(p<0.001).

5.5.2.2 Impact of chronic fluoxetine treatment on baclofen-induced hypothermia.

Although the acute administration of fluoxetine appears to have no effect on $GABA_B$ receptors function, blunted $GABA_B$-receptor-induced effects (including electrophysiological and signalling responses) have been demonstrated in the DRN of mice lacking the 5-HT transporter that is the molecular substrate for SSRI antidepressants (Mannoury la Cour et al., 2004) suggesting that chronic treatment with SSRI may affect $GABA_B$ receptor function. In order to corroborate these results in vivo, we investigated the effect of chronic fluoxetine treatment (20 mg/kg/day during 21 days.) on the hypothermic response elicited by baclofen. Fig.5.11A showed the effect of chronic treatment with fluoxetine on baclofen-induced hypothermia 24 hours after the last injection of fluoxetine. One-way repeated measures ANOVA revealed a significant effect of fluoxetine treatment [$F(1,22) = 47.247$, $P < 0.001$],, of time [$F(3,66) = 80.429$, $P < 0.001$] and time X fluoxetine interaction [$F(3,66) = 37.717$, $P < 0.001$]. Although baclofen elicited a marked hypothermia in vehicle-treated animals, this hypothermic response was totally blunted in mice treated chronically with fluoxetine. Similarly, we also observed this phenomenon 48 hours and 72 hours after the last injection of fluoxetine (Fig 5.11B and C, respectively). 48 hours later the last injection of fluoxetine, the one-way repeated measures ANOVA revealed a significant effect of fluoxetine treatment [$F(1,9) = 20.650$, $P = 0.0014$], of time [$F(2,18) = 16.032$, $P < 0.001$] and time X fluoxetine interaction [$F(2,18) = 29.933$, $P < 0.001$]. Finally, 72 hours after the last injection of fluoxetine, the one-way repeated measures ANOVA revealed a significant effect of fluoxetine treatment [$F(1,10) = 11.890$, $P = 0.0062$],, of time [$F(2,20) = 9.562$, $P = 0.0012$] and time X fluoxetine interaction [$F(2,20) = 9.733$, $P = 0.0011$]. In summary, we demonstrated that chronic, but not acute treatement with fluoxetine blocked hypothermic response elicited by

baclofen, suggesting that chronic blockade of 5-HT transporter alter GABA$_B$ receptor function.

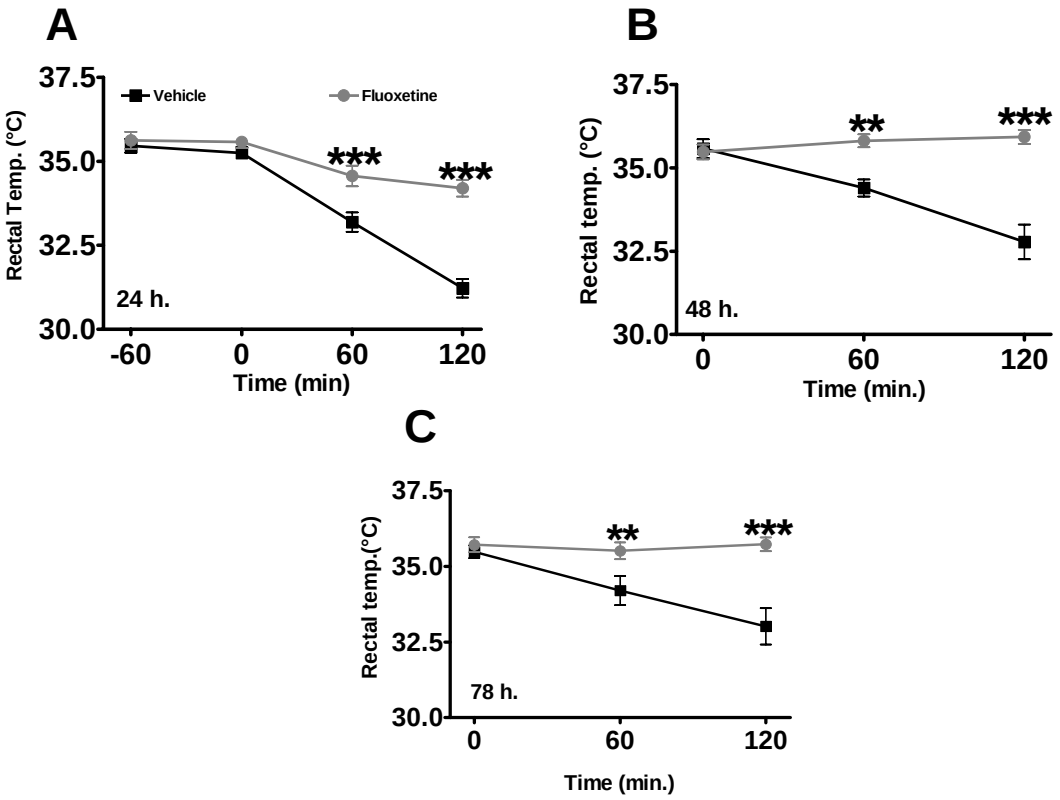

Fig.5.11. Effect of chronic fluoxetine treatment on baclofen-induced hypothermia.

Chronic administration of fluoxetine (20mg/kg/day during 21 days) blunted totally the hypothermic response triggered by baclofen , 24 hours (A), 48h hours (B) and 72 hours (C) after the last injection of fluoxetine. N = 12 per treatment group for the experiment made 24 hour after the last injection of fluoxetine and N =5-6 per treatment group for experiment made 48h and 72h after the last injection of fluoxetine. All data points represent mean ± SEM values..**, ***Groups that differed significantly from to vehicle treated animals ($p<0.01$ and $p<0.001$, respectively).

5.5.2.3 Effects of targeted deletion of serotonin transporter on baclofen-induced hypothermia.

In an attempt to confirm the results obtained after chronic treatment with fluoxetine, we also investigated the effect of genetic inactivation of serotonin transporter on the

hypothermic response induced by baclofen. The one-way repeated measures ANOVA revealed a significant effect of genotype treatment [F(1,19) = 14.492, P = 0.0012], of time [F(5,95) = 157.990, P <0.001] and time X genotype interaction [F(5,95) = 6.507, P < 0.001]. Intriguingly, post-hoc analysis revealed that 5-HTT$^{-/-}$ mice exhibited more pronounced hypothermia that wild-type animals. Interestingly, we demonstrated that chronic pharmacological blockade and genetic inactivation of serotonin transporter modulate hypothermic response elicited by baclofen in opposite manners. Nevertheless, it is important to note that compensatory changes in 5-HTT$^{-/-}$ mice cannot be excluded.

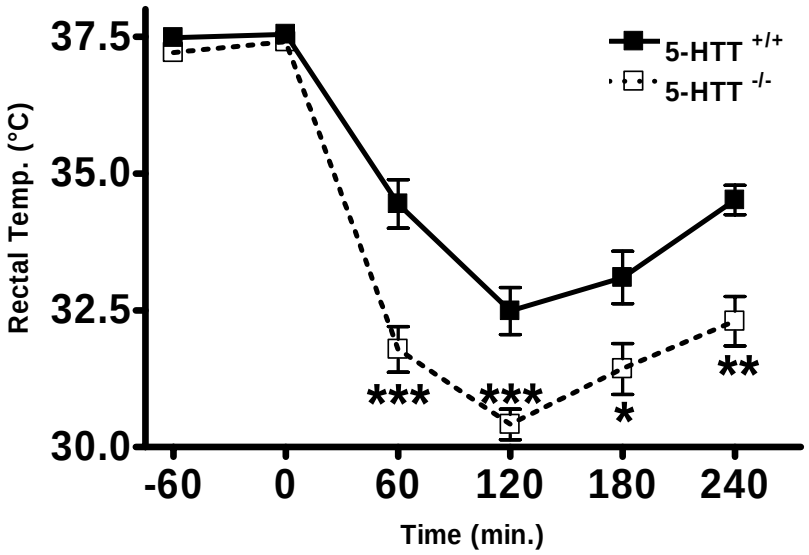

Fig.5.12. Effect of targeted deletion of serotonin transporter on baclofen-induced hypothermia.
Acute administration of (+/-)baclofen (12 mg/kg) elicited a pronounced hypothermia in both genotype. Nevertheless the amplitude of hypothermic response was more marked in 5-HTT$^{-/-}$ mice (N=14) compared to wild-type animals (N=7).. All data points represent mean ± SEM values.. *, **, ***Groups that differed significantly from to vehicle treated animals (p<0.05, p<0.001 and p<0.001, respectively).

5.5.3 Effects of GABA$_B$ receptors ligands and antidepressants on downregulation of hippocampal 5-HT$_{1A}$ receptors mRNA induced by chronic handling stress.

We hypothesized that $GABA_B$ receptor ligands, by interacting with serotonergic neurotransmission, would counteract the downregulation of hippocampal 5-HT_{1A} receptor mRNA induced by handling-stress. In order to confirm this hypothesis, we investigated the effects of chronic handling on 5-HT_{1A} mRNA receptor in hippocampus and attempted to reverse these effects with antidepressants and $GABA_B$ receptor ligands.

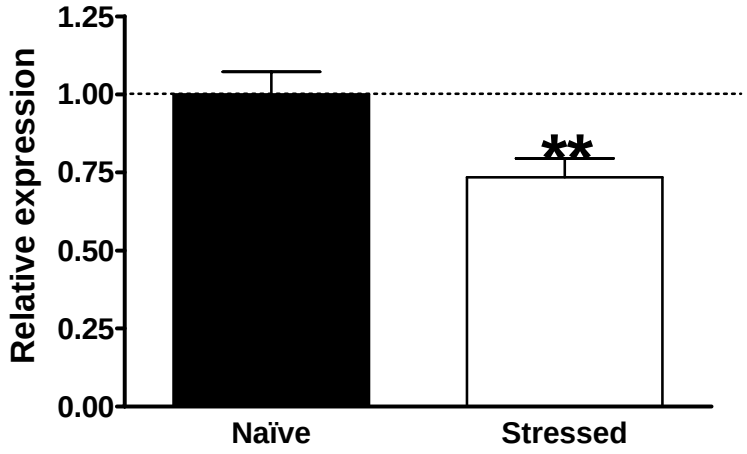

Fig.5.13. Effect of chronic handling and injections of vehicle on 5-HT_{1A} receptor mRNA in hippocampus of BALB/c mice.
Data represent mean ± s.e.m. Student's t-test was performed where ** P < 0.01 represents statistical difference compared with vehicle.

As shown in Fig.5.13, we observed a downregulation of 5-HT_{1A} receptor mRNA (-26.6 %) in the hippocampus after chronic handling (combined with injection) compared to naïve animals (p = 0.012). In contrast, as shown in Fig.5.14A, acute administration of fluoxetine increased 5-HT_{1A} mRNA expression in the hippocampus compared to vehicle treated animals. Similarly, sub-chronic and chronic fluoxetine treatment up-regulated hippocampal 5-HT_{1A} receptor mRNA (+31.6 %, p= 0.028 and +20.4%, p=0.06 respectively). Although, neither acute nor sub-chronic treatment with desipramine up-regulated 5-HT_{1A} mRNA, we observed that 21 days treatment with desipramine significantly increased 5-HT_{1A} mRNA expression in hippocampus (+35%, p=0.003, Fig. 2B).

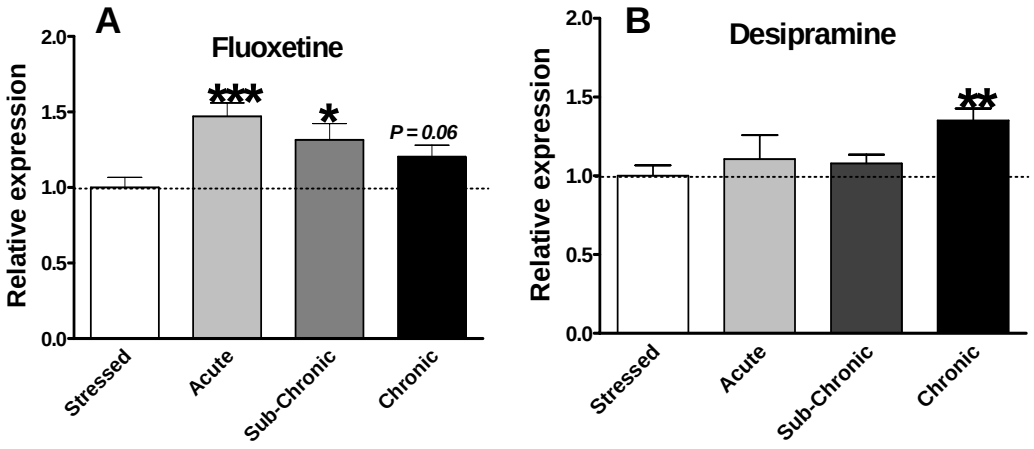

Fig.5.14. Effects of (A) fluoxetine (20 mg/kg p.o) and (B) desipramine (15 mg/kg, p.o) treatments (acute , sub-chronic and chronic) on 5-HT$_{1A}$ receptor mRNA in hippocampus of BALB/c mice.

Data represent mean ± s.e.m. Student's t-test was performed where * P<0.05, ** P < 0.01 and *** P < 0.001 represent statistical difference compared with vehicle.

As shown in figure 5.15, 5-HT$_{1A}$ receptors mRNA level were significantly higher in all baclofen treated groups. There was a 42% increase of 5-HT$_{1A}$ receptor mRNA after acute treatment with baclofen, 53% increase of 5-HT$_{1A}$ receptor mRNA level after sub-chronic baclofen treatment whereas chronic baclofen treatment increased expression level by 34.3% (p=0.0028, p=0.0005 and p=0.031 respectively) (Fig.5.15 A). Similarly, GS39783, the novel GABA$_B$ receptor positive modulator, significantly increased 5-HT$_{1A}$ expression in hippocampus acutely, sub-chronically and chronically (+53%, p=0.005; +39%, p=0.0024 and +44 %, p=0.003 respectively, Fig.5.15B). We also observed that CGP564333A, a GABA$_B$ receptor antagonist, failed to change 5-HT$_{1A}$ mRNA expression after acute and sub-chronic treatment (Fig.5.15 C), while 21 days treatment with CGP56433A increased significantly hippocampal 5-HT$_{1A}$ mRNA level (+30.7, p=0.015). To conclude, we demonstrate that even a relatively minor stressor, such as daily handling and injections can induce marked changes in hippocampal 5-HT$_{1A}$ receptor expression levels of BALB/c mice. These data confirm, and expand upon, the results obtained from previous preclinical and clinical studies which demonstrated that chronic unpredictable stress downregulates, whereas antidepressant

treatments up regulates hippocampal 5-HT$_{1A}$ receptors (Lopez et al., 1998). Furthermore, we demonstrate that antidepressants and GABA$_B$ receptor ligands, can counteract this phenomenon

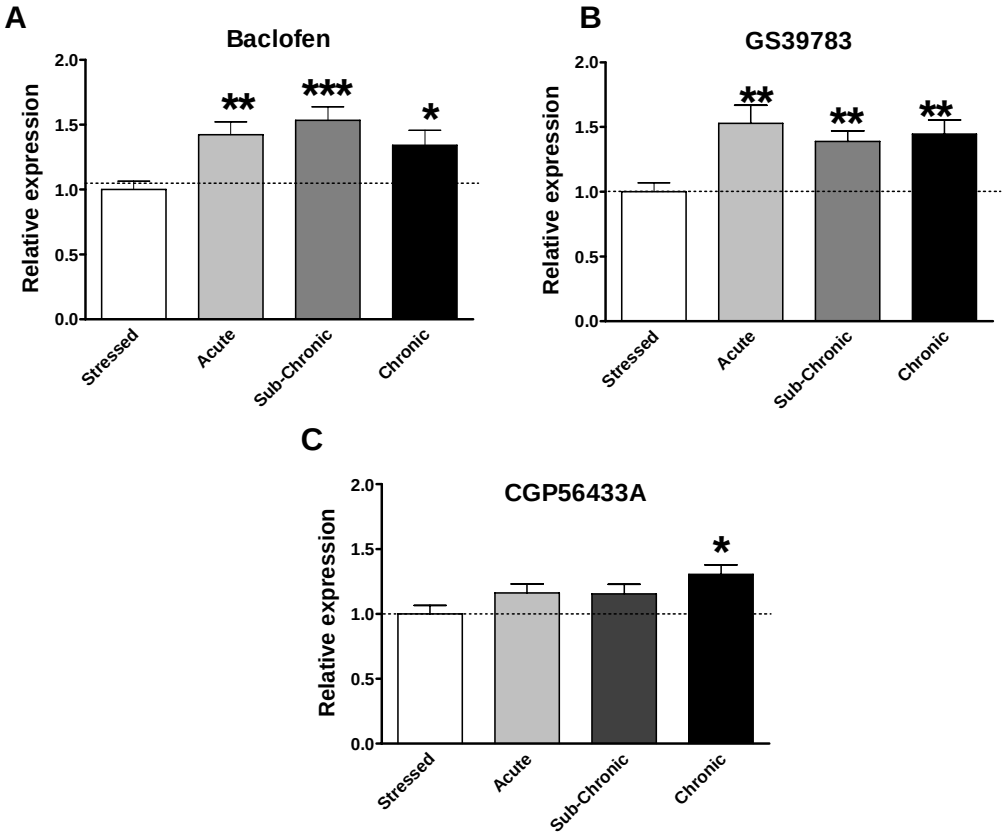

Fig. 5.15. Effects of (A) baclofen (5 mg/kg p.o.) , (B) GS39783 (10 mg/kg p.o.) and (C) CGP56433A (6 mg/kg p.o.) treatments (acute , sub-chronic and chronic) on 5-HT$_{1A}$ receptor mRNA in hippocampus of BALB/c mice.
Data represent mean ± s.e.m. Student's t-test was performed where * P<0.05, ** P < 0.01 and *** P < 0.001 represent statistical difference compared with vehicle

5.6 Discussion.

In the present chapter, we demonstrated that both genetic inactivation and pharmacological blockade of GABA$_B$ receptors, results in the same antidepressant-like effect in the forced swim test but not in tail suspension paradigm. Conversely, we also demonstrated that GABA$_B$ receptor activation elicited depressant-like effect in the forced swim test. In

order to elucidate the interaction between serotoninergic system and $GABA_B$ receptors and their involvement in antidepressant like effect, we assessed the effect of $GABA_B$ receptor antagonist on the anti-immobility effect of fluoxetine in the forced swim test. Somewhat surprisingly, CGP56433A failed to potentiate the antidepressant-like effect elicited by fluoxetine. Secondly, we assessed the effect of several pharmacological and genetic manipulation of serotonin transporter on well characterized index of $GABA_B$ receptor function baclofen-induced hypothermia. Although acutely fluoxetine failed to affect hypothermic response elicited by baclofen, chronically, fluoxetine totally blocked this response, suggesting an alteration of $GABA_B$ receptor after chronic pharmacological blockade of serotonin transporter. Intriguingly, we observed an enhancement of hypothermic response induced by baclofen in 5-$HTT^{-/-}$ mice. Finally, we demonstrated that both antidepressants and $GABA_B$ receptor ligands, including agonist, antagonist and positive modulator; counteract the downregulation of hippocampal 5-HT_{1A} receptor mRNA elicited by chronic handling.

The mouse FST is the most widely used experimental paradigm for detecting antidepressant activity and to assess alterations in depression-like behaviour in genetically modified animals (Borsini and Meli, 1988; Cryan et al., 2002a; Cryan and Mombereau, 2004). The behavioural responses in the FST is thought to comprise a coping strategy (Thierry et al., 1984) in which immobility behaviours represent the psychological concept of "entrapment" described in clinical depression (Dixon, 1998; Gilbert and Allan, 1998; Lucki, 2001). Here we demonstrate that $GABA_{B(1)}^{-/-}$ mice have an antidepressant-like effect in the FST as indicated by significantly lower immobility than their wildtype controls. This effect is not due to hyperactivity per se, as a reduced locomotor response was observed in the very same mice after being placed in a novel locomotor activity chamber, with activity increasing over time. This is compatible with the anxious phenotype of $GABA_{B(1)}^{-/-}$ mice and suggests that they are more fearful upon being placed in a novel environment. In opposition to normal habituation

responses in a novel environment, locomotor activity in $GABA_{B(1)}^{-/-}$mice slowly increased with time indicating a disinhibition of their initial anxiety. Further, there was no correlation between activity in the FST and that in the locomotor activity apparatus (Fig. 8). This initial hypoactivity was unrelated to prior exposure to swim stress or age, as it was also evident (although not as pronounced) in experimentally naïve mice (Fig. 2). However, at later time-points these animals became somewhat more active than wildtype controls, which is in accordance with previous data (Schuler et al., 2001).

Interestingly, $GABA_{B(1)}^{-/-}$ mice behave similarly to their wildtype controls in the tail suspension test. The tail suspension test is another well characterized test for assessing depression-like and antidepressant-like activity (Porsolt, 2000; Cryan et al., 2002a; Cryan and Mombereau, 2004; Cryan et al., 2005). Although this test is similar to the FST in the constructs that it purports to assess (immobility) and for its ability to detect a broad spectrum of antidepressants (Steru et al., 1985), it is becoming clear that both tests are probably different from each other in terms of the biological substrates that underlie their observed behaviours (Bai et al., 2001; Renard et al., 2003; Cryan and Mombereau, 2004; Cryan et al., 2005). Accordingly, it is believed that using both paradigms can give complementary and/or converging information on activities of novel potential antidepressants or molecular pathways including those altered in genetically modified animals (Bai et al., 2001) (Conti et al., 2002; Cryan et al., 2005). The current data are among the first to show differential effects of a genetic modification in the FST and the tail suspension test and confirms the assertion of a differential neurochemical underpinning to each test.

In agreement with what we previously observed in the light-dark box test of anxiety, $GABA_{B(2)}^{-/-}$ mice also behaved similarly to $GABA_{B(1)}^{-/-}$ mice in the forced swim test. Indeed, targeted deletion of $GABA_{B(2)}$ receptor subunit induced a marked decrease in the time spent in immobility in the forced swim test. Consistent with the anxiety data, the $GABA_{B(1,2)}$

heterodimer appears to be required for the neurophysiologic processes involved in the forced swim test.

In order to confirm the antidepressant-like phenotype of the $GABA_{B(1)}^{-/-}$ mice and $GABA_{B(2)}^{-/-}$ mice pharmacologically, we assessed the effects of the $GABA_B$ receptor antagonist CGP56433A in the FST. Our data demonstrate that this $GABA_B$ receptor antagonist when administered acutely also decreases immobility in the FST without having any significant change in locomotor activity (Fig. 9). Chronic administration of CGP56433A also produced an antidepressant-like effect similar to that of the antidepressant desipramine (Fig. 10). These data corroborated previous results obtained in another depression paradigm, such as chronic mild stress (Bittiger et al., 1996) or learned helplessness (Nakagawa et al., 1999). Conversely, we observed that $GABA_B$ receptor agonist elicited depressant like effect in FST. These results are in line with previous observation describing that baclofen increased susceptibility to helplessness and attenuated the effects of antidepressants (Nakagawa et al., 1996a; 1996b). Furthermore, baclofen also reduced the efficacy of antidepressants in the FST (Nakagawa et al., 1996c). Taken together our current data support the contention that antagonism of $GABA_B$ receptors may be a suitable target for the development of antidepressant agents.

Although we demonstrated that $GABA_B$ receptors appear to be involved in the neuropathophysiolgy of depression, the mechanism implicated in the antidepressant-like effect of $GABA_B$ receptor antagonist are not clear. Nevertheless, several authors have speculated that antidepressant-like effects of $GABA_B$ receptor antagonist might be mediated via the serotoninergic system. Indeed, Slattery and coworkers have demonstrated that $GABA_B$ receptor antagonist increased swimming behaviour in the modified rat forced swim test (Slattery et al., 2005a). This behavioural pattern has been shown to be selectively affected by SSRIs and serotonergic agonists (Cryan et al., 2002a). Moreover, they also demonstrated that

serotonin depletion attenuates the increase in swimming induced by CGP564333A, confirming the involvement of serotonergic system in the antidepressant-like effect of $GABA_B$ receptor antagonists. Congruently, it has been shown that $GABA_B$ receptor stimulation in the dorsal raphé inhibits the firing of serotoninergic neurons (Innis et al., 1988). Thus, we hypothesized that $GABA_B$ receptor antagonist should potentiate the effect of SSRIs, via alleviation of the inhibition of firing serotoninergic neurons in raphé. Although both the $GABA_B$ receptor antagonist and fluoxetine induced an antidepressant-like effect in the forced swim test, we demonstrated that $GABA_B$ receptor blockade failed to potentiate the anti-immobility effect of fluoxetine in this test. Despite these negative data without neurochemical studies, we cannot definitively conclude that $GABA_B$ receptor antagonists do not enhance serotonin release induced by fluoxetine. Similarly, there is extensive data available which demonstrate that SSRI effects on extracellular 5-HT concentration (Gartside et al., 1995) might be increased by administration of $5\text{-}HT_{1A}$ autoreceptor antagonists, however they appear to be without effect on behavioural response elicited by SSRI (Moser and Sanger, 1999). Nevertheless, this hypothesis was recently validated using phaclofen and citalopram in the tail suspension. Indeed, Cremers et al. demonstrated that systemic administration of $GABA_B$ antagonist augment both anti-immobility effect and serotonin release elicited by citalopram SSRI injection (Jongsma et al., 2004). Taking this research into account, it appears that $GABA_B$ receptor antagonist might also be considered for accelerate the onset of action and increase the effectiveness of antidepressant. The use of animal model requiring chronic antidepressant treatment, such as chronic mild-stress or olfactory bulbectomy will confirm the reduction of onset of action of antidepressant by $GABA_B$ receptor. Thus, further studies, such as microdialysis studies, are clearly required in order to more appreciate the impact of $GABA_B$ receptor blockade on the antidepressant-like effect of SSRI and their associated release of serotonin.

As mentioned in the introduction, several studies investigated the impact of antidepressant on $GABA_B$ receptor function. Kasture and co-workers demonstrated that acute administration of fluoxetine reversed the catatonia induced by baclofen. In the present study, we demonstrated that acute administration of fluoxetine appears to attenuate, but not block, the baclofen-induced hypothermia (Kasture et al., 1996). Conversely, Gray and co-workers demonstrated that repeated treatment with amytriptyline, a tricyclic antidepressant, enhanced temperature and sedation induced by baclofen (Gray et al., 1987). Nevertheless, we demonstrated in the current study that chronic treatment with fluoxetine totally blunted the baclofen-induced hypothermia, suggesting that chronic blockade of serotonin transporter might induce a functional desensitization of $GABA_B$ receptor. These results are consistent with the findings that 5-$HTT^{-/-}$ mice exhibited altered $GABA_B$-induced responses (Mannoury la Cour et al., 2004). Specifically, these animals present a functional desensitization of $5\text{-}HT_{1A}$ autoreceptors in the dorsal raphé. Moreover, the $GABA_B$ receptor agonist inhibited 5-HT cell firing in the DRN, which occurred with a 30-fold decrease in potency in 5-HTT knock-out mice and was blocked by administration of $GABA_B$ receptor antagonist. In addition, [^{35}S]GTP-γ-S binding induced by baclofen was also significantly decreased in the DRN of 5-HTT knockout mice after baclofen administration. Altogether, the findings herein, combined with the data of Mannoury la Cour and collaborators, suggest that both chronic administration of SSRI or genetic ablation of serotonin transport might result in a concomitant functional desensitization of $5\text{-}HT_{1A}$ and $GABA_B$ receptor, mediated by alteration of common pool of G-proteins (Andrade et al., 1986).More recently, targeted deletion of GIRK2 has been shown to attenuated hypothermic response elicited by $5\text{-}HT_{1A}$ and $GABA_B$ receptors agonist (Costa et al., 2005).Given that $GABA_B$ and $5\text{-}HT_{1A}$ receptors are coupled to the same GIRK, we can also presume that modulation of their common GIRK channels might be also responsible of functional desensitization of both $5\text{-}HT_{1A}$ and $GABA_B$ receptors in the raphé nucleus. Nevertheless, further studies are required in order to understand the exact mechanism

underlying this downstream effect. Thus, it might be also interesting to recapitulate our present data using these techniques in effort to better understanding the potential adaptation of $GABA_B$ receptors induced by chronic administration of SSRI. However, we present findings were obtained using baclofen-induced hypothermia as an index of $GABA_B$ receptors and there is no clear evidence, at this time, for a real involvement of $GABA_B$ receptor of raphé in this response. Surprisingly, we also observed that targeted deletion of serotonin transporter potentiated the hypothermic response induced by baclofen. Nevertheless, we cannot exclude compensation mechanism in these mice. Previous studies have shown that SERT deficient animals have behavioural effects that are opposite to that observed following adult knockdown/inhibition of the transporter (Holmes et al., 2003b; Ansorge et al., 2004), which may be due to early life loss of the transporter.

Chronic stress has been shown to be implicated in the aetiology of depressive disorders (Charney and Manji, 2004). In the present studies, we demonstrated that a relatively minor stressor such as daily handling and injections induce marked changes in the hippocampal 5-HT_{1A} mRNA receptor expression of BALB/c mice. These data corroborate, and expand upon, the results obtained from previous preclinical and clinical studies which demonstrated that chronic stress downregulates 5-HT_{1A} receptor mRNA (Lopez et al., 1998). Interestingly, only few studies have investigated the impact of handling and saline injection on behavioural and neurochemical parameters. Saline administration have been shown to induced depressive-like behaviour in the tail-suspension test combined with a suppression of cAMP formation stimulate (Izumi et al., 1996), confirming that chronic handling might be considered as chronic mild stressor. Several other studies demonstrated that different regimen of stressor induced 5-HT_{1A} receptor adaptation in the hippocampus. For example, it has been shown that chronic restraint stress decreased [^{3}H]8-OHDPAT bindings sites in the hippocampus of rat (Watanabe et al., 1993) or tree shrew (Flugge, 1995). Taken together, this

result suggests that chronic mild stress might affect 5-HT_{1A} receptor in the hippocampus. Conversely, we observed that antidepressant, including the tricyclic desipramine and the SSRI fluoxetine, could counteract the effect of stress on hippocampal 5-HT_{1A} receptor mRNA. In agreement with these results, several electrophysiological studies have demonstrated a sensitization of hippocampal 5-HT_{1A} receptor function after long term antidepressant (see (Mongeau et al., 1997) for review). Nevertheless, the effects of chronic antidepressant treatment on the densities, bindings and expression of 5-HT1A receptor in hippocampus have drowned controversial results. Although we corroborated the fact that chronic desipramine treatment counteracted the downregulation of hippocampal 5-HT_{1A} receptor mRNA described by Lopez and coworkers (Lopez et al., 1998), further studies recapitulating these results on the 5-HT_{1A} receptor protein or function, are required to confirmed the role of postsynaptic 5-HT_{1A} receptor in depression an antidepressant-like effect. In addition to effects observed after sub-chronic and chronic treatement, we also observed that acute administration of fluoxetine up-regulated hippocampal 5-HT_{1A} receptor mRNA. Nevertheless, there is no information in the literature regarding this effect and the mechanism involved in this acute effect is still elusive. Finally, we demonstrated that $GABA_B$ activation, via systemic administration of $GABA_B$ receptor agonist and positive modulator, induced an increase in hippocampal 5-HT_{1A} receptor mRNA level following acute treatment that was sustained following sub-chronic and chronic treatment. Considering that bath application of the $GABA_B$ receptor agonist, baclofen, was found to cause a concentration-dependent inhibition of dorsal raphé nucleus 5-HT cell firing (Innis et al., 1988) , it is conceivable to believe that activation of $GABA_B$ receptor could decrease serotonin tone leading to a up-regulation of hippocampal 5-HT_{1A} receptor. Nevertheless, the effects of chronic $GABA_B$ receptor antagonist treatment on this parameter is more difficult to explain, we demonstrate that chronic treatment only, with CGP56433A upregulated 5-HT_{1A} receptors in stressed animals. However, the alleviation of the effects of stress by anxiolytics or antidepressants can be conceivable and we demonstrated that $GABA_B$

positive modulator and $GABA_B$ antagonist have respectively anxiolytic and antidepressant profile in several behavioural paradigms. Nevertheless, it would be simplistic to assume that this effect was induced by a direct action of $GABA_B$ receptor on serotoninergic system. Indeed, in a recent study, Mitsukawa and collaborators demonstrated a up-regulation of hippocampal $5\text{-}HT_{1A}$ mRNA in $mGlur7^{-/-}$(Mitsukawa et al., 2005) in this studies, the authors speculated that this adaptation might be a consequence of regulatory effect of HPA axis on post-synaptic $5\text{-}HT_{1A}$ receptors. Nevertheless, further studies are required to understand the differential onset of action of $GABA_B$ receptor antagonist vs $GABA_B$ positive modulator and to determinate their role in anxiolytic and antidepressant elicited by GS39783 and CGP56433A.

In summary, the present studies demonstrated that genetic inactivation or pharmacological blockade of $GABA_B$ receptors produced antidepressant-like effects in the forced swim test, confirming the role of $GABA_B$ receptor in the modulation of emotion. Although the antidepressant-like effects of $GABA_B$ receptor antagonist have been shown to be mediated by serotoninergic system, the desensitization of $GABA_B$ receptor observed after chronic SSRI treatment suggests that $GABA_B$ receptor might be involved in the therapeutic properties of SSRI. These data, combined with the alleviative effect of $GABA_B$ ligands on downregulation of $5\text{-}HT_{1A}$ receptor mRNA induced by chronic handling, confirm that $GABA_B$ receptors antagonists may represent a novel approach to the treatment of depression.

CHAPTER 6: THE ROLE OF $GABA_B$ RECEPTORS IN ADDICTION.

6.1 Introduction.

There is accumulating evidence indicating that $GABA_B$ receptor activation could be beneficial in the treatment of drug addiction. Baclofen has indeed shown efficacy in human clinical trials in reducing cocaine, opiate and alcohol craving (Brebner et al., 2002). Baclofen also inhibits the rewarding properties of cocaine (Brebner et al., 2000; Di Ciano and Everitt, 2003; Hotsenpiller and Wolf, 2003), amphetamine (Brebner et al., 2005), nicotine (Corrigall et al., 2000) and opiates (Tsuji et al., 1996; Xi and Stein, 1999) in a variety of preclinical models.

Exposure to cocaine elicits specific biochemical, physiological and behavioural modifications which are hypothesized to be responsible for the development of addictive behaviours, such as craving and relapse (Nestler and Aghajanian, 1997). Acutely, cocaine elicits motor stimulant effects which are thought to be mediated via an increase in dopaminergic transmission in the mesocorticolimbic system. Cocaine inhibits dopamine (DA), norepinephrine and serotonin reuptake and thereby causes an increase in synaptic concentrations of these neurotransmitters. In the nucleus accumbens (NAc) elevated DA levels cause a dysregulation of D1/D2-like DA receptor signalling. This in turn leads to the upregulation of several molecular markers of DA signalling, mainly through activation of the adenylyl cyclase/ protein kinase A (PKA) pathway (for review see (Anderson and Pierce, 2005). Fos is a marker of cell activation and is upregulated in the striatum by acute cocaine (Graybiel et al., 1990).In addition, repeated administration of cocaine has been shown to elicit behavioural sensitization. As mentioned in the introduction, cocaine sensitization is though to underlie some facets of cocaine addiction, including craving and relapse (Kalivas et al., 1993;

Robinson and Berridge, 1993). Furthermore, repeated administration of cocaine has been shown to induce ΔFosB, a truncated form of FosB, which slowly accumulates in NAc and dorsal striatum during chronic drug exposure (Hope et al., 1994). Acute and chronic cocaine administration also increases the expression of Ca^{2+}-and cAMP-response element binding protein (CREB) and dopamine-and-cAMP-regulated-phosphoprotein of 32 kD (DARPP-32) in the NAc(Terwilliger et al., 1991; Kano et al., 1995; Bibb et al., 1999; Nishi et al., 1999a). Therefore, such increases are thought to play a role in addiction, since viral-mediated overexpression of CREB in this region decreases the reinforcing properties of cocaine (Carlezon et al., 1998). Moreover, mice expressing specifically ΔfosB in the nucleus accumbens and dorsal striatum exhibited sensitized behavioural response to cocaine (Kelz and Nestler, 2000).

Nicotine is the primary psychostimulant component of the tobacco smoke and has pharmacological properties leading to a progressive and long-lasting dependence, as shown in clinical and preclinical studies (Stolerman and Jarvis, 1995). Indeed, the positive reinforcing properties of nicotine has been shown in a wide variety of species (Goldberg et al., 1989; Donny et al., 1998; Picciotto, 1998; Stolerman, 1999) in self-administration paradigms. In addition, nicotine was also found to increase the rewarding efficacy of intracranial self-stimulation (Panagis et al., 2000; Harrison et al., 2002). Place conditioning paradigms, conditioned place preference and conditioned place aversion (CPP and CPA, respectively) are based upon the principle that animals, like humans, would learn to seek or avoid environmental stimuli which have been previously associated with rewarding or aversive events, respectively (Carr et al., 1989)(see section 1.3.3.2). Further, most drugs of abuse, such as cocaine, amphetamine, morphine are effective in supporting place preference (Tzschentke, 1998). Furthermore, several studies demonstrated that nicotine induces conditioned place

preference, making it an ideal model to assess nicotine-induced reinforcement (Tzschentke, 1998; Pierce and Kumaresan, 2005).

Baclofen's mechanism of action as an anti-addictive agent likely involves modulation of dopamine (DA) activity in the VTA. $GABA_B$ receptors are highly expressed in the limbic system and can have both pre- and postsynaptic localizations (Bettler et al., 2004). Activation of postsynaptic $GABA_B$ receptors hyperpolarizes the resting membrane potential through activation of potassium channels whereas activation of presynaptic receptors, via Ca^{2+} channel inhibition, reduces the release of neurotransmitters such as DA, glutamate and GABA. In the VTA, $GABA_B$ receptors are expressed on dopaminergic cell bodies and presynaptically on glutamatergic terminals (Johnson and North, 1992; Fadda et al., 2003). Activation of these receptors would hyperpolarize DA neurons and/or lead to a reduction in excitatory inputs, respectively, thereby functionally counteracting the effects of cocaine. Supporting this hypothesis, micro-injection of the $GABA_B$ receptor agonist baclofen into the VTA decreases DA release in the NAc in a model of heroin self-administration (Xi and Stein, 1999). Further, Fadda et al (2003) have also shown that baclofen antagonizes nicotine, cocaine and morphine induced dopamine release in the NAc in rats.

Nevertheless, baclofen induces unwanted side effects such as muscle relaxation, hypothermia and sedation. Conversely, positive $GABA_B$ receptor modulators such as GS39783 are active only in presence of GABA and are devoid of the sedative and muscle relaxant effects of full agonists (Urwyler et al., 2001; Cryan et al., 2004). GS39783 reduces cocaine self-administration in rodent models (Smith et al., 2004) and inhibits the rewarding properties of acute cocaine in an intracranial self-stimulation paradigm (Slattery et al., 2005b). Although mice lacking $GABA_B$ receptor would provide a powerful tool in effort to study the role of $GABA_B$ receptor in addictive disorders, there are several potential caveats associated with their used in animal model of addiction. First, $GABA_B$ receptor mice are generated on

BALB/c background. Indeed, BALB/c mice have been shown to be insensitive to psychostimulant-properties of drugs of abuse such as amphetamine (Logan et al., 1988), cocaine (Seale and Carney, 1991) and to phencylciclidine (Freed et al., 1984).Secondly, the pro-convulsant properties of cocaine (Grabarits et al., 1966) might enhance the frequency of spontaneous seizures observed in $GABA_B$ knockout mice (Schuler et al., 2001; Gassmann et al., 2004), that could interfere with their locomotor activity pattern

The goals of the present chapter were to assess the efficacy of $GABA_B$ receptor activation, via administration of $GABA_B$ receptor agonist or positive modulator, in modulating the reinforcing properties of cocaine and nicotine. First, we investigated the effect of baclofen and GS39783 on both locomotor activity induced by a single administration of cocaine and on its associated striatal Fos upregulation. In addition, we assessed the effects of GS39783 on behavioural sensitization elicited by repeated administration of cocaine. In order to identify the molecular pathways underlying the anti-addictive properties of $GABA_B$ receptor positive modulation, we also examined the effects of GS39783 on specific molecular markers of dopamine signalling, such as CREB, DARPP-32 or ΔFosB. We also investigated the effects of the novel $GABA_B$ positive modulator GS39783 on the reinforcing properties of nicotine, using an unbiased place conditioning paradigm. We first evaluated the intrinsic reinforcing properties of GS389783 in rats. We then assessed the effect of GS39783 on the establishment and the expression of nicotine-induced place preference. Finally, we examined whether that $GABA_B$ positive modulation could alter ΔFosB accumulation induced by repeated administration of nicotine associated with place preference paradigm.

6.2. Experimental Design.

6.2.1 Effects of GABA$_B$ receptor activation on cocaine-related behaviours and their associated molecular markers.

Concerning the effects of baclofen and GS39783 on the hyperlocomotion elicited by a single administration of cocaine, male C57BL/6J mice (18-20g) were individually placed in locomotor test chambers (see chapter 2 for details). After 30 min of habituation GS39783 (10, 30, 100 mg/kg p.o.), baclofen (3, 6 mg/kg p.o.) or methylcellulose was applied and the locomotor activity recorded. 30 min later mice were injected with cocaine (10 mg/kg i.p.) or saline and locomotor activity was recorded for additional 60 min. Doses of GS39783 were selected based on previous studies showing activity in anxiety models at this dose range (Mombereau et al., 2004). The doses of baclofen were selected based on it being maximal doses before behavioural inhibition occurs (Cryan et al., 2004; Jacobson and Cryan, 2005).. The dose of cocaine was selected as it produced a robust hyperactivity in previous studies (Cryan et al., unpublished). In order to assess the effects of a GABA$_B$ receptor agonist and a positive modulator on Fos induction triggered by cocaine, mice were sacrificed 1 hour after cocaine application and the brains were quickly removed, chilled in ice-cold phosphate-buffered saline (PBS) and cut in 1-mm thick slices using a mouse brain matrix (RBM 2000C, Asi Instruments). NAc and dorsal striatum were dissected on an ice-chilled glass plate and flash-frozen in dry ice. Subsequently, c-fos expression was analyzed using the method described in section 2.8.

In studies addressing the effect of GS39783 on behavioural sensitization-induced by cocaine, we used the design described in Chapter 2, using male C57BL/6J mice (18-20g).The mice were habituated to the test environment for three days and basal locomotor activity was measured. After an intraperitoneal injection of saline the mice were placed in the test cages (as above) for 30 min and locomotor activity was recorded. From days 4-10, mice were

injected with cocaine (20 mg/kg i.p.) or saline and locomotor activity was recorded. To assess the effects of GABA$_B$ receptor positive modulation on the acquisition of behavioural sensitization to cocaine, GS39783 (30 mg/kg p.o.) or vehicle (0.5% methylcellulose) was applied 30 minutes before each cocaine injection. This period of acquisition of sensitization was followed by 14 days without drug treatment. In order to investigate the effect of GS39783 on the expression of cocaine sensitization, we designed a challenge trial. On day 23 (challenge day), mice were administrated a dose of 10 mg/kg i.p. cocaine. In order to assess the effect of GABA$_B$ receptor positive modulator on the expression of sensitization, GS39783 (30 mg/kg p.o.) or methylcellulose was applied 30 min prior to the cocaine injection. Separate groups of animals were used for molecular studies. The same regimen of cocaine administration was employed as in behavioural studies. 24 hours after the last injection of cocaine animals were killed and brains were quickly removed, chilled in ice-cold phosphate-buffered saline (PBS) and cut in 1-mm thick slices using a mouse brain matrix (RBM 2000C, Asi Instruments). NAc and dorsal striatum were dissected on an ice-chilled glass plate and flash-frozen in dry ice. ΔFosB , CREB, phospho-CREB and DARPP-32 was analyzed using techniques described in section 2.8.

6.2.2 Effects of GABA$_B$ receptor activation on nicotine-related behaviours and their associated molecular markers.

We chose to investigate the effects of GS39783 on the behavioural effects of nicotine in rats as a robust CPP procedure had been previously validated in our laboratories (Forget et al., 2005). All experiments were carried out on experimentally naïve Male Wistars (Iffa Credo, F-69592 L'Arblesle, Cedex France) weighing 180-220g at the beginning of the experiments. They were housed four per cage (55x33x19 cm) in a humidity- and temperature-controlled room under a 12 h light/dark cycle (lights on at 0700). One week prior

to the beginning of the experiments, all animals were food-restricted (20 g/day) until the end of the study.

In the study addressing the intrinsic reinforcing\aversive properties of $GABA_B$ receptor positive modulator, naive rats were given GS39783 (10, 30 and 100 mg/kg, i.p.), or its vehicle for the control group, 30 minutes prior to the drug-paired conditioning sessions. All of the rats received vehicle before the vehicle-paired sessions. A single test session took place the day following the last conditioning session, i.e. 48 h after the last injection of GS39783. Rats were given no injection before this session.

Concerning the effect of GS39783 on the acquisition of place preference to nicotine, naïve rats were handled and pre-exposed to the conditioning dose of nicotine (0.06 mg/kg, s.c., o.d.) or saline for associated control group, on each of 5 days of the week prior the first conditioning session. For the four drugs-paired sessions, rats received nicotine (0.06 mg/kg s.c.) or saline immediately before the sessions. 30 minutes prior to the drug-paired conditioning session, nicotine-treated rats were given GS39783 (30 and 100 mg/kg; p.o.) or its vehicle. In addition, non-nicotine treated received vehicle 30 minutes before drugs-paired conditioning session. During the four vehicle-paired sessions, all the rats received saline and vehicle (immediately before session and 30 min. prior session respectively). A single test session was conducted the day following the last conditioning session, i.e. 48 h after the last injection of nicotine.

Regarding the effects of GS39783 on the expression of conditioned place preference to nicotine, naïve rats were handled and pre-exposed to the conditioning dose of nicotine (0.06 mg/kg, s.c., o.d.) or saline for associated control group on each of 5 days of the week prior the first conditioning session. During the four drug-paired sessions, rats received nicotine (0.06 mg/kg s.c.) or saline immediately before the sessions, where all the rats received saline before the four vehicle-paired sessions. GS39783 (30 and 100 mg/kg, p.o.), or

its vehicle (methylcellulose) for the associated control group, was administered only once, 45 min before a single test session, which was conducted the day following the last conditioning session, i.e. 48 h after the last.

In order to assess the effects of GS39783 and nicotine regimen on ΔFosB, animals were decapitated immediately after the test phase of place conditioning paradigm. Brains were quickly removed, chilled in ice-cold phosphate-buffered saline (PBS) and cut in 1-mm thick slices using a brain matrix. NAc and Dorsal Striatum were dissected on ice-chilled glass plate and flash-frozen in dry ice. Subsequently, ΔFosB expression was analyzed using the method described in section 2.8.

6.3 Effects of GABA$_B$ receptors activation on specific cocaine-induced behavioural and molecular alterations.

6.3.1 GABA$_B$ receptor activation decreases selective molecular and behavioural effects of acute cocaine administration.

6.3.1.1 GABA$_B$ receptor activation attenuates cocaine-induced hyperlocomotion.

In rodents, a behavioural consequence of acute cocaine administration is increased locomotor activity (see section 1.3.3.2). We used locomotor activity as readout to assess the behavioural effects of baclofen and GS39783 on cocaine exposure. A single injection of cocaine (10 mg/kg i.p.) resulted in a marked increase in ambulatory activity, compared with administration of saline (Fig.6.1). *Post hoc* analysis revealed that baclofen at 6 mg/kg lowered spontaneous locomotor activity at 0 to 10 minutes time point in animals injected with saline ($p < 0.05$; Fig.6.1A). Unexpectedly, animals that had been administered baclofen (3 mg/kg) 30 minutes prior to saline injection had a brief, exaggerated increase in locomotor activity immediately after saline injection which normalized 10 minutes later (Fig.6.1A). In contrast to

the effects of baclofen, GS39783 (at 10, 30, 100 mg/kg) did not affect spontaneous locomotor activity (Fig. 6.1C).

The effects of baclofen and GS39783 on cocaine-induced locomotor activity are shown in Fig. 6.1B and 1D. *Post hoc* analysis revealed that both GS39783 and baclofen blunted the stimulatory effect of cocaine. Although baclofen significantly attenuated cocaine-induced hyperactivity at all doses investigated (Fig.6.1B), interpretation of the effect obtained with the higher dose (6 mg/kg) is confounded by baclofen's sedative properties as evidenced by reduced basal activity at this dose (Fig.6.1A, B). The $GABA_B$ receptor positive modulator GS39783 significantly attenuated hyperlocomotion between 10 and 60 minutes after cocaine administration (Fig.6.1D). However, in contrast to baclofen, GS39783 did not affect basal locomotor activity. Taken together, these data suggested that activation of $GABA_B$ receptors with the agonist baclofen or with the positive modulator GS39783 can attenuate the locomotor-stimulation induced by a single administration of cocaine. Further, we confirmed previous observations showing that GS39783 is devoid of sedative properties of the $GABA_B$ receptor agonist baclofen (Cryan et al., 2004).

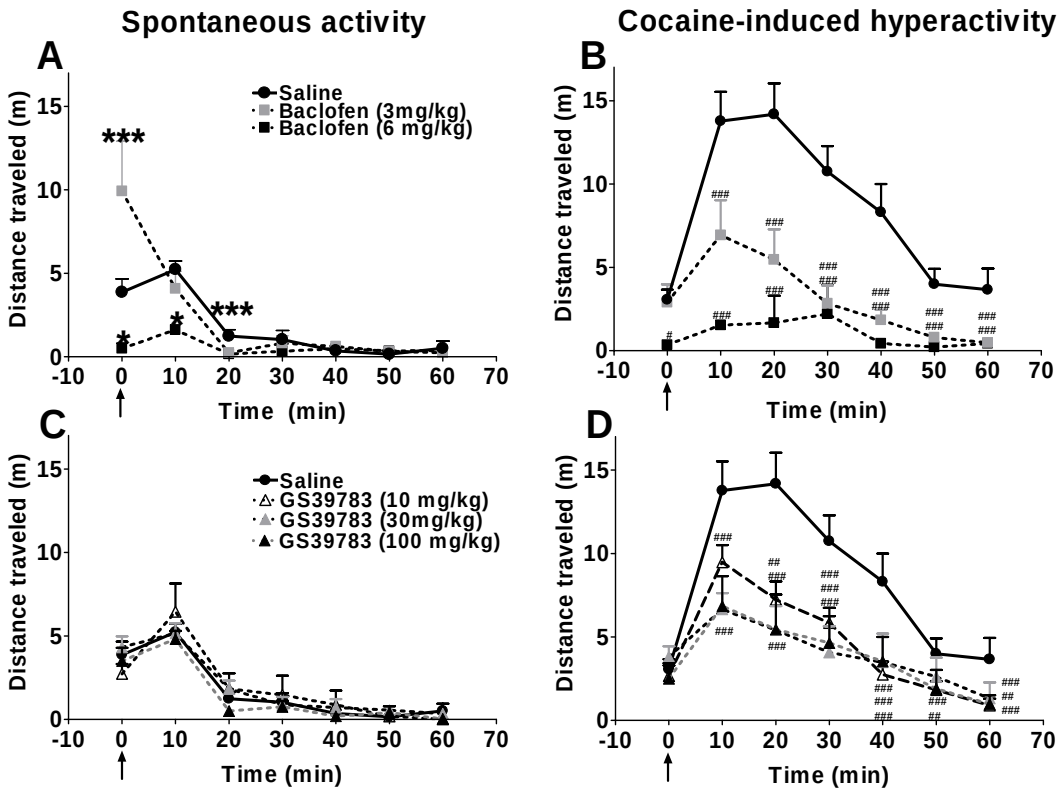

Fig.6.1. GABA$_B$ receptor activation attenuates cocaine-induced hyperlocomotion.

A, C; Effects of baclofen and GS39783 on locomotor activity in mice (n = 12) during a period of 60 minutes after saline injection. Values are means ± S.E.M. *, ***, groups that differed significantly from vehicle-treated animals ($p < 0.05$ and $p < 0.001$, respectively). B, D, effects of baclofen and GS39783 on cocaine induced hyperactivity (10 mg/kg, i.p.) in mice (n = 12) during a period of 60 minutes after cocaine injection. Values are means ± S.E.M. #, ## and ###, groups that differed significantly from vehicle-treated animals ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). The arrows indicate the time point of saline or cocaine injection; baclofen or GS39783 were applied 30 min before saline/ cocaine.

6.3.1.2 Striatal fos upregulation by acute cocaine is inhibited by GABA$_B$ receptor activation.

To date, in the context of drug addiction only few studies have focused on the investigation of the molecular mechanisms affected by potential therapeutic strategies, including those focused on GABA$_B$ receptors. One of the most robust responses to acute cocaine is the activation of immediate early gene expression, most notably Fos, the product of the immediate early gene cfos in the NAc and dorsal striatum (Graybiel et al., 1990; Curran et al., 1996). To investigate a possible effect of GABA$_B$ receptor activation on cocaine-induced

Fos upregulation we conducted an experiment with a separate group of animals. The mice were treated with baclofen, GS39783 or saline 30 min prior to cocaine or saline injection. Fos expression was detected by immunoblots on dorsal striatum and NAc samples and normalized to actin controls (Fig.6.2). Treatment with cocaine triggered a robust upregulation of Fos expression in both dorsal striatum and NAc ($p < 0.001$ versus saline; Fig.6.2C-F). Baclofen dose-dependently attenuated cocaine-induced Fos expression in both structures ($p < 0.001$, 6 mg/kg baclofen) but did not affect basal Fos levels ($p > 0.1$; Fig.6.2C, D). Similarly to baclofen, GS39783 dose-dependently inhibited Fos induction in both dorsal striatum and NAc ($p < 0.001$; 30, 100 mg/ kg GS39783; Fig.6.2E, F) without affecting basal Fos expression at any dose used ($p > 0.1$). Taken together these data show that $GABA_B$ receptor activation by baclofen and GS39783 attenuated acute cocaine-induced Fos expression.

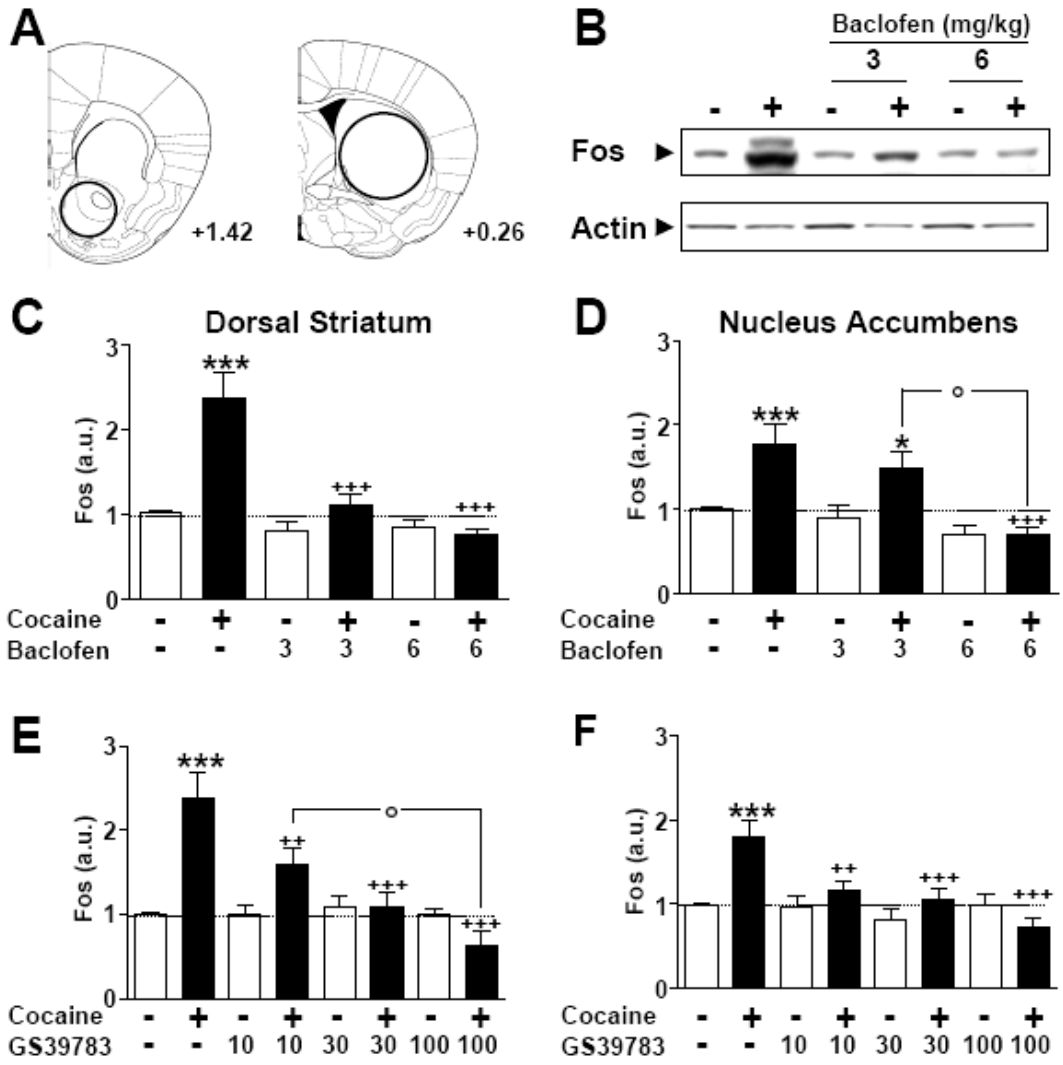

Fig.6.2. GABA$_B$ receptor activation inhibits cocaine-induced Fos accumulation: Baclofen and GS39783 were applied 30 min prior to cocaine and the mice were sacrificed 2 hrs after cocaine injection (20mg/ kg i.p; n = 5 for each group). A, circles in schematic drawing after Paxinos and Franklin (2001) indicate dissected brain regions (NAc, left panel; Dorsal Striatum, right panel; bregma coordinates are given). Fos was detected by immunoblot in Dorsal Striatum (B, C, E) and NAc (D, E) samples. B, representative Fos immunoblot obtained from Dorsal Striatum samples, with its corresponding actin control. Mice were either injected with saline or cocaine in absence or presence of Baclofen (3 or 6 mg/kg); S, saline; C, cocaine. C, D, Effect of baclofen on Fos-upregulation by cocaine. Averaged densitometric values obtained from Dorsal striatum (C) and NAc (D) samples are shown. E, F, GS39783 attenuates cocaine-induced Fos upregulation in Dorsal striatum (E) and NAc (F) samples. *, + indicate differences to saline controls or cocaine groups, respectively; o indicates differences within treatment groups. *, +, o, $p < 0.05$; **, ++, $p < 0.01$; ***, +++, $p < 0.001$; a.u., arbitrary units.

6.3.2 GABA$_B$ receptor activation alters behavioural sensitization to cocaine and associated specific molecular markers.

6.3.2.1 GABA$_B$ receptor positive modulation alters the acquisition of cocaine sensitization without affecting its expression.

Chronic cocaine induces locomotor sensitization, which results in an enduring enhancement of behavioural responses during repeated drug administration. In behavioural sensitization studies at least two different phases are recognized, acquisition and expression (for a review, (Kalivas et al., 1993)). Briefly, acquisition is the phase in which behavioural and physiological changes develop due to repeated, intermittent exposure to psychostimulants. The expression phase is the resulting long-term behavioural changes that are the result of underlying neuroadaptations.

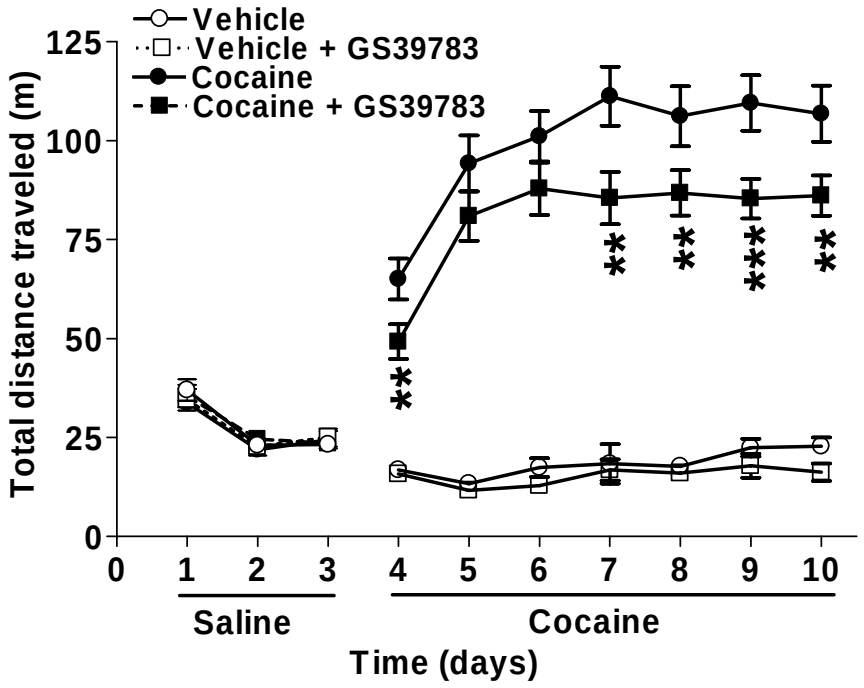

Fig.6.3. The GABA$_B$ receptor positive modulator GS39783 attenuates the acquisition of cocaine sensitization.
Mice (n = 12) were habituated to the locomotor activity chambers during three daily sessions (days 1-3) of 30 minutes after receiving intraperitoneal saline injection. GS39783 (30 mg/kg p.o.) or vehicle were administrated 30 minutes before cocaine injection (20 mg/kg i.p.) on 7 consecutives days (days 4-10). Locomotor activity was recorded immediately after cocaine injection for 30 minutes. Values are means (n = 10 per group) ± S.E.M. of the total distance traveled during the total 30 minutes of daily session. Two-way repeated measures ANOVA revealed a significant effects of GS39783 ($F_{1,68}$ = 8.632; p = 0.005), of cocaine ($F_{1,68}$ = 378.082; p < 0.001) and a significant interaction GS39783 x cocaine ($F_{1,68}$ = 4.446; p = 0.039). Statistical analysis demonstrated an effect of time ($F_{6,408}$ = 23.966; p < 0.001) and interaction cocaine x time ($F_{6,408}$ = 19.626; p < 0.001). Groups that differed significantly from cocaine treated animals are indicated (**, p < 0.01; ***, p < 0.001).

In order to assess the effect of GS39783 on the acquisition of behavioural sensitization to cocaine, we measured locomotor activity of mice immediately after daily cocaine injection for 30 min during the acquisition phase (Fig.6.3). Statistical analysis (see legend) suggested that behavioural sensitization to cocaine occurred. Furthermore, as in the experiments shown in Fig. 1D, GS39783 attenuated the hyperlocomotion induced by a single administration of cocaine (Fig.6.3, day 4; p < 0.01). In addition, *post hoc* analysis revealed that mice treated daily with cocaine and GS39783 exhibited less hyperactivity than mice treated with cocaine alone (days 7 to 10; Fig.6.3). These data suggested that GS39783 attenuated the acquisition of

cocaine sensitization. Repeated treatments did not affect locomotor activity compared with the vehicle treated group, confirming the absence of sedative properties of GS39783.

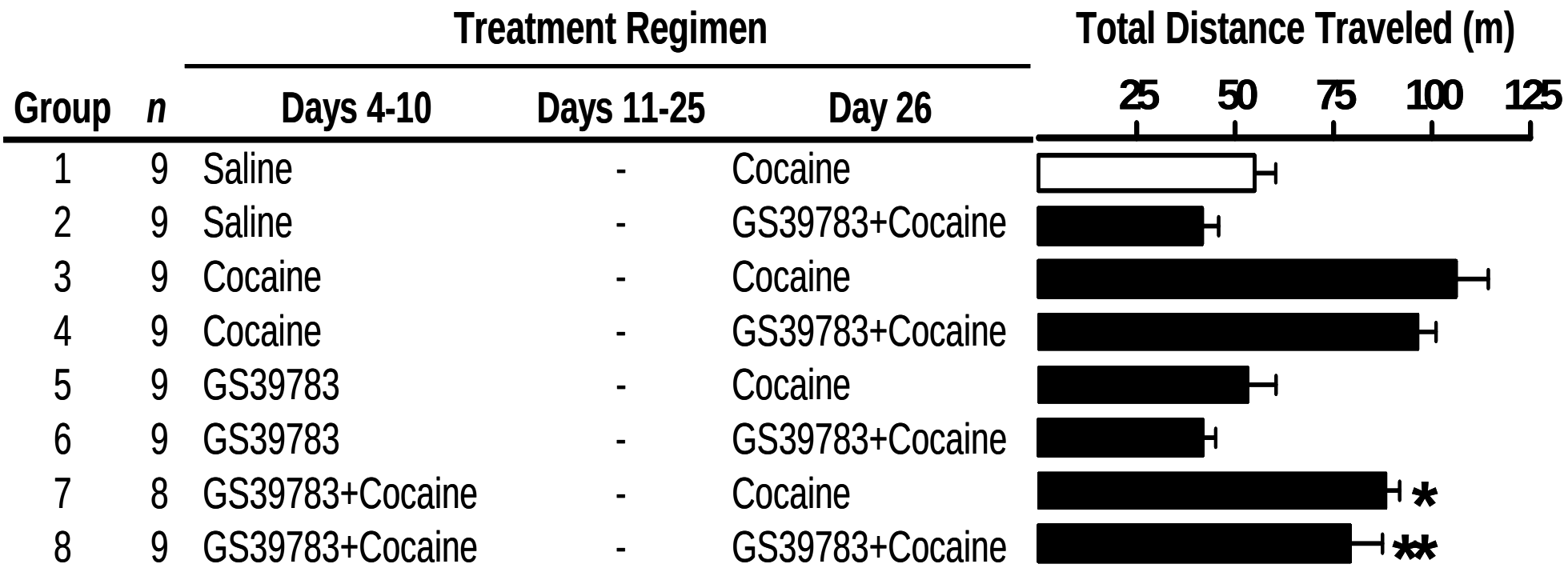

Fig.6.4. GS39783 does not attenuate the expression of cocaine sensitization.
The effects of different GS39783 regimen (30 mg/kg p.o.) on hyperactivity induced by challenging dose of cocaine (10 mg/kg i.p., day 26) injected after 14 days of drug-free period are shown. Treatment during the acquisition phase (days 4-10) was as decribed in Fig. 3. Three-way ANOVA revealed significant effects of repeated cocaine treatment ($F_{1,63}$ = 113.408; p < 0.001). There was an effect of GS39783 administration both during the acquisition period ($F_{1,63}$ = 4.708; p = 0.034) and prior to the challenge ($F_{1,63}$ = 6.795; p = 0.005), and an interaction between repeated cocaine treatment and GS39783 administrated during the acquisition phase ($F_{1,63}$ = 4.022; p = 0.049).Bar graphs show means ± S.E.M. of the total distance traveled during the 30 minutes of challenge session. ** and ***, groups that differed significantly from cocaine sensitized animals (p < 0.05 and p < 0.01, respectively).

To investigate the effect of GS39783 on the expression of cocaine sensitization, we designed a challenge trial, 14 days after the last cocaine injection (Kalivas and Stewart, 1991). During this challenge, all mice received 10 mg/kg of cocaine. As expected, mice treated repeatedly with cocaine exhibited an enhancement of total distance traveled compared to mice treated only with the challenging dose of cocaine (Fig.6.4, groups 3 versus 1; $p < 0.001$). These data confirmed the presence of behavioural cocaine sensitization. Cocaine sensitized mice pretreated with GS39783 before the cocaine challenge did not differ significantly in their locomotor response compared with mice receiving only the challenging dose of cocaine (Fig. 6.4, groups 4 versus 3) indicating that GS39783 does not affect the expression of sensitization. Mice treated concomitantly with GS39783 and cocaine during the acquisition phase of exhibited significantly less locomotor activity compared to mice treated only with cocaine (Fig.6.4, groups 7 versus 3; $p < 0.05$), confirming the data shown in Fig.6.3. Dual administration of GS39783 during the acquisition and prior to the challenge reduced locomotor activity (Fig.6.4, groups 8 versus 3). Altogether, these data suggest that GS39783 attenuated the acquisition of behavioural sensitization to cocaine without affecting its expression.

6.3.2.2 GS39783 blunts chronic cocaine-associated ΔFosB upregulation in dorsal striatum.

In the mesolimbic circuit, chronic cocaine administration triggers the accumulation of ΔFosB, which is thought to play a pivotal role in long-lasting effects of a variety of drugs of abuse including cocaine. Over-expression of ΔFosB increases the sensitivity to cocaine and the motivational aspects of reward (Kelz et al., 1999; Colby et al., 2003). We studied ΔFosB expression after 7-days of daily treatment with saline/ cocaine or GS39783/ cocaine (Fig.6.5). The animals were sacrificed 24 hours after the last administration. A separate group of animals from those used in behavioural studies were used. ΔFosB expression was measured using semi-quantitative Western blot analysis employing selective antibodies (Zhang et al.,

2002; Muller and Unterwald, 2005). Cocaine stimulated a robust increase in ΔFosB expression in dorsal striatum (Fig. 6.5B; $p < 0.001$ versus saline) that was partially blocked by GS39783 ($p < 0.05$). In NAc, ΔFosB levels were also upregulated by chronic cocaine (Fig.6. 5A; $p < 0.001$) however, in this brain region GS39783 failed to modulate ΔFosB induction by cocaine ($p = 0.92$). Basal levels of ΔFosB expression were not affected in either structure ($p > 0.1$). During protracted withdrawal ΔFosB levels decrease to basal levels, i.e. 10-12 days after cessation of chronic cocaine treatment (Hope et al., 1994). In line with these observations we did not detect ΔFosB 14 days after cessation of cocaine repeated administration (Fig.6.5C). Chronic treatment with GS39783 before withdrawal had no effect on ΔFosB levels. In summary, our data therefore suggest that the mode of action of GS39783 has a modest impact on cocaine-modulated ΔFosB expression in the dorsal striatum but not in NAc.

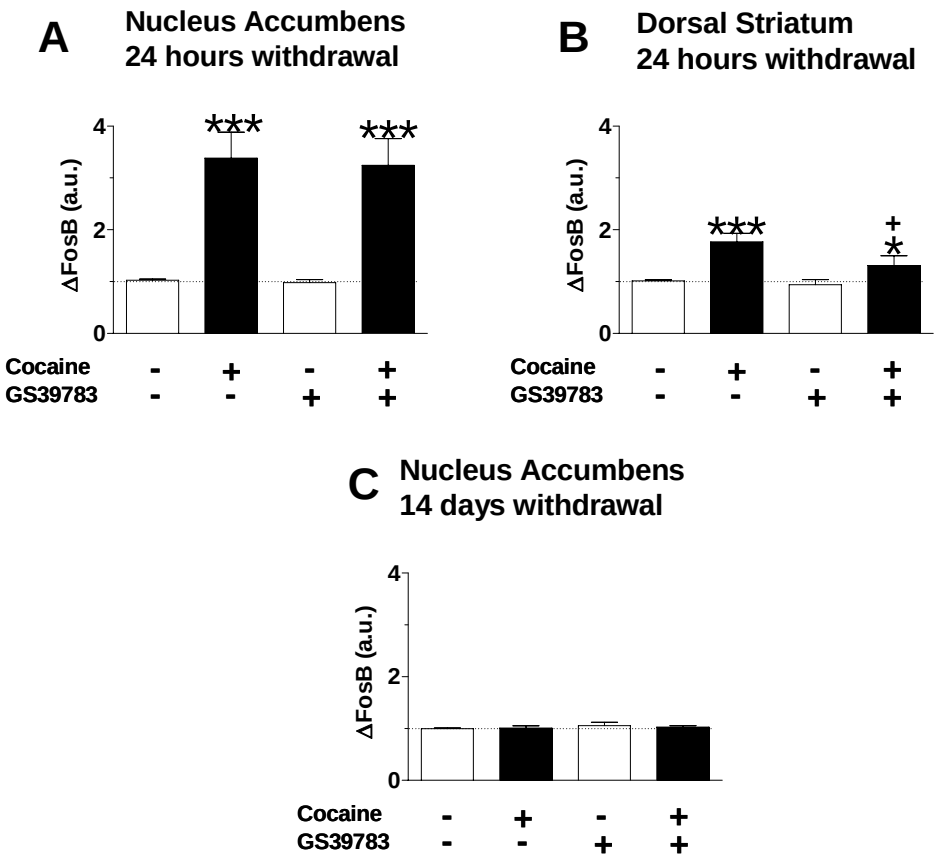

Fig.6.5. GS39783 has a weak inhibitory effect on ΔFosB induction by chronic cocaine.

Mice (n = 5-10 animals/experimental group) were treated daily with cocaine, (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (-) and respective combinations as indicated on 7 consecutive days. 24 hours after the last treatment the mice were sacrificed and NAc and dorsal striatum dissected and processed for immunoblot analysis. Averaged densitometry values (bottom panels) are shown. A, B, Cocaine induces ΔFosB upregulation in NAc and dorsal striatum. GS39783 partially inhibits ΔFosB induction in dorsal striatum (B) but not in NAc (A). C, Mice (9 animals/group) were administered saline/cocaine for 7 days, after which cocaine exposure was stopped for 14 days. Then the mice received a cocaine challenge (20 mg/kg i.p.) 24 hours after which NAc samples were processed for immunoblotting. ΔFosB protein levels are high immediately after repeated cocaine treatment (24 hours of withdrawal) whereas expression levels decline to basal levels after 14 days of cessation of chronic cocaine. * and + represent differences to saline or cocaine groups, respectively; *, +, $p < 0.05$; ***, +++, $p < 0.001$.

6.3.2.3 GS39783 blocks chronic cocaine-induced upregulation and activation of DARPP32 and CREB.

Previous studies have shown that chronic cocaine induces a strong activation of DARPP-32 and CREB through increased DA signaling (Kano et al., 1995). We therefore examined whether such changes on DARPP-32 and CREB are modulated by GS39783 (Fig.6.6, Fig.6.7). An experimental setup identical to the ΔFosB studies as described above was used. In the dorsal striatum a trend towards a decrease of DARPP-32 expression by chronic cocaine as well as GS39783 treatments was observed ($F_{3,50}$ = 2.44; p = 0.07; Fig.6.6). In NAc however, chronic cocaine stimulated DARPP-32 expression. In agreement with previous studies (Lin et al., 2002; Hu et al., 2005) chronic cocaine administration increased DARPP-32 expression in NAc (p < 0.001), but not in dorsal striatum. DARPP-32 upregulation was not observed when GS39783 was applied 30 minutes prior to each daily cocaine administration (p < 0.001). GS39783 did not affect basal DARPP-32 levels (p > 0.1; Fig.6.6B).

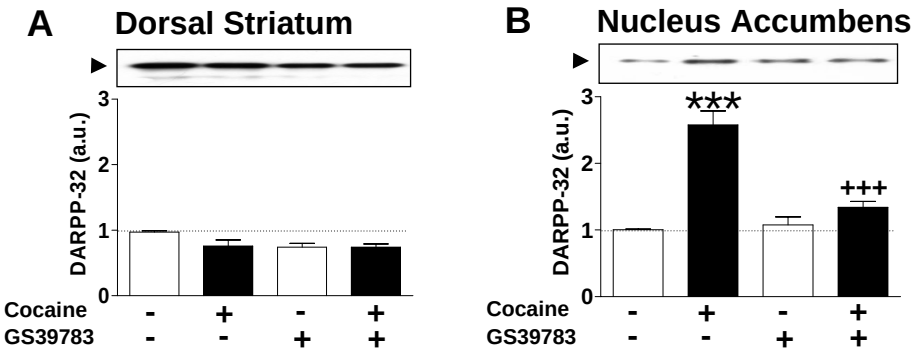

Fig.6.6. GS39783 inhibits DARPP-32 upregulation by chronic cocaine.
Dorsal striatum and NAc samples were prepared 24 hours after cessation of repeated cocaine treatment, as described in Fig. 4. Treatments with cocaine (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (-) and respective combinations are indicated. Representative immunoblots (top panels) and averaged densitometry values (bottom panels, n = 5-10) are shown. A, GS39783 does not affect DARPP-32 expression in dorsal striatum. B, DARPP-32 upregulation in NAc by repeated cocaine is inhibited by GS39783. * and + indicate differences to saline or cocaine groups, respectively. ***, +++, p < 0.001.

CREB is activated by phosphorylation and this active form is shuttled to the nucleus where it drives expression of its target genes (Shaywitz and Greenberg, 1999). In addition to total CREB we therefore investigated the levels of phosphorylated CREB (pCREB), and determined the pCREB/CREB ratios. In dorsal striatum, cocaine and GS39783 had a minor effect and the ratio of pCREB/CREB remained unmodified (Fig.6.7A; $F_{3,81} = 1.75$; $p = 0.16$. In the NAc chronic cocaine increased CREB activation as evidenced from the pCREB/CREB ratio, confirming previous studies (Fig.6.7B; $p < 0.01$) (Terwilliger et al., 1991; Kano et al., 1995). Calculation of the pCREB/CREB ratios demonstrated that GS39783 effectively inhibited chronic cocaine-induced CREB stimulation.

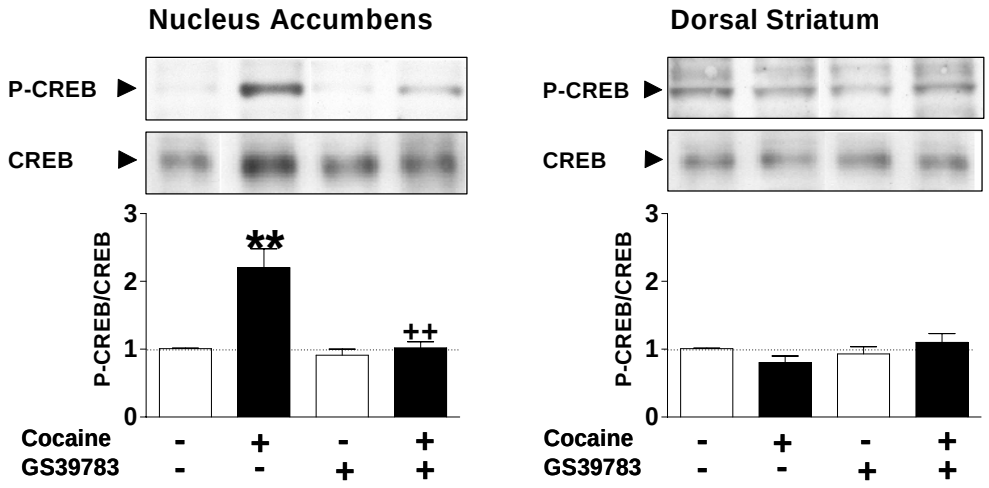

Fig.6.7. GS39783 inhibits CREB activation by chronic cocaine.
Mice were treated for 7 days with daily combinations of cocaine (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (-) and respective combinations as indicated. dorsal striatum and NAc samples were dissected 24 hours after the end of treatment. P-CREB/CREB ratios were calculated from immunoblots (n = 5-10); representative blots are shown (top panels). GS39783 does not modify CREB expression in dorsal striatum (B) but inhibits cocaine-induced CREB activation in NAc (A).* and + mark differences to saline or cocaine groups, respectively; **, ++, $p < 0.01$.

6.4 Effects of GABA$_B$ receptors positive modulation in a model of nicotine reinforcement.

6.4.1 GABA$_B$ receptor activation blocks the establishment of nicotine-induced place preference but not its expression.

6.4.1.1 Intrinsic reinforcing properties of GS39783

On average, control animals (given saline before both drugs-paired sessions and vehicle-paired sessions) did not significantly exhibit preference for either floor texture, demonstrating that there was no unconditioned environmental preference. One-way ANOVA revealed a significant effect of GS39783 on the time spent in paired quadrant [F(3,47) = 4.771, P = 0.006]. Subsequently, post-hoc analysis indicates a significant reduction of the time spent in modulator-paired quadrants in animals treated with 100 mg/kg of GS39783, when compared to control group (p<0.001).In contrast, at 10 mg/kg and 30 mg/kg, GS39783 did not affect the time spent in drug-paired quadrants (Fig.6.8).Together, these results suggest that although GS39783 supports conditioned place aversion at 100mg/kg , both lower doses tested are hedonically neutral.

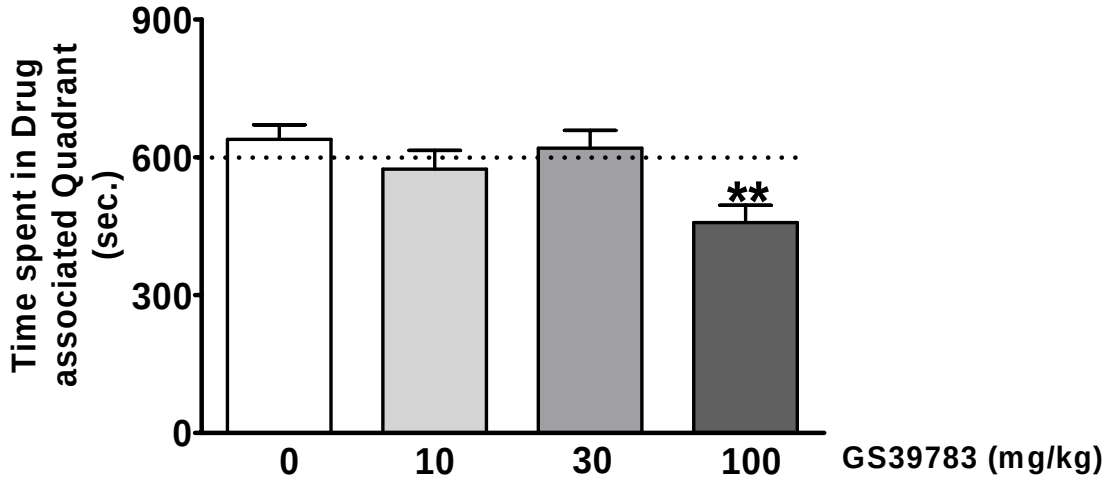

Fig.6.8. Intrinsic reinforcing properties of GS39783 in the place conditioning paradigm.
At 100 mg/kg, GS39783 elicits a significant place aversion. Each bar represents the mean (n = 12 per group) ± S.E.M. of the time spent in drugs associated quadrants. *, **, groups that differed significantly from vehicle treated animals (p < 0.05 and p < 0.01, respectively).

6.4.1.2 Effects of GS39783 on the establishment of nicotine-induced place preference.

In order to test the effect of $GABA_B$ receptor positive modulation on the establishment of nicotine place conditioning, GS39783 was administered 30 min prior to the nicotine injection during the acquisition phase. Regarding the effect of GS39783 on the establishment of nicotine-induced place preference, one-way ANOVA revealed an overall effect of treatment [$F(3,40) = 3.524$, $P = 0.023$] and subsequent post-hoc test revealed a significant effect of nicotine on time spent in drug-paired quadrants (Fig.6.9). Indeed, rats given nicotine at the 0.06 mg/kg, during the conditioning phase spent significantly more time on the paired nicotine-paired quadrants, compared to control animals ($p = 0.044$). This result suggested that 0.06 mg/kg of nicotine supports conditioned place preference as previously reported (Forget et al., 2005). Post-hoc analysis revealed that GS39783 at 30 mg/kg and 100 mg/kg , when it was given 30 min prior each drug-paired session, decreased significantly the amount of time the animals spent in nicotine-paired quadrants compared to animals treated only with nicotine (30 mg/kg, $p = 0.005$ and 100 mg/kg, $p = 0.013$). Consequently, these data show that $GABA_B$ receptor positive modulation during the conditioning phase antagonizes the establishment of nicotine-induced place preference

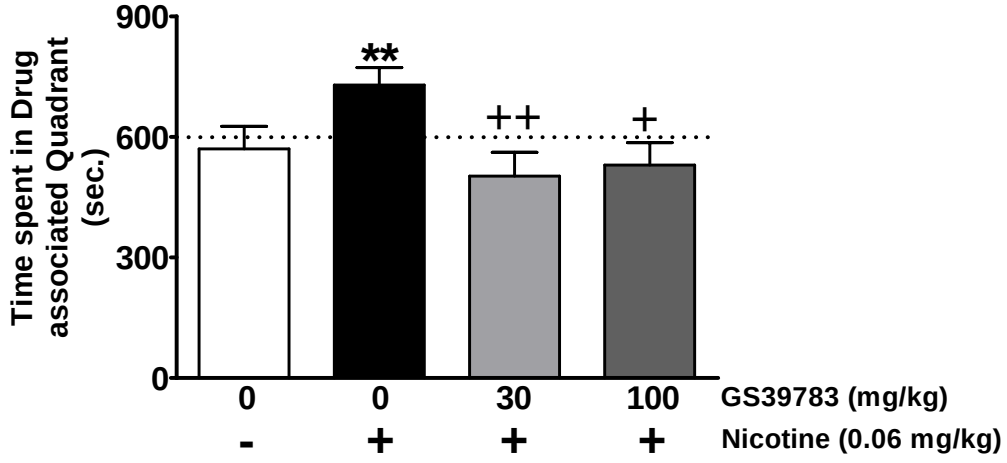

Fig.6.9. Effects of GS39783 on the acquisition of the nicotine-induced place preference.

Nicotine, at 0.06 mg/kg s.c, elicits an significant place preference (black bar) that could be blocked by administration of GS39783, at both 30 and 100 mg/kg, during the conditioning phase(Grey Bars). Each bar represents the mean (n = 11 per group) ± S.E.M. of the time spent in drugs associated quadrants. * and + represent differences to saline or nicotine groups, respectively; **, $p < 0.01$; +, $p < 0.05$ and ++, $p < 0.01$.

6.4.1.3 Effects of GS39783 on the expression of nicotine-induced place preference.

The effects of GS39783 on the expression of nicotine-induced place preference are shown in Fig.6.10. The one-way ANOVA revealed a overall effect of treatments on the time spent in drugs associated quadrants [F(3,40) =3.352, P = 0.028]. As shown in Fig.6.10., nicotine supported conditioned place preference (p = 0.03 , compared to control group). Post-hoc analysis however, revealed that GS39783 at 30 mg/kg and 100mg/kg , when given 45 min prior to the testing session, failed to decrease significantly the amount of time spent in nicotine-paired quadrant compared to animals treated only with nicotine (p=0.558 and p=0.167, respectively). However, the time spent on the nicotine-paired texture by rats given GS39783 (100mg/kg) did not differ from the control level (p =0.408). Consequently, although GS39783 seems to affect the acquisition of nicotine-induced place preference, it appears to be without effect on its expression.

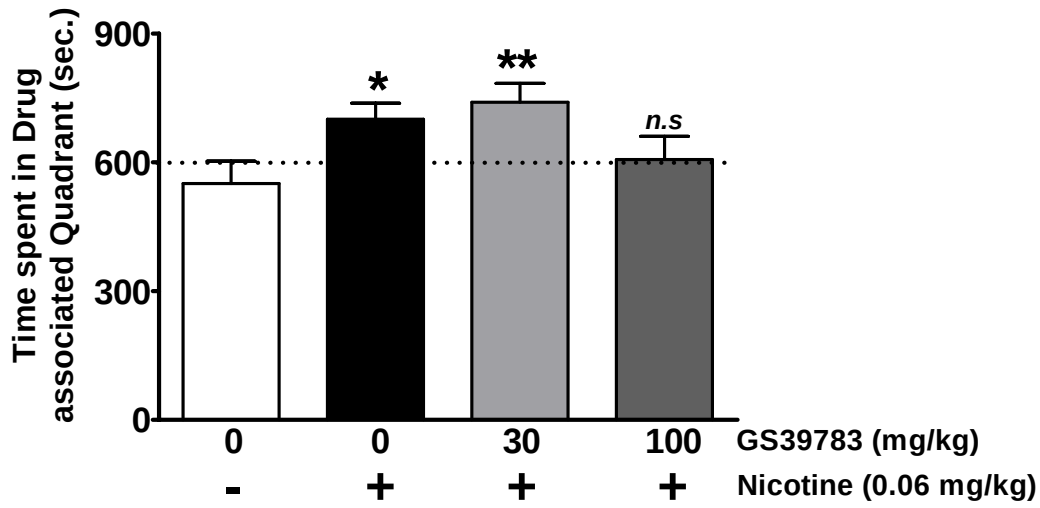

Fig.6.10. Effects of GS39783 on the expression of nicotine-induced place preference.

Nicotine, at 0.06 mg/kg s.c, induces an significant place preference (black bar) but a single administration of GS39783 (Grey bars) prior test phase failed to block the nicotine-induced conditioned place preference. Each bar represents the mean (n = 11 per group) ± S.E.M. of the time spent in drugs associated quadrants. *, **, groups that differed significantly from vehicle treated animals ($p < 0.05$ and $p < 0.01$, respectively).

6.4.2 Effects of GABA$_B$ positive modulator on selective molecular marker associated with nicotine administration.

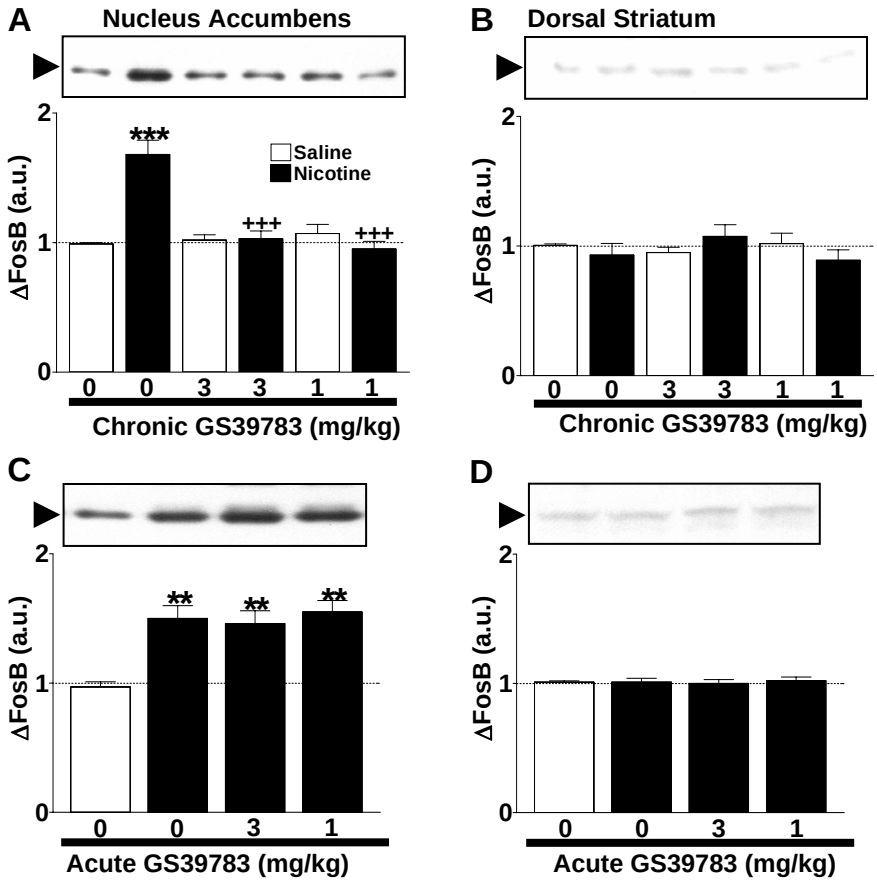

Fig.6.11. GS39783 inhibits ΔFosB induction in the NAc by chronic nicotine but not acute.

Effects of chronic GS39783 administration on ΔFosB accumulation (A, B). Rats (n = 5-8 animals/experimental group) were treated daily with saline (white bars) or nicotine, (0.06mg/ kg i.p; black bars) during the 5 days of pre-exposure and the acquisition phase of place preference. During conditioning, GS39783 was injected (at indicated concentrations, p.o.) 30 minutes prior to nicotine/saline administration. Animals were sacrificed 48 hours after final test (no nicotine, no GS39783). NAc and Dorsal striatum dissected and processed for immunoblot analysis. Representative immunoblots (top panels) and averaged densitometry values (bottom panels) are shown. Nicotine induces ΔFosB upregulation in NAc (A) but not in Dorsal Striatum (B). Further, GS39783 completely inhibits ΔFosB induction in NAc at both doses used (A). C and D, lack of effect of a single administration of GS39783 on ΔFosB accumulation. Rats (n=10 animals/group) underwent nicotine pre-conditioning and conditioning without exposure to GS39783. The modulator was administered 30 min prior to final test at the doses indicated. ΔFosB protein levels are upregulated by nicotine pre-treatment in NAc (C) and not in dorsal striatum (D). Moreover acute GS39783 treatment fails to inhibit this upregulation in NAc. * and + represent differences to saline or nicotine groups, respectively; **, $p < 0.05$; ***, +++ $p < 0.001$.

Given that ΔFosB was the most robust change in cocaine experiment, we studied the influence of both repeated and single administration of GS39783 on ΔFosB expression in NAc and dorsal striatum by semi-quantitative Western blot analysis immediately after

completion of the final test. Chronic nicotine stimulated a robust increase in ΔFosB expression in NAc (Fig.6.11A; $p < 0.001$ versus saline). This upregulation was completely blocked by GS39783 ($p < 0.001$ versus nicotine) at both doses used, when daily injected during the acquisition phase. It should also be noted that both doses of GS39783 did not have any intrinsic effect on basal ΔFosB levels. In NAc, a single administration of GS39783 before the final CPP testing failed to inhibit ΔFosB induction at both doses (Fig.6.11C). One potential concern of the experimental procedure we used is that the ΔFosB signal observed might be a direct consequence of the last CPP test, as opposed to a slow build-up of the protein during chronic nicotine exposure. This hypothesis can however be ruled out since 1) only chronic treatment with GS39783 resulted in inhibition of ΔFosB induction and 2) in experiment 2, the last GS39783 administration was performed 48 hours before CPP testing. In dorsal striatum, ΔFosB levels were not altered by chronic nicotine, chronic or acute GS39783 (Fig.6.11B, D; $p > 0.05$ versus saline). Finally, fig.6.12 shows a plot of the data obtained from individually paired behavioural and biochemical data. A significant positive correlation between CPP scores and levels of ΔFosB expression can be observed ($p < 0.0001$, $R^2=0.38$).

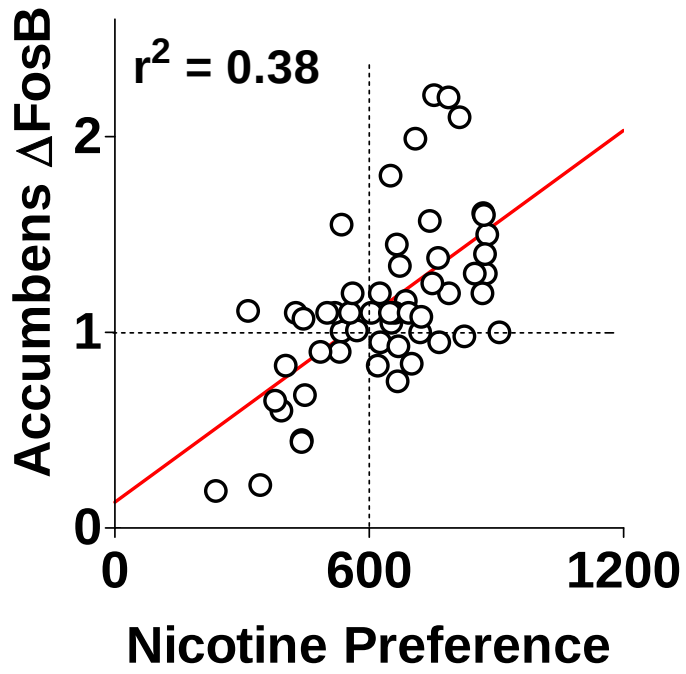

Fig.6.12. ΔFosB induction is correlated to nicotine reinforcement.
All data available for animals in both behavioural and biochemical analyses are included. Accumbal ΔFosB relative abundance is plotted against the subject's CPP score. All experimental groups were mixed and no animals for which both variables were available were discarded. The red line represents the best fit with a least-square methods. Dashed lines indicate chance level (x-axis) and ΔFosB basal level (y-axis).

6.4 Discussion.

Although molecular adaptations to repeated administration of drugs of abuse have been hypothesized to play a major role in the manifestation of dependence (Nestler and Aghajanian, 1997) very few studies to date have investigated the ability of potential therapeutic agents to modulate such responses. In the present chapter, we demonstrated that GABA$_B$ receptor positive modulator attenuates both behavioural effects, such as psychostimulant effects, sensitization or place preference, elicited by drugs of abuse and is also effective in preventing the induction of several molecular markers triggered by single or repeated administration of these substances.

Effects of GABA$_B$ receptor activation on cocaine-related behaviour and molecular adaptations. Both the GABA$_B$ receptor agonist baclofen and the positive modulator GS39783 attenuated the hyperlocomotion induced by a single administration of cocaine. Further, we have shown that GS39783 affects the acquisition but not the expression of cocaine sensitization. These data are in line with studies demonstrating that intra-prefrontal injection of the GABA$_B$ agonist baclofen alters the induction of cocaine sensitization without affecting its expression (Steketee and Beyer, 2005). On the other hand, intra-VTA baclofen administration attenuated both the induction and the expression of behavioural sensitization to opiates (Leite-Morris et al., 2004) and a single systemic administration of baclofen attenuated the expression of sensitization to locomotor stimulant effect of amphetamine (Bartoletti et al., 2005).

GS39783 induced similar effects as a non-sedative dose of baclofen (3mg/kg) in attenuating acute cocaine-induced hyperlocomotion (Fig.6.1), which is relevant as clinical studies suggest efficacy of baclofen in cocaine dependence (Shoptaw et al., 2003). The observations that GS39783, in contrast to baclofen, does not alter baseline locomotor activity is in agreement with the lack of effects in other behavioural tests in both rats and mice which are sensitive to baclofen administration; these include the rotarod motor co-ordination task, cognitive tasks and hypothermia measurements (Cryan et al., 2004; Jacobson and Cryan, 2005). Of note, the effects of GABA$_B$ receptor ligands on cocaine-induced hyperactivity appear not as robust as that reported with D1 selective DA receptor antagonists such as SCH23390, which almost completely block the locomotor stimulant effects of cocaine (O'Neill and Shaw, 1999; Adams et al., 2001). However, a thorough evaluation of GABA$_B$ receptor positive modulators in comparison to other drugs attenuating locomotor stimulant effects of cocaine requires side-by-side experiments and a careful investigation of their side effect profile which may influence locomotor-based behavioural readouts.

Locomotor activity is critically dependent on activation of dopaminergic neurotransmission (Kelly et al., 1975). A pivotal role of VTA DA neurons in mediating the hyperlocomotor effects of DA has been described (Kalivas and Stewart, 1991). VTA DA neurons project to numerous limbic loci including the NAc and the prefrontal cortex. Activation of $GABA_B$ receptors on the cell bodies of VTA DA neurons reduce their excitability which in turn leads to a reduction in DA release in the NAc (Olpe et al., 1977; Lacey et al., 1988; Westerink et al., 1996; Wirtshafter and Sheppard, 2001). In addition, the activity of VTA DA neurons is modulated by excitatory (glutamatergic) and inhibitory (GABAergic) afferents. Activation of $GABA_B$ receptors on glutamatergic afferents would reduce excitatory inputs into the VTA (Johnson and North, 1992; Wu and Saggau, 1997; Hotsenpiller and Wolf, 2003). In support of a key role of $GABA_B$ receptors in the VTA in blocking cocaine-induced hyperlocomotion, baclofen pretreatment dose dependently reduced nicotine-, morphine, and cocaine-evoked DA release in the NAc (Fadda et al., 2003). Furthermore, baclofen injection into the VTA blocked an increase in firing of NAc neurons induced by reward-predictive cues (Yun et al., 2004). Further, Brebner et al. (2005) demonstrated that pretreatment with baclofen dose-dependently reduces d-amphetamine self-administration and blunts associated elevation of DA release in NAc(Brebner et al., 2005). Taken together these data provide evidence that $GABA_B$ receptor activation reduces VTA DA neuron excitability and thereby functionally antagonizes effects of cocaine. However, contributions of other components of the mesocorticolimbic circuitry such as the ventral pallidum or the prefrontal cortex are also possible (Gong et al., 1998; Kalivas and Volkow, 2005). Interestingly, Liu et al. (2005) recently reported that cocaine exposure in vivo facilitates LTP formation in midbrain DA neurons and that drug induced synaptic plasticity could be prevented by enhanced GABAergic inhibition (Liu et al., 2005). The consequence of $GABA_B$ receptor activation on the formation of drug-associated memories however, has not yet been investigated.

$GABA_B$ receptor positive modulators such as GS39783 are devoid of intrinsic agonistic activity (Urwyler et al., 2003; Cryan et al., 2004). Their action therefore is dependent on the presence of GABA (i.e. synaptically released GABA). Several lines of evidence suggest that VTA DA neurons are under tonic inhibitory control by $GABA_B$ receptors. Intra-VTA application of the selective $GABA_B$ receptor antagonist CGP55845A increased extracellular DA levels (Giorgetti et al., 2002). Furthermore, systemic application of the $GABA_B$ receptor antagonist CGP35348 increased firing of VTA DA neurons (Erhardt et al., 2002) whereas the $GABA_B$ receptor positive modulator CGP7930 decreased the firing frequency of VTA DA neurons in midbrain slice preparations (Chen et al., 2005). Taken together with the data for baclofen as described above these studies suggest that $GABA_B$ receptor positive modulators such as GS39783 may attenuate effects of cocaine by decreasing VTA DA neuron excitability.

We have demonstrated that the modulation of several molecular markers by chronic cocaine exposure is attenuated by GS39783. In the NAc GS39783 blocked the induction of both CREB (as evidenced by the ratio of pCREB/CREB) and DARPP-32, without affecting basal levels (Fig.6.6, Fig.6.7). Cocaine treatment increases synaptic DA concentrations which in turn causes a dys-regulation of DA receptor signaling. In the NAc this leads to an upregulation of the adenylyl cyclase signalling pathway, via increased D1-like DA receptor activity (Anderson and Pierce, 2005). Increased cAMP pathway activation in turn augments the expression and activation by phosphorylation of CREB and DARPP-32 (Terwilliger et al., 1991; Bibb et al., 1999). Therefore, most likely GS39783 attenuated CREB and DARPP-32 induction through $GABA_B$ receptor mediated reduction of DA neuron excitability, thus preventing selective cocaine-induced changes in DA receptor signaling. Furthermore, $GABA_B$ receptors negatively couple to adenylyl cyclase (Bettler et al., 2004). GS39783 mediated inhibition of cAMP formation would be expected to decrease the effect of DA receptor

signalling induced upregulation of the adenylyl cyclase pathway, in addition to reducing DA neuron excitability.

Fos expression can be stimulated by a variety of regulators including CREB. After acute treatment Fos is induced in NAc and striatum by several drugs of abuse, including cocaine (Graybiel et al., 1990; Young et al., 1991; Nye and Nestler, 1996; Zhang et al., 2002; Zhang et al., 2004). Leite-Morris et al. (2002) provided evidence that baclofen treatment in the VTA blocks Fos immunoreactivity in the NAc, by inhibiting the activation of dopaminergic neurons. We have shown that systemic applications of either baclofen or GS39783 effectively blocked cocaine-induced Fos induction. These data are in line with the observed attenuation of behavioural effects of cocaine and support the hypothesis that $GABA_B$ receptor activation inhibits the activation of dopaminergic neurons after cocaine treatment.

ΔFosB is accumulated in different brain regions in response to various chronic stimuli including cocaine. We observed an increase of ΔFosB levels subsequent to a chronic cocaine treatment regimen in NAc, and to a lesser extent in dorsal striatum (Fig.6.5), in agreement with previous studies (Hope et al., 1994; Nye et al., 1995; McClung et al., 2004). Interestingly, GS39783 did not significantly affect ΔFosB upregulation in NAc but attenuated the induction in the dorsal striatum. ΔFosB induction is D1-like DA receptor dependent but very little information is available about the downstream signaling cascades/transcription factors responsible for its induction (Nye et al., 1995). It is conceivable that ΔFosB expression is regulated via several tissue specific signaling pathways, which could explain the differential effects of GS39783 on ΔFosB induction in NAc versus dorsal striatum.

Effects of $GABA_B$ receptor positive modulator on place preference induced by nicotine and its associated molecular markers. Although it appears that GS39783, at 100 mg/kg, elicited a place aversion in the present study which can be interpreted as an aversive or

anhedonic property of the compound at this relatively high dose (Fig.6.8). It should be noted that the same dose of GS39783 has been shown to have no effect on baseline intracranial self stimulation (ICSS) thresholds (Slattery et al., 2005b) and spontaneous locomotor activity (Cryan et al., 2004). Nevertheless, the place conditioning protocol employed here requires four administrations of GS39783 versus one for the ICSS and locomotor activity. Although these dissimilarities in the regimen of GS39783 could explain the differences between the studies, the processes involved in the aversive effect of $GABA_B$ positive modulator in the present paradigm remain elusive. Furthermore, we cannot rule out a somatic or peripheral mediation of this aversive behaviour as the solubility of GS39783 is poor and the texture of the gavage treatment may have some taste component that induces aversion. ICSS directly targets the reward pathway and thus bypasses sensory inputs such as taste (Wise et al., 1992). Further, studies using conditioned taste aversion protocols may help in the elucidation of such intrinsic behavioural effects of GS39783. In addition, future studies using $GABA_B$ receptor antagonist might clarify if this effect is totally $GABA_B$ receptor dependent. Regardless of theses considerations, we clearly demonstrated that GS39783, when administrated during conditioning phase, blocked conditioned place preference elicited by nicotine at both doses used (Fig.6.9) suggesting that $GABA_B$ positive modulation blocked the acquisition of nicotine-induced place preference, even at doses that were without intrinsic activity. Thus, we can deduce that administration of GS39783 prior each drug pairing session attenuates reinforcing properties of nicotine during the conditioning phase. As discussed in the previous section, we can assume that GS39783 might attenuate the increase of dopamine release, via its action dopaminergic neurons of the VTA, resulting in a reduction of the salience of rewarding stimulus. Thus, this reduction might limit the establishment of the association between nicotine and paired cues. These data are in line with the ability of GS39783 to decrease rewarding properties of cocaine in both self administration and ICSS paradigms (Smith et al., 2004; Slattery et al., 2005b). Correspondingly, it has been shown also that $GABA_B$ receptor

activation, via agonist administration, decreases also nicotine self-administration (Corrigall et al., 2000; Paterson et al., 2004, 2005). Interestingly, we also observed that a single administration of GS39783 prior the test failed to significantly affect the expression of nicotine-induced place preference. These present results are consistent with previous data showing that $GABA_B$ receptor positive modulator blocked the establishment but not the expression of behavioural sensitization to cocaine (see Fig.6.4.). This absence of effects of GS39783 on the expression of CPP might be attributed to the inability of $GABA_B$ receptor positive modulator to affect conditioned motivational properties of nicotine-paired cues. Nevertheless, it has been shown that CGP44532, a $GABA_B$ agonist has been shown to block cue-induced reinstatement of nicotine-seeking in rats (Paterson et al., 2005). Moreover, a number reported that baclofen affects the expression of place preference elicited by ethanol, methamphetamine or morphine (Li et al., 2001; Bechtholt and Cunningham, 2005). Thus, it is might be postulated that these response require agonistic activity and that enhancement of $GABA_B$ tone induced by GS39783 is not sufficient to affect the expression of place preference elicited by nicotine. Nevertheless, further studies are required in order confirm this hypothesis.

Moreover, as mentioned in the introduction, the major caveat of place conditioning paradigm consists in its large learning component. While the $GABA_B$ receptor agonist baclofen have been shown to induce memory impairment in several paradigm, such as passive avoidance or water-maze (Swartzwelder et al., 1987; Nakagawa and Takashima, 1997), there is no evidence for amnesic-like effects of GS39783. Indeed, GS39783 did not have any deleterious effects on cognitive performance in the passive avoidance task(Cryan et al., 2004). However, these results were obtained with only one injection of GS39783 before a single session of training whereas GS39783 was administrated before each conditioning session in the present study. Thus, it might be relevant to evaluate the effect of GS39783 in more

sensitive paradigm like water-maze, for spatial memory or delayed non-matching to position, for working memory, requiring administration of the drugs before each training session.

Repeated nicotine self-administration have been also shown to induce a strong accumulation of ΔFosB in the NAc, but not in dorsal striatum (Pich et al., 1997) Here, we confirm these results in our model of nicotine reinforcement (Fig.6.9). Indeed, we observed that GS39783 was effective in inhibiting ΔFosB accumulation in the nucleus accumbens when injected chronically during the acquisition phase of place preference. Nevertheless, we observed , in the previous section, that GS39783 attenuated cocaine-induced induction of ΔFosB in dorsal striatum, but not in nucleus accumbens. This discrepancy might be explained by the fact that cocaine has higher reinforcing efficacy than nicotine (Risner and Goldberg, 1983). Moreover, Pich and collaborators demonstrated that cocaine elicited a greater accumulation of ΔFosB in nucleus accumbens than nicotine, at doses known to maintain self-administration (Pich et al., 1997).Conversely, a single administration of GS39783 before the final test failed to block nicotine-induced ΔFosB accumulation. Taken together, these results suggested that administration of $GABA_B$ receptor positive modulator may limit the gradual accumulation of ΔFosB occurring during the acquisition phase of conditioned place preference to nicotine. Furthermore, we took advantage of the fact that both behavioural and biochemical tests in this study were performed on the same group of animals to compare both data. We found a strong positive correlation between ΔFosB expression and preference for nicotine. Genetic overexpression of ΔFosB in striatal tissues enhances cocaine place preference at low doses (Kelz et al., 1999) while, mice carrying an inactivating mutation of FosB show reduced preference for cocaine (Hiroi et al., 1997). Our data therefore strengthen this existing body of literature suggesting strong associations between the accumulation of ΔFosB and the manifestation of reinforcing properties of drugs of abuse.

Conclusions. In the present chapter, we demonstrated that $GABA_B$ receptor positive modulator attenuated both molecular and behavioural changes induced by cocaine and nicotine, supporting a beneficial role of $GABA_B$ receptor modulation as a pharmacotherapeutic strategy for addictive disorders. Nevertheless, the observation that GS39783 attenuated the acquisition but not the expression of both behavioural sensitization and conditioned place preference elicited by cocaine and nicotine, may raise concerns on the potential clinically efficacy of $GABA_B$ positive modulators, as patients are already sensitized or conditioned at the time of treatment. However, in a recent study in rodents also baclofen failed to affect the expression of sensitization in a recent study in rodents (Steketee and Beyer, 2005), despite having clinical efficacy (Addolorato et al., 2002b; Brebner et al., 2002). Further, our data demonstrate that several molecular adaptations after repeated cocaine and nicotine are attenuated by GS39783 are significant in this respect. It seems evident that additional facets to the addiction process need to be addressed in order to develop successful pharmacotherapeutic strategies. Therefore, interventions should not be limited to inhibiting the rewarding effects of a drug, but should also include strategies to enhance the saliency value of natural reinforcers, strengthen inhibitory control, decrease conditioned responses and improve withdrawal-induced deficits in mood and anxiety (Volkow and Li, 2004). The fact that $GABA_B$ receptor positive modulators reduce anxiety in preclinical paradigms (Chapter 3; Cryan et al., 2004) suggests that they may assist in the treatment of addiction beyond simply reducing the primary rewarding effects of the reinforcer.

CHAPTER 7: GENERAL DISCUSSION.

The studies in the current thesis are focused on addressing a broad hypothesis that $GABA_B$ receptors play a role in the manifestation of psychiatric disorders. More specifically, we investigated the involvement of $GABA_B$ receptors in anxiety, depression and addictive disorders. The resurgence in interest in $GABA_B$ receptors biology can be dated to the cloning of $GABA_{B(1)}$ receptor subunit in 1997 (Kaupmann et al., 1997). Thus, we firstly evaluated the impact of targeted deletion of either $GABA_B$ receptor subunit on $GABA_B$ receptor function and confirmed the conventional view that functional $GABA_B$ receptors assemble from two subunit $GABA_{B(1)}$ and $GABA_{B(2)}$. Further, we demonstrated that these mice are useful tools for assessing $GABA_B$ receptor function in vivo. Secondly, we focused on the involvement of $GABA_B$ receptors in anxiety disorders, using both genetic and pharmacological approaches. In the third part of the present thesis, we assessed the effect of both genetic ablation and pharmacological blockade of $GABA_B$ receptor in animal models of antidepressant-activity. In an effort to gain a better understanding of processes underlying the antidepressant properties of $GABA_B$ receptor antagonist, we explored the interaction between $GABA_B$ receptor and serotoninergic system. Thus, we assessed the impact of $GABA_B$ receptors ligands on behavioural effects of SSRIs and the expression of 5-HT_{1A} receptor mRNA in the hippocampus. Conversely, we also investigated the influence of blockade of serotonin transporter on $GABA_B$ function, using baclofen-induced hypothermia as an index of $GABA_B$ receptor function. Finally, the last part of the present thesis addressed the issue of a role of $GABA_B$ receptors in addiction by evaluating the effect of $GABA_B$ receptor stimulation, via $GABA_B$ agonist or positive modulator, on both molecular changes and behaviours elicited by administration of drugs of abuse, such as cocaine or nicotine.

In addition to these topics which have been individually discussed at the end of the individual chapter there are a number of other issues arising from the studies in this thesis that are worthy of further elaboration and which are detailed below.

7.1 Heterodimerization: A prerequisite for $GABA_B$ receptor function?

As mentioned in the introduction, molecular biological and biochemical approaches have suggested that, in contrast to the established dogma for the G protein coupled receptor superfamily, the $GABA_B$ receptor exists as a heterodimer rather than as a single subunit (Calver et al., 2002). In the heteromeric $GABA_B$ receptor, $GABA_{B(1)}$ subunit have been demonstrated to be responsible for binding of GABA, whereas $GABA_{B(2)}$ subunit is necessary for surface trafficking and the initiation of G-protein signalling (Bettler et al., 2004). Challenging this theory, several researchers hypothesized that functional $GABA_B$ receptors may exists in neurons that naturally lack $GABA_{B(2)}$ receptor subunits. Specifically, $GABA_{B(1)}$ subunit exhibits a more widespread distribution throughout the neuroaxis compared with $GABA_{B(2)}$ subunit (Ng and Yung, 2001). Additionally, $GABA_{B(1)}$ have been shown to yield infrequent electrophysiological biochemical responses, when expressed alone in heterologous cells (Kaupmann et al., 1998a; Kaupmann et al., 1998b).

Taking such research into account, we investigated the impact of targeted deletion of $GABA_{B(1)}$ and $GABA_{B(2)}$ subunit on $GABA_B$ receptor function. In chapter 3, we demonstrated that both $GABA_{B(1)}$ and $GABA_{B(2)}$ knockout animals exhibited a blunted hypothermic response to baclofen, suggesting that heteromeric $GABA_{B(1,2)}$ receptor is required for $GABA_B$ function involved in thermoregulation, in agreement with the general view that heterodimerization is a prerequisite for $GABA_B$ function. Correspondingly, we also observed absence of impairment of motor coordination induced by baclofen in both knock-out lines. Similarly, we also demonstrated that genetic ablation of one of the two receptor subunit abolished totally the hypothermic response associated with GHB, response that have been

demonstrated to be mediated by $GABA_B$ receptor. Additionally, we also demonstrated that $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice exhibited similar behavioural profiles in anxiety and depression-related paradigm. More specifically, both lines showed both anxious phenotype and antidepressant-like behaviour in several paradigms (Chapter 4 and Chapter 5 respectively). Altogether, these results agrees with the general view that most of functions mediated by $GABA_B$ receptor require the assembly of two $GABA_B$ receptor subunits and that potential $GABA_{B(1)}$ homodimer or monomer are not involved in the modulation of emotions, thermoregulation and motor coordination.

Although our studies failed to demonstrated differences in the baseline (FST, TST, light-dark box) and $GABA_B$ receptor agonist-induced (baclofen-induced hypothermia) behavioural trait of both lines, electrophysiological and molecular studies have shown atypical electrophysiological $GABA_B$ responses in hippocampal slices of $GABA_{B(2)}^{-/-}$ mice and a redistribution of $GABA_{B(1)}$ receptor subunit in $GABA_{B(2)}^{-/-}$ neurons from distal neuronal sites to the soma and proximal dendrites respectively(Gassmann et al., 2004). These observations imply that neurons naturally lack $GABA_{B(2)}$ receptor subunit have the potential to express functional $GABA_B$ receptors at membrane surface. While in the general view $GABA_{B(2)}$ receptor subunit appears to be essential for the correctly trafficking of $GABA_{B(1)}$ receptor subunit to cell surface, several studies have pointed that $GABA_{B(1)}$ could also exported by another protein. For example, GABAA receptor gamma2S, when expressed with $GABA_{B(1)}$ receptor subunit have be shown to promote cell surface expression of the $GABA_{B(1)}$ receptor subunit (Balasubramanian et al., 2004). Nevertheless, the $GABA_{B(1)}$/gamma2S complex is not detectably functional when expressed alone, as assessed in both ERK (extracellular regulated kinase) activation assays and physiological analyses in oocytes. Additionally, coexpression of mGluR4 and $GABA_{B(1)}$ receptor subunit have also been demonstrated to led assist plasma membrane expression of $GABA_{B(1)}$ receptor, which appears,

unfortunately, not functional (Sullivan et al., 2000). Consequently, we can predict that $GABA_{B(1)}$ receptor might be expressed at the membrane level, nevertheless, the effector mechanism underlying atypical current is still elusive. Altogether, we demonstrated that the majority of $GABA_B$ functions in CNS are mediated by $GABA_{B(1,2)}$ assemblies and, although electrophysiological studies suggest that $GABA_{B(1)}$ monomers could be functional on is own, their physiological relevance are still unclear and therefore difficult to assess their pertinence if any in the context of psychiatric disorders.

7.2 $GABA_B$ receptors: pharmacological target of GHB?

Given both the therapeutic effects and emerging public health issues related to the use of GHB (Snead), it was particularly crucial to investigate the physiological processes involved in GHB mediated response. GHB have been shown to be effective in the treatment of sleep disorders and alcohol dependence (Agabio et al., 1998). In addition to these therapeutic properties, GHB is readily self-administrated and has been recognized as a drug with a strong abuse potential (Martellotta et al., 1998a; Nicholson and Balster, 2001). Nevertheless, the processes underlying these responses are a matter of debate. Some evidence suggests that GHB acts as a weak agonist at $GABA_B$ receptors (Mathivet et al., 1997; Lingenhoehl et al., 1999), whereas other studies indicate that GHB binds to specific receptor (Maitre, 1997; Snead, 2000). In the present thesis, we took the advantage of recently generated mice lacking $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor in order to assess the involvement of $GABA_B$ receptor in the well characterized GHB-elicited hypothermic responses.

In chapter 3, we observed that targeted deletion of $GABA_{B(1)}$ receptor subunit totally abolished the hypothermic responses induced by oral administration of GHB. Interestingly, these observations combined with the lack of GHB-elicited sedation and the absence of GBL-induced delta waves in $GABA_{B(1)}^{-/-}$ mice (Kaupmann et al., 2003), suggested that most of pharmacological effects of GHB might be $GABA_B$ receptor dependent. Nevertheless, in the

same study, Kaupmann and collaborators demonstrated specific binding sites in the brains of $GABA_{B(1)}^{-/-}$ mice for GHB and NCS-382, a GHB antagonist, confirming the previous notion that $GABA_B$ receptor do not significantly contribute to GHB-binding sites in the brain (Bernasconi et al., 1999). Given that the expression pattern in the brain of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunits do not fully overlap, it is also conceivable that the $GABA_{B(2)}$ receptor subunit might contribute to the binding of GHB. Although the absence of hypothermic response induced by GHB observed in $GABA_{B(2)}^{-/-}$ mice cannot exclude this last theory, these results confirmed that GHB require a functional $GABA_{B(1,2)}$ receptor assembly in order to elicit its hypothermic responses.

Thus, our data combined with data assessed in collaboration with the laboratory of Kaupmann and collaborators, provided physiological evidence for a contribution of $GABA_B$ receptors in the majority of response induced by GHB and that GHB might be a weak agonist of $GABA_B$ receptor. Although GHB and $GABA_B$ receptor agonists share a large number of their in vivo effects, including sedation, hypothermia and also beneficial effects in certain conditions of drug dependence (Gallimberti et al., 1994; Addolorato et al., 2002a), GHB itself may cause physical dependence whereas no abuse potential has been reported for baclofen after more than 30 years of clinical use. One possible explanation for this is provided by the recent elegant study of Cruz et al., (2004) suggesting that GHB, acts like low-affinity $GABA_B$ ligands and inhibits mesolimbic dopamine system whereas baclofen and other high-affinity agonists, will inhibit the system ((Cruz et al., 2004), Fig.7.1). Nevertheless, we cannot exclude that some specific GHB receptors might participate also to these differential effects. Indeed, the recent development of GHB analogs sharing some, but not all, effect of GHB and $GABA_B$ receptors agonists offer a new opportunity to dissect GHB receptor mediated effect from those mediated by $GABA_B$ receptors (Carter et al., 2004). Given that ataxic effects of these GHB analogs are not counteracted by $GABA_B$ receptor antagonist, it might be

interesting to evaluate these compounds in mice lacking $GABA_B$ receptor. Moreover, these novel GHB analogs represent also exciting new tools for studying the involvement of GHB receptors in addictive or anti-addictive properties of GHB.

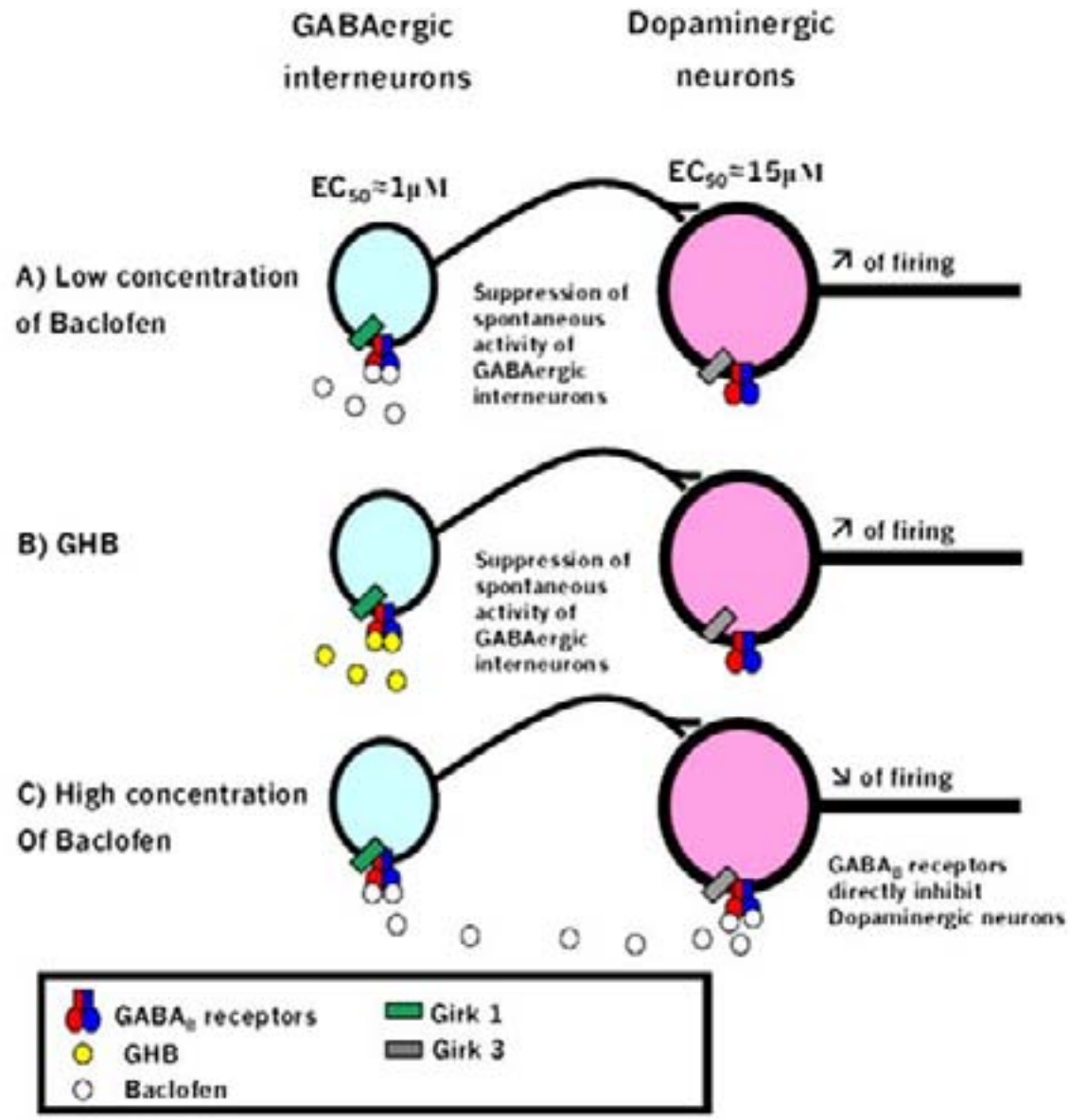

Fig.7.1. Bi-directional effects of $GABA_B$ receptor agonists on the mesolimbic dopamine system.

At low concentration, baclofen stimulate preferentially $GABA_B$ receptor included in GABAergic interneuron leading to disinhibition of dopaminergic. Similarly, GHB, as a weak $GABA_B$ receptor agonist induced the same effect than low concentration of baclofen. C) $GABA_B$ agonist at high dose stimulates $GABA_B$ receptors in dopaminergic neurons of VTA, inhibited mesolimbic system. (based on Cruz et al., 2004)

7.3 Pharmacological vs genetic approaches to investigate role of $GABA_B$ receptors in anxiety.

The results of the section 4.3 and 4.4 provided evidence that targeted deletion of either subunit of $GABA_B$ receptor induced exaggerated anxious phenotype compared to wild-type animals. This was clearly illustrated in the light-dark box for both transgenic lines, and in staircase test and elevated-zero maze for $GABA_{B(1)}^{-/-}$ mice. Supporting earlier findings suggesting that $GABA_B$ receptors stimulation decreases anxiety in several paradigm (Cryan and Kaupmann, 2005), the present data confirm the key role of GABAB receptor in the neurophysiology of anxiety. However, we also observed that GABAB receptor antagonist CGP56433A failed to induce anxiogenic effects in light-dark box paradigm, acutely and chronically. The observed mismatch between the phenotype of knockout animals and the effects of antagonist in normal animals could be explained in three ways.

- First, one can assume that $GABA_{B(1)}$ or $GABA_{B(2)}$ disruption activated compensatory changes during development, and these may have affected behaviour in addition to gene disruption. For example, the loss of sensitivity to classical benzodiazepine observed in $GABA_{B(1)}^{-/-}$ mice suggested that lack of $GABA_B$ receptor during the course of early development may result in alterations in $GABA_A$ system.

- Alternatively, we can presume that spontaneous seizures observed in both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice may contribute to their hyper-anxious phenotype. Indeed, both preclinical and clinical studies suggested that repeated episodes of seizures can result in anxiety (Sayin et al., 2004; Beyenburg et al., 2005).

- Thirdly, we also observed that both pharmacological blockade and genetic ablation of $GABA_B$ receptor result in the same antidepressant effects in mouse forced swim test (Chapter 5), suggesting that the anxiogenic and antidepressant-like effect might recruit different $GABA_B$ receptors population in brain. Indeed, it is conceivable that CGP56433A might bind preferentially at post-or pre-synaptic level resulting in antidepressant-like effect but not in anxiogenic effects.

In order to elucidate the contribution of both genetic compensation and seizures, it would be interesting in future experiments to use conditional knock-down approaches, using cre-lox or siRNA approaches, in the same anxiety paradigm (Haller et al., 2004; Thakker et al., 2005). Moreover, the development of $GABA_B$ receptor antagonists able to bind specifically to pre-or post synaptic receptor would provide a powerful tools in order to investigate the impact of $GABA_B$ receptor blockade on anxiety and depression.

Regardless of the interpretation problems surrounding the data observed using $GABA_B$ knockout animals and $GABA_B$ receptor antagonists, we also demonstrated that $GABA_B$ activation, via administration of recently developed $GABA_B$ receptor positive modulator GS39783, elicits a clear anxiolytic effect in several animal models of anxiety, including ethological tests such as the novelty-induced hypophagia, the elevated plus maze and the light–dark box. Moreover the anxiolytic effects of chronic treatment with GS39783 persist over 21 days, suggesting that there is no obvious tolerance. Moreover, earlier studies indicated that GS39783 is devoid of side-effects associated to both $GABA_B$ full agonist and benzodiazepines (Cryan et al., 2004). Altogether, these findings implicate that $GABA_B$ receptor positive modulation might represent a novel approach in the treatment of anxiety disorder and support the putative role of $GABA_B$ receptors in anxiety disorders. Nevertheless, the behavioural characterization of other $GABA_B$ receptor positive modulator, including

CGP7930 and CGP13501 (Urwyler et al., 2001; Kerr et al., 2002; Urwyler et al., 2003) is required , before it can be definitively stated that they might represent a novel class of anxiolytic with a superior side-effect profile than benzodiazepines.

While we demonstrated that genetic inactivation or pharmacological activation of $GABA_B$ receptor modulates anxiety in opposite manners, the mechanisms underlying the influence of $GABA_B$ receptors on anxiety behaviour are not well understood at present. Firstly, $GABA_B$ receptor has been shown to modulate several monoaminergic systems involved in anxious behaviours. As suggested in Chapter 5, $GABA_B$ receptors strongly interact with serotoninergic system in raphé nuclei. Indeed, it has been shown that $GABA_B$ receptors are densely localized on serotoninergic neurons of the dorsal raphé and their activation results in the inhibition of firing of these neurons (Innis et al., 1988; Serrats et al., 2003). In the context of anxiety, Andrews and File reported that baclofen reversed both anxiogenic response elicited by benzodiazepine withdrawal and its associated increased of hippocampal 5-HT release (Andrews and File, 1993). In line with these studies, we demonstrated in the section 5.5.3 that both $GABA_B$ receptor positive modulator and $GABA_B$ receptor agonist counteracted the downregulation of hippocampal 5-HT_{1A} receptor mRNA-induced by a chronic handling. Thus, it might be conceivable that $GABA_B$ receptor positive modulator could attenuate the elevation of 5-HT in hippocampus induced by stress via their inhibiting action on the firing of serotoninergic neurons in the raphé. However, it would be simplistic to assume that anxiolytic effect of $GABA_B$ agonists can be attributed to a direct suppression of serotoninergic transmission. Indeed, $GABA_B$ receptors have been also shown to modulate of noradrenalin that could also participate in their influence on anxious states (Olpe et al., 1988; Losada, 1991). Secondly, $GABA_B$ receptor are broadly distribute in several structure involved in the regulation of anxiety states, such as prefrontal cortex , hippocampus, dorsal periaqueductal grey matter and amygdala (Bischoff et al., 1999; Margeta-Mitrovic et

al., 1999). Thus, local injection of $GABA_B$ receptor agonist or positive modulator may provide more insight into the physiological processes and structure involved in the anxiolytic properties of $GABA_B$ receptor stimulation. For example, recent studies demonstrated a panicolytic-like effect of stimulation of $GABA_B$ receptors in the dorsal periaqueductal grey of rats (Bueno et al., 2005).

7.4 $GABA_B$ receptor blockade a novel therapeutic strategy for antidepressant development ?

One major purpose of the present thesis was to evaluate the therapeutic benefits of altering $GABA_B$ receptor function in animal models of antidepressant-like behaviour. As reported previously, several studies have demonstrated that $GABA_B$ receptor blockade was effective in several animal models of depression (Cryan and Kaupmann, 2005) Given the emerging evidence for a role of serotonergic system in the antidepressant-like effect associated to $GABA_B$ receptor blockade (Slattery et al., 2005a); it appeared essential to investigated these interactions using appropriate tools.

In section 5.3, we observed an antidepressant-like phenotype in both $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice and pharmacological antagonism in the forced swim test but not the tail suspension test. In contrast of data observed in anxiety sections, we demonstrated that both genetic inactivation and pharmacological blockade of $GABA_B$ receptor result in the same effect. Consequently, we can exclude a potential involvement of compensatory changes in the antidepressant-like effect observed in both knockout lines. Nevertheless, more studies using paradigms other than the forced swim test, (e.g. the chronic mild stress or in olfactory bulbectomy paradigm) are required to confirm the putative role of $GABA_B$ receptors in depression. For example, Bilkei-Gorzo and collaborators illustrated these needs in a recent study (Bilkei-Gorzo et al., 2002). Although they demonstrated an effect of targeted deletion of *Tac-1* a gene involved in Neurokinin metabolism, in both the forced swim test and tail

suspension, they also observed that this genetic manipulation rescued behavioural changes elicited by bulbectomy. Regardless of its effect in behaviour, it might be also intuitive to explore the effects of $GABA_B$ receptors ablation on physiological aspects of depression (Cryan and Mombereau, 2004). For example, we demonstrated recently that a clear hypersensitivity to dexamethasone suppression test in $mGlur7^{-/-}$ mice (Mitsukawa et al., 2005). Interestingly, this enhanced suppression is opposite to that seen in many depressed patient (Holsboer et al., 1980), corroborating their antidepressant-like phenotype observed previously in forced swim test and tail suspension (Cryan et al., 2003c).

In the second part of chapter 5, we recapitulated the antidepressant-like effects of genetic ablation of $GABA_B$ receptor using $GABA_B$ receptor antagonist CGP56433A. Although the influence of $GABA_B$ receptor on serotoninergic system is probably still the most parsimonious mechanism so far to account for antidepressant-like of $GABA_B$ receptor antagonist (Chapter 5), $GABA_B$ receptors are also to known to alter other neurotransmitter. As mentioned previously, $GABA_B$ receptors actively inhibit the firing in the locus coeruleus (Olpe et al., 1988). Thus, it is therefore possible that $GABA_B$ receptors antagonist would enhance noradrenergic activity, resulting in potential antidepressant-like effect. Nevertheless, the absence of effect of $GABA_B$ receptor antagonist CGP56433A of climbing behaviour in the modified rat forced swim test led to exclude a potential involvement of noradrenergic system in antidepressant-like effect mediated by $GABA_B$ receptor antagonist (Slattery et al., 2005a). Indeed, this pattern of behaviour have been shown to be modulated by catecholamine compounds (Detke et al., 1995; Lucki, 1997; Cryan et al., 2002a). Consistent with this idea, microdialysis studies reported that injection of $GABA_B$ receptor antagonist in the locus coeruleus does not affect the noradrenergic release in frontal cortex (Kawahara et al., 1999). In addition, prior studies combined with the findings of chapter 6 provided evidence for a modulatory effect of $GABA_B$ receptor on dopaminergic system that could participate in

antidepressant action of $GABA_B$ receptor antagonist. Indeed, anhedonic component of depression have been propose to be mediated by dysfunction of brain reward circuitry (Gambarana et al., 2001; Tremblay et al., 2005). Given the fact that $GABA_B$ receptor blockade in the ventral tegmental area elicits increased of local dopamine release (Giorgetti et al, 2002), it is conceivable that antidepressant-like potential of $GABA_B$ receptors antagonists may be due in part to an increase of dopaminergic transmission in brain reward circuitry, which may help to alleviate anhedonic-like symptoms. Moreover, $GABA_B$ receptor stimulation have been demonstrated to increase release of corticosterone and ACTH in rats, suggesting a role of HPA axis in the antidepressant-like effect of $GABA_B$ receptor (Hausler et al., 1993). Based on the fact that behavioural immobility in the FST seems to be contingent upon the presence of corticosterone, as elimination of the effects of corticosterone through an adrenalectomy (Jefferys and Funder, 1996), inhibition of corticosterone synthesis (Baez and Volosin, 1994) alter immobility in this test. It is, therefore, conceivable that the effect observed in the present thesis might be partially due to the influence of $GABA_B$ antagonist of HPA axis activation. Conversely, the pro- depressant effect of CGP44532 observed in the present thesis could be also mediated by an enhancement of corticosterone release during the forced swim test. As mentioned in the introduction, there is growing body of evidence for an involvement of cell survival pathway in therapeutic action of antidepressant (Nestler et al., 2002). Thus, the recent findings suggesting that $GABA_B$ receptor antagonist increase the concentration of BDNF in the hippocampus and cortex, adds another facet to the complexity in the process of understanding mechanism involved in antidepressant-like effect of $GABA_B$ receptors antagonist (Heese et al., 2000). Actually, this elevation of BDNF represents a possible mechanism which may contribute to the antidepressant-like effects of $GABA_B$ receptors antagonist in the forced swim test, as local injection of BDNF in hippocampus decreases time spent in the forced swim test (Shirayama et al., 2002).Together, the above neurochemical and behavioural findings provide clear evidence for a antidepressant-like

effect of $GABA_B$ receptor antagonist. Nevertheless, the exact mechanism underlying this effect is still unclear and the evaluation pharmacological or genetic manipulation of these on the antidepressant-like effect of $GABA_B$ receptor will new insights on how $GABA_B$ receptors influence depression states and antidepressant action.

7.5 Therapeutic relevance of $GABA_B$ receptor activation and blockade in the context of co-morbidity of anxiety and depression.

Superficially at least, it may seem counterintuitive that modulation of a given receptor may induce a differential effect on anxiety and depression-like behaviours, given the extensive co-morbidity of such disorders clinically (Moller, 2002). However, $GABA_B$ receptors are localized both pre- and post-synaptically, and the elucidation of the relative contribution of these individual receptor populations to behavioural phenotypes is currently not possible. It should be noted that there are a number of other receptor systems which genetic and/or pharmacological manipulation gives opposite effects in animal models of depression and anxiety. Interestingly, mice lacking the 65-kDa isoform of glutamic acid decarboxylase (GAD65), which plays an essential role for GABA synthesis, have a similar phenotype to $GABA_B$ knockout mice (increased anxiety and decreased depression-related behaviour; (Stork et al., 2000; Stork et al., 2003)). $GAD65^{-/-}$ mice have a deficit in the temporal increase in GABA synthesis which occurs postnatally in wildtype animals. It is tempting to speculate that the phenotype of these mice may be in part related to insufficient agonist occupancy at $GABA_B$ receptors especially during critical postnatal periods. Also of note is the fact that such a behavioural pattern is also observed for mice lacking the $5\text{-}HT_{1A}$ receptor (Ramboz et al., 1998).These mice have been demonstrated to display more anxious-like behaviour in open-field and elevated-plus maze and antidepressant-like phenotype in the forced swim test. Although these phenotypes might seem paradoxical, since anxiety and depression are often associated in humans, there is also evidence that serotonin can modulate

anxiety and depression in opposite manners, with high serotoninergic activity being associated with anxiety and low activity with depression (Graeff et al., 1996). Based of this idea, it is conceivable that the opposite action of $GABA_B$ receptor stimulation and blockade on serotoninergic system might contribute to the contradictory phenotype of mice lacking $GABA_B$ receptor and to the anxiolytic and antidepressant-like effect of $GABA_B$ receptor positive modulator and antagonist. On the other hand, targeted deletion of serotonin transporter induced both antidepressant-like effect and anxiogenic effect while chronic SSRI have been shown to be effective as treatment for anxiety disorders both preclinically and clinically (Holmes et al., 2003b).Therefore, recent study demonstrated that blockade of serotonin transporter during the early-life a might contribute to the anxious phenotype observed in 5-$HTT^{-/-}$ mice (Ansorge et al., 2004). Consequently, we can hypothesize that anxious phenotype observed in mice lacking $GABA_B$ knock-out animals might be caused by the alleviation of inhibition of $GABA_B$ receptor on serotoninergic neuron of raphé during early-life phase. Considering this, it would be also pertinent to evaluate the effect of chronic pharmacological blockade of $GABA_B$ receptor in anxiety paradigm sensitive to SSRI , such as novelty-induced hypophagia (Dulawa et al., 2004; Dulawa and Hen, 2005) in mature organism, considering the putative enhancement of serotoninergic system induced $GABA_B$ receptor antagonist.

Another receptor system of interest is the nociceptin (aka Orphanin FQ, NOP) which has been implicated in anxiety and depression(Jenck et al., 2000; Gavioli et al., 2003; Gavioli et al., 2004). As in the case with $GABA_B$ receptors most of the available literature data suggest that the activation of the noceceptin receptor produces anxiolytic-like effects, while its blockade elicit antidepressant-like actions. Interestingly, several other biological features are shared by these two receptors: i) both NOP and $GABA_B$ receptors are coupled with the same type of G-proteins i.e. the Gi/o and reduce neurotransmitter release at presynaptic sites

and cellular excitability at postsynaptic sites (Calo et al., 2000; Bowery et al., 2002) ii) both receptors are widely expressed in the brain, and densely located in 5-HTergic neurons of the dorsal raphe nucleus (Serrats et al., 2003; Le Maitre et al., 2005); iii) both nociceptin and $GABA_B$ seems to exert a similar and powerful control of the 5-HT system functions by activating K+ conductance in the dorsal raphe nucleus neurons (Williams et al., 1988; Vaughan and Christie, 1996) and reducing 5-HT release in the cerebral cortex (Schlicker et al., 1984; Siniscalchi et al., 1999; Sbrenna et al., 2000). The similar pattern of anatomical distribution, transductional mechanisms, effects in models of affective disorders, and ability to modulate 5-HT neuron functions displayed by nociceptin and $GABA_B$ receptors are certainly impressive and strongly suggest that serotonergic signalling represents the target of NOP and $GABA_B$ mediated actions on anxiety and depression. As stated previously, the fact that 5-HT has long been linked with the pathophysiology of depression and anxiety and particularly that clinical and pharmacological studies point to dysfunctions in the dorsal raphe 5-HT system as critical for the pathophysiology of affective disorders (Valentino and Commons, 2005) strongly corroborate this view.

7.6 $GABA_B$ receptors activation, a novel therapeutic approach to treat addictive disorders?

Prior to the emergence of new genetic and pharmacogical tools, there had been a decreased interest in examining the role of $GABA_B$ receptor in anxiety and depression, however at this time (mid 1990's) researchers began to shown positive effects of baclofen in animal model of addiction (Roberts et al., 1996). Over the past decade, $GABA_B$ receptor activation has emerged as the most interesting therapeutic strategies for the treatment of dependence to drugs such as cocaine and nicotine. Further, the last aim of the present thesis was to evaluate the putative anti-addictive properties of $GABA_B$ receptor stimulation. We focused particularly on elucidating both behavioural and molecular changes following chronic

exposure to either cocaine or nicotine. Our data suggest that $GABA_B$ receptor activation (either directly or via positive modulation) can reduce the rewarding aspects of abused drugs at both the molecular and behavioural level. Nevertheless, we have to be particularly cautious regarding the linkage between the effects observed in behaviour and molecular changes. Indeed, the inhibition of DARPP-32 upregulation by GS39783 does not intuitively agree with the reduced locomotor activity observed upon the cocaine challenge trial. Although, in line with our findings, total DARPP-32 has been shown to be increased after repeated administration of cocaine (Hu et al., 2005). The majority of studies investigating DARPP-32's role in addiction are focused on its phosphorylation, and more specifically, on its phosphorylation on Thr-75. Since DARPP-32 is preferentially phosphorylated on Thr-75 by repeated administration of cocaine and DARPP-32 Thr-75 mutant mice exhibit attenuation of cocaine sensitization, study of phosphorylation state of DARPP-32 after cocaine and GS39783 treatments could help clarify this matter. While $CREB^{\alpha\Delta}$ mutant mice, have been shown to be more responsive to the reinforcing effects of cocaine compared to wild-type littermates (Walters and Blendy, 2001), CREB activation have been further presumed as regulating the aversive such as tolerance and dysphoria (Carlezon et al., 1998). Nevertheless, the reduction of ΔFosB induction observed in CPU could be related to the blunting effect of GS39783 on cocaine sensitization. Indeed, mice overexpressing ΔFosB have heightened cocaine-induced locomotor activity during the sensitization(Kelz et al., 1999).

In order to further confirm the putative anti-addictive properties of $GABA_B$ receptor stimulation, we also assessed the effect of GS39783 on reinforcing properties of nicotine in the place conditioning paradigm. Interestingly, GS39783 attenuated the acquisition but not the expression of place preference triggered by nicotine. Similarly with data obtained with cocaine, we also observed that $GABA_B$ receptor activation via application of GS39783, blocked also the accumulation of ΔFosB in the nucleus accumbens only when is injected prior

the test. Although it seems difficult and perhaps premature to clearly link these molecular adaptations to the behaviours observed, the findings provide in this thesis provide evidence for the ability of GS39783 to have anti-addictive potential.

However, the exact mechanisms underlying these effects are not clear and can only be speculated upon at this point. According to the hypothesis that mesolimbic dopaminergic pathway involved in reward, we can presume that $GABA_B$ receptor might modulate dopaminergic release in several structures. As mentioned in chapter 6, the more simplistic scenario would be that $GABA_B$ receptor positive modulators enhance the stimulation of $GABA_B$ receptor on dopaminergic neurons in the VTA. Nevertheless, this response might recruit $GABA_B$ receptor from other structure such as prefrontal cortex or ventral pallidum. $GABA_B$ receptor antagonists have been shown to increase ventral pallidal dopamine release (Gong et al., 1998). Given the fact that dopaminergic lesions of the ventral pallidal terminals impairs the development of place preference elicited by cocaine (Gong et al., 1997), we can assume that $GABA_B$ receptor positive modulators might also recruit $GABA_B$ receptors within the ventral pallidum to attenuate the reinforcing properties of nicotine as observed in the studies presented in Chapter 6 of this present thesis. On the other hand, we can also speculate that the $GABA_B$ receptor positive modulator effect might also recruit $GABA_B$ receptors within the prefrontal cortex. Indeed, local $GABA_B$ receptor stimulation in the median prefrontal cortex have been shown to attenuate the hyperactivity induced by a single administration of cocaine, and the establishment of behavioural sensitization to cocaine (Steketee and Beyer, 2005). Similarly, Doherty and Gratton demonstrated that local injection of baclofen in this area inhibits accumbal dopamine release triggered by stress (Doherty and Gratton, 1999). Further studies, using local administration of GS39783 in these different structures may help to deduce a schema of the neuroanatomical pathways where $GABA_B$ receptors recruited in the anti-addictive response of GS39783.

Similar complexity is observed in order to explain the ability of $GABA_B$ receptor positive modulator to counteract the activation of CREB, DARPP-32 and the induction of ΔfosB triggered by repeated administration of nicotine or cocaine. As mentioned in chapter 6, we presume that this downstream effect is principally mediated by the inhibition of adenylyl cyclase by $GABA_B$ receptor. Indeed, repeated exposure to stimulant increases activity of cAMP-PKA pathway within the nucleus accumbens resulting in an activation of CREB and DARPP-32, via the stimulation of D1 receptors. This action might be partially inhibited by inhibitory action of $GABA_B$ receptor on adenylyl cyclase. Nevertheless, we cannot exclude that the effect elicited by GS39783 could be simply the reduction of dopaminergic tone at D1-receptor elicited by the stimulation of $GABA_B$ receptors in the ventral tegmental area. Thus, it might be relevant to investigate these processes electrophysiologically in nucleus accumbens slices, in order to limit the interfering effect of $GABA_B$-mediate dopaminergic regulation from the VTA.

Additionally, the multiplicity of pathway mediating the activation of CREB, DARPP-32 and ΔfosB, add a real complexity in the effort to understand the mechanism of action of GS39783 at cellular level. For example, CREB activation have been shown to be modulate by three different pathways (see fig.7.2): the cyclic AMP-dependant protein kinase pathway (cAMP-PKA pathway), the calmodulin kinase pathway (CAMK pathway) and mitogen-activated protein kinase pathway (MAPK pathway, see Carlezon et al, 2005 for review). Although it has been postulated that repeated injection of drugs of abuse recruit mainly the cyclic cAMP-PKA pathway (Anderson and Pierce, 2005), a recent study demonstrated also an involvement of MAPK pathway in the phosphorylation of CREB elicited by repeated administration of cocaine (Mattson et al., 2005). Thus, it is likely, given that $GABA_B$ stimulation have been shown to decrease MAPK activation (Ren and Mody, 2003), that the effect of GS39783 on activation of CREB might be mediated via inhibition of MAPK

pathway. Thus, further studies on accumbal slices or cell culture might help to clarify all cellular pathway involved in the downstream effects of GS39783 on molecular changes elicited by administration of drugs of abuse.

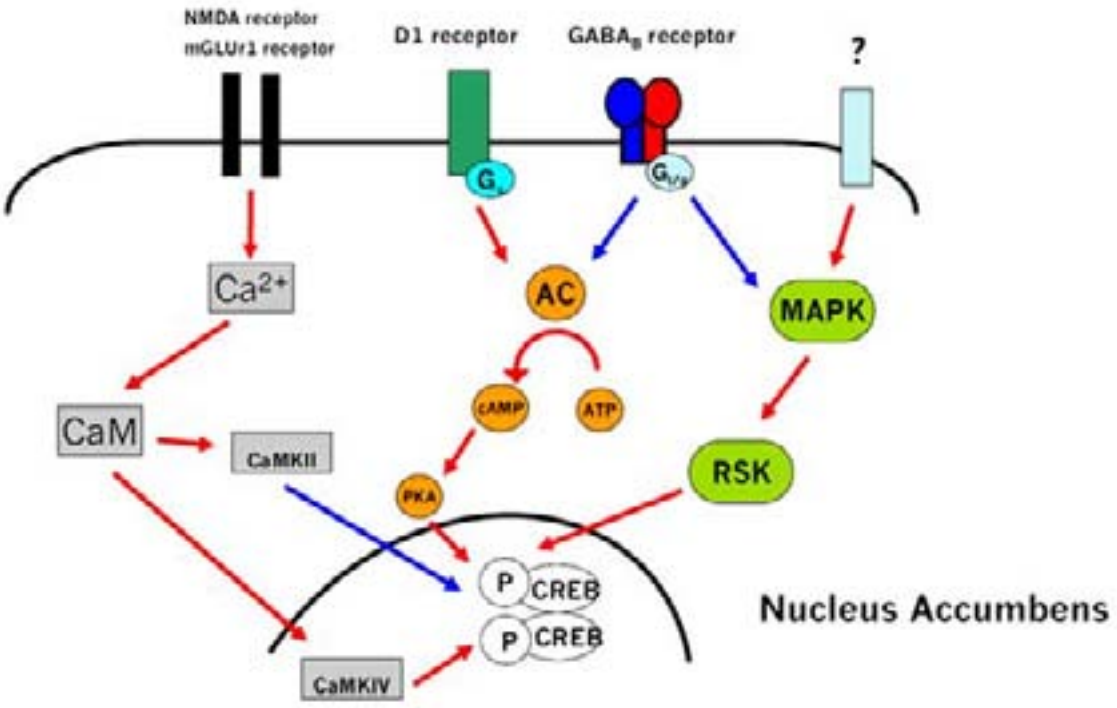

Fig.7.2 Cellular pathway involved in regulation of CREB activity.
There are three different pathways regulating CREB activity :the cAMP-PKA pathway (orange),the CAMK pathway (grey) and the MAPK pathway (Green). Repeated administrations of drugs have been shown to stimulate both cAMP-PKA pathway, via D1 stimulation, and MAPK pathway. $GABA_B$ receptors might counteract activation of CREB via : diminution of dopaminergic tone at D1 receptors, inhibition of adenylyl cyclase and inhibition of activation of MAPK. Red arrows signify activation. Blue arrows signify inhibition. Abbreviation: CaM, calmoduline; CaMK; Ca^{2+}Calmoduline dependent kinase; RSK, MAPK-activated ribosomal S6 kinases; AC, adenylyl cyclase; PKA ; cAMP dependent protein kinase.

In conclusion, the findings of the present thesis suggest a putative anti-addictive property of $GABA_B$ positive modulator. Indeed, administration of GS39783 attenuated both behavioural effect and molecular adaptation elicited by administration of drugs of abuse. Nevertheless, it should be noted that the major caveat of models used in the present thesis is that drugs were passively administrated by the animal. Given the fact that passive vs active

administration of drugs of abuse elicited common but also different molecular and physiological changes (Jacobs et al., 2003), it would be pertinent to assess the effect of GS39783 in self-administration paradigms in order to compare with the present findings. Additionally, addiction is a multifaceted disorder including different states such as craving or withdrawal. Although we demonstrated that GS39783 might attenuate the reinforcing properties of drugs of abuse, it would be appropriate to investigate its effect in paradigm relevant to these syndromes. Considering that it has been postulated that sensitization plays a major role in some of the persistent features of drug abuse, such as drug craving and compulsive drug-seeking behaviour (Robinson and Berridge, 1993), it would be conceivable that GS39783 might reduce craving behaviour and drugs seeking. This hypothesis is supported by the fact that $GABA_B$ receptor agonist, namely baclofen and CGP44532, have been shown to inhibit cue-triggered relapse to nicotine and cocaine-seeking behaviour. On the other hand, it might be also essential to assess the effect of GS39783 in animal model of withdrawal. Considering that baclofen has been shown to decrease the abstinence sign of morphine, alcohol and barbital (Sandoval and Palermo-Neto, 1985; File et al., 1992; Kemmling et al., 2002), we can speculate a putative effect of GS39783 in these models. Taken together, these findings suggest that $GABA_B$ receptor activation, and more specifically, $GABA_B$ receptor positive modulation might be considered as a novel approach to treat addictive disorder. Moreover, its effectiveness in counteracting the effects of a wide spectrum of drugs of abuse and in different symptoms of addictive disorder leads one to consider the $GABA_B$ receptor as one of the most promising therapeutic targets for treating addiction.

7.7 Concluding remarks

Since its cloning, $GABA_B$ receptor research has undergone a renaissance over the past decade. Through the development and subsequent access to novel pharmacological and genetic tools, such as $GABA_B$ positive modulator and mice lacking $GABA_B$ receptor, I have

generated strong evidence that $GABA_B$ receptors play a key role in psychiatric disorders, such as anxiety, depression or addiction. The exact physiological and molecular mechanisms by which modulation of $GABA_B$ receptor system contribute to these disorders are still elusive. Nevertheless, the emergence new technology such as conditional knockdown (Haller et al., 2004) or RNA interference (Thakker et al., 2005) will help to dissect the brain structure recruited in the beneficial properties of $GABA_B$ ligands. Moreover, the relative contribution of presynaptic and postsynaptic receptors to behavioural effects of $GABA_B$ receptors ligands needs to be clarified. To this purpose, the group of Bernard Bettler (University of Basel), in collaboration with colleagues at Novartis, generated mice lacking $GABA_{B(1a)}$ or $GABA_{B(1b)}$ isoforms. Electrophysiology in the CA1 region of mutant mice demonstrates that $GABAB_{(1a,2)}$ receptors and $GABAB_{(1b,2)}$ receptors fulfil distinct pre- and postsynaptic roles. This novel advance give the opportunity to explore the involvement of these two isoforms the modulation of emotion and reward system (Biermann et al., 2005; Jacobson et al., 2005).

Additionally, the recent initiation of mapping of GS39783 positive modulator binding site at the $GABA_B$ receptor heterodimer (Binet et al., 2004; Dupuis et al., 2004) will hopefully provide new tools, such as antagonist of $GABA_B$ positive modulator or mutant mice lacking $GABA_B$ positive modulation allosteric site. Consequently, these approaches may help clarify the localization of $GABA_B$ receptor recruited in anxiolytic and anti-addictive effects of $GABA_B$ positive modulator. Finally, although we focused this thesis on these three psychiatric disorders, $GABA_B$ receptor system might be involved in other pathology such as cognitive dysfunction, epilepsy or schizophrenia, pain food intake (Patel et al., 2001; Treiman, 2001; Mizukami et al., 2002; Froestl et al., 2004; Bowery, 2005; Buda-Levin et al., 2005; Foltin, 2005). The use of the novel tools employed in this thesis should also help to further delineate the therapeutic potential of $GABA_B$ receptor activation in animal models of these diseases.

Taken together, it's hoped that these studies presented here have, and will continue to, renew interest in the behavioural pharmacology of $GABA_B$ receptor. Further, we inspire that these data will serve as a primer for the development of novel therapies for anxiety, depression and drugs dependence where is a huge unmet medical need.

References:

Abellan MT, Adell A, Honrubia MA, Mengod G and Artigas F (2000) GABAB-RI receptors in serotonergic neurons: effects of baclofen on 5-HT output in rat brain. *Neuroreport* **11**:941-945.

Adams JU, Careri JM, Efferen TR and Rotrosen J (2001) Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. *Behav Pharmacol* **12**:603-611.

Addolorato G, Armuzzi A, Gasbarrini G, De Lorenzi G, Ancona C, Abenavoli L, Parente A, Leggio L, Capristo E, Greco AV, Janiri L, Pozzi G, Taranto C, Caputo F, Bernardi M, Stefanini GF and Foschi FG (2002a) Pharmacological approaches to the management of alcohol addiction. *Eur Rev Med Pharmacol Sci* **6**:89-97.

Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL and Gasbarrini G (2000) Ability of baclofen in reducing alcohol craving and intake: II--Preliminary clinical evidence. *Alcohol Clin Exp Res* **24**:67-71.

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, Agabio R, Colombo G, Gessa GL and Gasbarrini G (2002b) Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. *Alcohol Alcohol* **37**:504-508.

Adinoff B, Kramer GL and Petty F (1995) Levels of gamma-aminobutyric acid in cerebrospinal fluid and plasma during alcohol withdrawal. *Psychiatry Res* **59**:137-144.

Agabio R, Colombo G, Loche A, Lobina C, Pani ML, Reali R and Gessa GL (1998) Gamma-hydroxybutyric acid reducing effect on ethanol intake: evidence in favour of a substitution mechanism. *Alcohol Alcohol* **33**:465-474.

Agmo A, Pruneda R, Guzman M and Gutierrez M (1991) GABAergic drugs and conflict behavior in the rat: lack of similarities with the actions of benzodiazepines. *Naunyn Schmiedebergs Arch Pharmacol* **344**:314-322.

Alcaro A, Cabib S, Ventura R and Puglisi-Allegra S (2002) Genotype- and experience-dependent susceptibility to depressive-like responses in the forced-swimming test. *Psychopharmacology (Berl)* **164**:138-143.

Aley KO and Kulkarni SK (1990) Effect of baclofen, a GABAB-agonist, on forced swimming-induced immobility in mice. *Arch Int Pharmacodyn Ther* **307**:18-31.

Altar CA (1999) Neurotrophins and depression. *Trends Pharmacol Sci* **20**:59-61.

Ameisen O (2005) Complete and prolonged suppression of symptoms and consequences of alcohol-dependence using high-dose baclofen: a self-case report of a physician. *Alcohol Alcohol* **40**:147-150.

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders,* 4th edn. American Psychiatric Press: Washington, DC, 1994.

Amico C, Marchetti C, Nobile M and Usai C (1995) Pharmacological types of calcium channels and their modulation by baclofen in cerebellar granules. *J Neurosci* **15**:2839-2848.

Anderson SM and Pierce RC (2005) Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. *Pharmacol Ther* **106**:389-403.

Andlin-Sobocki P and Wittchen HU (2005) Cost of anxiety disorders in Europe. *Eur J Neurol* **12 Suppl 1**:39-44.

Andrade R, Malenka RC and Nicoll RA (1986) A G protein couples serotonin and GABAB receptors to the same channels in hippocampus. *Science* **234**:1261-1265.

Andrews N and File SE (1993) Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioural evidence. *Psychopharmacology (Berl)* **112**:21-25.

Anisman H, DeCatanzaro D and Remington G (1978) Escape performance following exposure to inescapable shock: Deficits in motor response maintenance. *J. Exp. Psychol. Anim. Behav. Process* **4**:197-218.

Anisman H, Irwin J and Sklar LS (1979) Deficits of escape performance following catecholamine depletion: implications for behavioral deficits induced by uncontrollable stress. *Psychopharmacology (Berl)* **64**:163-170.

Anraku T, Ikegaya Y, Matsuki N and Nishiyama N (2001) Withdrawal from chronic morphine administration causes prolonged enhancement of immobility in rat forced swimming test. *Psychopharmacology (Berl)* **157**:217-220.

Ansorge MS, Zhou M, Lira A, Hen R and Gingrich JA (2004) Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* **306**:879-881.

Arnold JM and Roberts DC (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* **57**:441-447.

Asano T and Ogasawara N (1986) Uncoupling of gamma-aminobutyric acid B receptors from GTP-binding proteins by N-ethylmaleimide: effect of N-ethylmaleimide on purified GTP-binding proteins. *Mol Pharmacol* **29**:244-249.

Asano T, Ui M and Ogasawara N (1985) Prevention of the agonist binding to gamma-aminobutyric acid B receptors by guanine nucleotides and islet-activating protein, pertussis toxin, in bovine cerebral cortex. Possible coupling of the toxin-sensitive GTP-binding proteins to receptors. *J Biol Chem* **260**:12653-12658.

Assadi SM, Radgoodarzi R and Ahmadi-Abhari SA (2003) Baclofen for maintenance treatment of opioid dependence: a randomized double-blind placebo-controlled clinical trial [ISRCTN32121581]. *BMC Psychiatry* **3**:16.

Awapara J, Landua AJ, Fuerst R and Seale B (1950) Free gamma-aminobutyric acid in brain. *J Biol Chem* **187**:35-39.

Baez M and Volosin M (1994) Corticosterone influences forced swim-induced immobility. *Pharmacol Biochem Behav* **49**:729-736.

Bai F, Li X, Clay M, Lindstrom T and Skolnick P (2001) Intra- and interstrain differences in models of "behavioral despair". *Pharmacol Biochem Behav* **70**:187-192.

Balasubramanian S, Teissere JA, Raju DV and Hall RA (2004) Hetero-oligomerization between GABAA and GABAB receptors regulates GABAB receptor trafficking. *J Biol Chem* **279**:18840-18850.

Bale TL and Vale WW (2003) Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. *J Neurosci* **23**:5295-5301.

Ballard TM, Pauly-Evers M, Higgins GA, Ouagazzal AM, Mutel V, Borroni E, Kemp JA, Bluethmann H and Kew JN (2002) Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J Neurosci* **22**:6713-6723.

Bardo MT and Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* **153**:31-43.

Barlow DH (2001) *Anxiety and its disorders. The nature and treatment of anxiety and panic.*, New York.

Barr AM and Phillips AG (1999) Withdrawal following repeated exposure to d-amphetamine decreases responding for a sucrose solution as measured by a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl)* **141**:99-106.

Barr AM, Zis AP and Phillips AG (2002) Repeated electroconvulsive shock attenuates the depressive-like effects of d-amphetamine withdrawal on brain reward function in rats. *Psychopharmacology (Berl)* **159**:196-202.

Barthel F, Kienlen Campard P, Demeneix BA, Feltz P and Loeffler JP (1996) GABAB receptors negatively regulate transcription in cerebellar granular neurons through cyclic AMP responsive element binding protein-dependent mechanisms. *Neuroscience* **70**:417-427.

Bartoletti M, Gubellini C, Ricci F and Gaiardi M (2005) Baclofen blocks the development of sensitization to the locomotor stimulant effect of amphetamine. *Behav Pharmacol* **16**:553-558.

Bassareo V, Tanda G, Petromilli P, Giua C and Di Chiara G (1996) Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. *Psychopharmacology (Berl)* **124**:293-299.

Bechtholt AJ and Cunningham CL (2005) Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behav Neurosci* **119**:213-223.

Belzung C and Griebel G (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* **125**:141-149.

Benavides DR and Bibb JA (2004) Role of Cdk5 in drug abuse and plasticity. *Ann N Y Acad Sci* **1025**:335-344.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. *Brain Res* **287**:173-196.

Bernasconi R, Mathivet P, Bischoff S and Marescaux C (1999) Gamma-hydroxybutyric acid: an endogenous neuromodulator with abuse potential? *Trends Pharmacol Sci* **20**:135-141.

Bernstein S (1957) Serial observations on the physiological and psychological changes in patients reacting with a depression to reserpine. *J Mt Sinai Hosp N Y* **24**:89-96.

Berthele A, Platzer S, Weis S, Conrad B and Tolle TR (2001) Expression of GABA(B1) and GABA(B2) mRNA in the human brain. *Neuroreport* **12**:3269-3275.

Bettler B, Kaupmann K and Bowery N (1998) GABAB receptors: drugs meet clones. *Curr Opin Neurobiol* **8**:345-350.

Bettler B, Kaupmann K, Mosbacher J and Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev* **84**:835-867.

Beyenburg S, Mitchell AJ, Schmidt D, Elger CE and Reuber M (2005) Anxiety in patients with epilepsy: systematic review and suggestions for clinical management. *Epilepsy Behav* **7**:161-171.

Biala G and Kotlinska J (1999) Blockade of the acquisition of ethanol-induced conditioned place preference by N-methyl-D-aspartate receptor antagonists. *Alcohol Alcohol* **34**:175-182.

Bibb JA, Snyder GL, Nishi A, Yan Z, Meijer L, Fienberg AA, Tsai LH, Kwon YT, Girault JA, Czernik AJ, Huganir RL, Hemmings HC, Jr., Nairn AC and Greengard P (1999) Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* **402**:669-671.

Biermann B, Vigot R, Bradaia A, Barbieri S, Abdel Aziz S, Gassmann M, Kapfhammer JP, Müller M, Bräuner-Osborne H, Kaupmann K and Bettler B (2005) A role of sushi repates in GABAB receptor comparmentalisation., in *Society for Neuroscience,* Washington D.C.

Bilkei-Gorzo A, Racz I, Michel K and Zimmer A (2002) Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac1 gene. *J Neurosci* **22**:10046-10052.

Binet V, Goudet C, Brajon C, Le Corre L, Acher F, Pin JP and Prezeau L (2004) Molecular mechanisms of GABA(B) receptor activation: new insights from the mechanism of action of CGP7930, a positive allosteric modulator. *Biochem Soc Trans* **32**:871-872.

Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K and Bettler B (1999) Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *J Comp Neurol* **412**:1-16.

Bittiger H, Froestl W, Gentsch C, Jaekel J, Mickel S, Mondadori C, Olpe H and Schmutz M (1996) GABAB receptor antagonists: potential therapeutic applications, in *GABA: Receptors, Transporters and Metabolism* (NG TCaB ed) pp 297-305, Birkhaeuser Verlag, Basel.

Black J and Guilleminault C (2001) Medications for the treatment of narcolepsy. *Expert Opin Emerg Drugs* **6**:239-247.

Blampied NM and Kirk RC (1983) Defensive burying: effects of diazepam and oxprenolol measured in extinction. *Life Sci* **33**:695-699.

Blanchard DC, Griebel G and Blanchard RJ (2003) The Mouse Defense Test Battery: pharmacological and behavioral assays for anxiety and panic. *Eur J Pharmacol* **463**:97-116.

Blanchard RJ, Griebel G, Henrie JA and Blanchard DC (1997) Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neurosci Biobehav Rev* **21**:783-789.

Blanton MG, Shen JM and Kriegstein AR (1987) Evidence for the inhibitory neurotransmitter gamma-aminobutyric acid in aspiny and sparsely spiny nonpyramidal neurons of the turtle dorsal cortex. *J Comp Neurol* **259**:277-297.

Blaxter TJ, Carlen PL, Davies MF and Kujtan PW (1986) gamma-Aminobutyric acid hyperpolarizes rat hippocampal pyramidal cells through a calcium-dependent potassium conductance. *J Physiol* **373**:181-194.

Blendy JA and Maldonado R (1998) Genetic analysis of drug addiction: the role of cAMP response element binding protein. *J Mol Med* **76**:104-110.

Boistel J and Fatt P (1958) Membrane permeability change during inhibitory transmitter action in crustacean muscle. *J Physiol* **144**:176-191.

Bormann J (1988) Electrophysiology of GABAA and GABAB receptor subtypes. *Trends Neurosci* **11**:112-116.

Bormann J (2000) The 'ABC' of GABA receptors. *Trends Pharmacol Sci* **21**:16-19.

Borsini F, Giuliani S and Meli A (1986) Functional evidence for altered activity of GABAergic receptors following chronic desipramine treatment in rats. *J Pharm Pharmacol* **38**:934-935.

Borsini F, Giuliani S and Meli A (1988) Functional responses to baclofen and 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol (THIP) in rats repeatedly treated with desipramine. *Pharmacol Biochem Behav* **29**:189-191.

Borsini F and Meli A (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl)* **94**:147-160.

Borsini F, Podhorna J and Marazziti D (2002) Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl)* **163**:121-141.

Bourin M and Hascoet M (2003) The mouse light/dark box test. *Eur J Pharmacol* **463**:55-65.

Boutrel B, Franc B, Hen R, Hamon M and Adrien J (1999) Key role of 5-HT1B receptors in the regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. *J Neurosci* **19**:3204-3212.

Boutrel B, Monaca C, Hen R, Hamon M and Adrien J (2002) Involvement of 5-HT1A receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT1A knock-out mice. *J Neurosci* **22**:4686-4692.

Bowery NG (2005) GABA(B) receptor: a site of therapeutic benefit. *Curr Opin Pharmacol*.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI and Enna SJ (2002) International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* **54**:247-264.

Bowery NG, Doble A, Hill DR, Hudson AL, Turnbull MJ and Warrington R (1981) Structure/activity studies at a baclofen-sensitive, bicuculline-insensitive GABA receptor. *Adv Biochem Psychopharmacol* **29**:333-341.

Bowery NG, Hill DR and Hudson AL (1983) Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. *Br J Pharmacol* **78**:191-206.

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J and Turnbull M (1980) (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283**:92-94.

Bowery NG, Hudson AL and Price GW (1987) GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* **20**:365-383.

Brambilla P, Perez J, Barale F, Schettini G and Soares JC (2003) GABAergic dysfunction in mood disorders. *Mol Psychiatry* **8**:721-737, 715.

Brebner K, Ahn S and Phillips AG (2005) Attenuation of d-amphetamine self-administration by baclofen in the rat: behavioral and neurochemical correlates. *Psychopharmacology (Berl)* **177**:409-417.

Brebner K, Childress AR and Roberts DC (2002) A potential role for GABA(B) agonists in the treatment of psychostimulant addiction. *Alcohol Alcohol* **37**:478-484.

Brebner K, Phelan R and Roberts DC (2000) Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology (Berl)* **148**:314-321.

Bremner JD (2004) Brain imaging in anxiety disorders. *Expert Rev Neurother.* **4**:275-284.

Bremner JD, Innis RB, White T, Fujita M, Silbersweig D, Goddard AW, Staib L, Stern E, Cappiello A, Woods S, Baldwin R and Charney DS (2000a) SPECT [I-123]iomazenil measurement of the benzodiazepine receptor in panic disorder. *Biol Psychiatry* **47**:96-106.

Bremner JD, Krystal JH, Southwick SM and Charney DS (1996) Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse* **23**:28-38.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL and Charney DS (2000b) Hippocampal volume reduction in major depression. *Am J Psychiatry* **157**:115-118.

Breslow MF, Fankhauser MP, Potter RL, Meredith KE, Misiaszek J and Hope DG, Jr. (1989) Role of gamma-aminobutyric acid in antipanic drug efficacy. *Am J Psychiatry* **146**:353-356.

Broadbent J and Harless WE (1999) Differential effects of GABA(A) and GABA(B) agonists on sensitization to the locomotor stimulant effects of ethanol in DBA/2 J mice. *Psychopharmacology (Berl)* **141**:197-205.

Brody DL and Yue DT (2000) Relief of G-protein inhibition of calcium channels and short-term synaptic facilitation in cultured hippocampal neurons. *J Neurosci* **20**:889-898.

Brogden RN, Speight TM and Avery GS (1974) Baclofen: a preliminary report of its pharmacological properties and therapeutic efficacy in spasticity. *Drugs* **8**:1-14.

Buda-Levin A, Wojnicki FH and Corwin RL (2005) Baclofen reduces fat intake under binge-type conditions. *Physiol Behav* **86**:176-184.

Bueno CH, Zangrossi H, Jr., Nogueira RL, Soares VP and Viana MB (2005) Panicolytic-like effect induced by the stimulation of GABAA and GABAB receptors in the dorsal periaqueductal grey of rats. *Eur J Pharmacol* **516**:239-246.

Bunney WE, Jr. and Davis JM (1965) Norepinephrine in depressive reactions. A review. *Arch Gen Psychiatry* **13**:483-494.

Burman KJ, Ige AO, White JH, Marshall FH, Pangalos MN, Emson PC, Minson JB and Llewellyn-Smith IJ (2003) GABAB receptor subunits, R1 and R2, in brainstem catecholamine and serotonin neurons. *Brain Res* **970**:35-46.

Cadoni C and Di Chiara G (1999) Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. *Neuroscience* **90**:447-455.

Calo G, Guerrini R, Rizzi A, Salvadori S and Regoli D (2000) Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br J Pharmacol* **129**:1261-1283.

Calver AR, Davies CH and Pangalos M (2002) GABA(B) receptors: from monogamy to promiscuity. *Neurosignals* **11**:299-314.

Campbell UC, Lac ST and Carroll ME (1999) Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)* **143**:209-214.

Campbell V, Berrow N and Dolphin AC (1993) GABAB receptor modulation of Ca2+ currents in rat sensory neurones by the G protein G(0): antisense oligonucleotide studies. *J Physiol* **470**:1-11.

Carai MA, Colombo G, Brunetti G, Melis S, Serra S, Vacca G, Mastinu S, Pistuddi AM, Solinas C, Cignarella G, Minardi G and Gessa GL (2001) Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. *Eur J Pharmacol* **428**:315-321.

Carlezon WA, Jr., Thome J, Olson VG, Lane-Ladd SB, Brodkin ES, Hiroi N, Duman RS, Neve RL and Nestler EJ (1998) Regulation of cocaine reward by CREB. *Science* **282**:2272-2275.

Abellan MT, Adell A, Honrubia MA, Mengod G and Artigas F (2000) GABAB-RI receptors in serotonergic neurons: effects of baclofen on 5-HT output in rat brain. *Neuroreport* **11**:941-945.

Adams JU, Careri JM, Efferen TR and Rotrosen J (2001) Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. *Behav Pharmacol* **12**:603-611.

Addolorato G, Armuzzi A, Gasbarrini G, De Lorenzi G, Ancona C, Abenavoli L, Parente A, Leggio L, Capristo E, Greco AV, Janiri L, Pozzi G, Taranto C, Caputo F, Bernardi M, Stefanini GF and Foschi FG (2002a) Pharmacological approaches to the management of alcohol addiction. *Eur Rev Med Pharmacol Sci* **6**:89-97.

Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL and Gasbarrini G (2000) Ability of baclofen in reducing alcohol craving and intake: II--Preliminary clinical evidence. *Alcohol Clin Exp Res* **24**:67-71.

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, Agabio R, Colombo G, Gessa GL and Gasbarrini G (2002b) Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. *Alcohol Alcohol* **37**:504-508.

Adinoff B, Kramer GL and Petty F (1995) Levels of gamma-aminobutyric acid in cerebrospinal fluid and plasma during alcohol withdrawal. *Psychiatry Res* **59**:137-144.

Agabio R, Colombo G, Loche A, Lobina C, Pani ML, Reali R and Gessa GL (1998) Gamma-hydroxybutyric acid reducing effect on ethanol intake: evidence in favour of a substitution mechanism. *Alcohol Alcohol* **33**:465-474.

Agmo A, Pruneda R, Guzman M and Gutierrez M (1991) GABAergic drugs and conflict behavior in the rat: lack of similarities with the actions of benzodiazepines. *Naunyn Schmiedebergs Arch Pharmacol* **344**:314-322.

Alcaro A, Cabib S, Ventura R and Puglisi-Allegra S (2002) Genotype- and experience-dependent susceptibility to depressive-like responses in the forced-swimming test. *Psychopharmacology (Berl)* **164**:138-143.

Aley KO and Kulkarni SK (1990) Effect of baclofen, a GABAB-agonist, on forced swimming-induced immobility in mice. *Arch Int Pharmacodyn Ther* **307**:18-31.

Altar CA (1999) Neurotrophins and depression. *Trends Pharmacol Sci* **20**:59-61.

Ameisen O (2005) Complete and prolonged suppression of symptoms and consequences of alcohol-dependence using high-dose baclofen: a self-case report of a physician. *Alcohol Alcohol* **40**:147-150.

Amico C, Marchetti C, Nobile M and Usai C (1995) Pharmacological types of calcium channels and their modulation by baclofen in cerebellar granules. *J Neurosci* **15**:2839-2848.

Anderson SM and Pierce RC (2005) Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. *Pharmacol Ther* **106**:389-403.

Andlin-Sobocki P and Wittchen HU (2005) Cost of anxiety disorders in Europe. *Eur J Neurol* **12 Suppl 1**:39-44.

Andrade R, Malenka RC and Nicoll RA (1986) A G protein couples serotonin and GABAB receptors to the same channels in hippocampus. *Science* **234**:1261-1265.

Andrews N and File SE (1993) Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioural evidence. *Psychopharmacology (Berl)* **112**:21-25.

Anisman H, DeCatanzaro D and Remington G (1978) Escape performance following exposure to inescapable shock: Deficits in motor response maintenance. *J. Exp. Psychol. Anim. Behav. Process* **4**:197-218.

Anisman H, Irwin J and Sklar LS (1979) Deficits of escape performance following catecholamine depletion: implications for behavioral deficits induced by uncontrollable stress. *Psychopharmacology (Berl)* **64**:163-170.

Anraku T, Ikegaya Y, Matsuki N and Nishiyama N (2001) Withdrawal from chronic morphine administration causes prolonged enhancement of immobility in rat forced swimming test. *Psychopharmacology (Berl)* **157**:217-220.

Ansorge MS, Zhou M, Lira A, Hen R and Gingrich JA (2004) Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* **306**:879-881.

Arnold JM and Roberts DC (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* **57**:441-447.

Asano T and Ogasawara N (1986) Uncoupling of gamma-aminobutyric acid B receptors from GTP-binding proteins by N-ethylmaleimide: effect of N-ethylmaleimide on purified GTP-binding proteins. *Mol Pharmacol* **29**:244-249.

Asano T, Ui M and Ogasawara N (1985) Prevention of the agonist binding to gamma-aminobutyric acid B receptors by guanine nucleotides and islet-activating protein, pertussis toxin, in bovine cerebral cortex. Possible coupling of the toxin-sensitive GTP-binding proteins to receptors. *J Biol Chem* **260**:12653-12658.

Assadi SM, Radgoodarzi R and Ahmadi-Abhari SA (2003) Baclofen for maintenance treatment of opioid dependence: a randomized double-blind placebo-controlled clinical trial [ISRCTN32121581]. *BMC Psychiatry* **3**:16.

Awapara J, Landua AJ, Fuerst R and Seale B (1950) Free gamma-aminobutyric acid in brain. *J Biol Chem* **187**:35-39.

Baez M and Volosin M (1994) Corticosterone influences forced swim-induced immobility. *Pharmacol Biochem Behav* **49**:729-736.

Bai F, Li X, Clay M, Lindstrom T and Skolnick P (2001) Intra- and interstrain differences in models of "behavioral despair". *Pharmacol Biochem Behav* **70**:187-192.

Balasubramanian S, Teissere JA, Raju DV and Hall RA (2004) Hetero-oligomerization between GABAA and GABAB receptors regulates GABAB receptor trafficking. *J Biol Chem* **279**:18840-18850.

Bale TL and Vale WW (2003) Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. *J Neurosci* **23**:5295-5301.

Ballard TM, Pauly-Evers M, Higgins GA, Ouagazzal AM, Mutel V, Borroni E, Kemp JA, Bluethmann H and Kew JN (2002) Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J Neurosci* **22**:6713-6723.

Bardo MT and Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* **153**:31-43.

Barlow DH (2001) *Anxiety and its disorders. The nature and treatment of anxiety and panic.*, New York.

Barr AM and Phillips AG (1999) Withdrawal following repeated exposure to d-amphetamine decreases responding for a sucrose solution as measured by a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl)* **141**:99-106.

Barr AM, Zis AP and Phillips AG (2002) Repeated electroconvulsive shock attenuates the depressive-like effects of d-amphetamine withdrawal on brain reward function in rats. *Psychopharmacology (Berl)* **159**:196-202.

Barthel F, Kienlen Campard P, Demeneix BA, Feltz P and Loeffler JP (1996) GABAB receptors negatively regulate transcription in cerebellar granular neurons through cyclic AMP responsive element binding protein-dependent mechanisms. *Neuroscience* **70**:417-427.

Bartoletti M, Gubellini C, Ricci F and Gaiardi M (2005) Baclofen blocks the development of sensitization to the locomotor stimulant effect of amphetamine. *Behav Pharmacol* **16**:553-558.

Bassareo V, Tanda G, Petromilli P, Giua C and Di Chiara G (1996) Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. *Psychopharmacology (Berl)* **124**:293-299.

Bechtholt AJ and Cunningham CL (2005) Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behav Neurosci* **119**:213-223.

Belzung C and Griebel G (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* **125**:141-149.

Benavides DR and Bibb JA (2004) Role of Cdk5 in drug abuse and plasticity. *Ann N Y Acad Sci* **1025**:335-344.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. *Brain Res* **287**:173-196.

Bernasconi R, Mathivet P, Bischoff S and Marescaux C (1999) Gamma-hydroxybutyric acid: an endogenous neuromodulator with abuse potential? *Trends Pharmacol Sci* **20**:135-141.

Bernstein S (1957) Serial observations on the physiological and psychological changes in patients reacting with a depression to reserpine. *J Mt Sinai Hosp N Y* **24**:89-96.

Berthele A, Platzer S, Weis S, Conrad B and Tolle TR (2001) Expression of GABA(B1) and GABA(B2) mRNA in the human brain. *Neuroreport* **12**:3269-3275.

Bettler B, Kaupmann K and Bowery N (1998) GABAB receptors: drugs meet clones. *Curr Opin Neurobiol* **8**:345-350.

Bettler B, Kaupmann K, Mosbacher J and Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev* **84**:835-867.

Beyenburg S, Mitchell AJ, Schmidt D, Elger CE and Reuber M (2005) Anxiety in patients with epilepsy: systematic review and suggestions for clinical management. *Epilepsy Behav* **7**:161-171.

Biala G and Kotlinska J (1999) Blockade of the acquisition of ethanol-induced conditioned place preference by N-methyl-D-aspartate receptor antagonists. *Alcohol Alcohol* **34**:175-182.

Bibb JA, Snyder GL, Nishi A, Yan Z, Meijer L, Fienberg AA, Tsai LH, Kwon YT, Girault JA, Czernik AJ, Huganir RL, Hemmings HC, Jr., Nairn AC and Greengard P (1999) Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* **402**:669-671.

Biermann B, Vigot R, Bradaia A, Barbieri S, Abdel Aziz S, Gassmann M, Kapfhammer JP, Müller M, Bräuner-Osborne H, Kaupmann K and Bettler B (2005) A role of sushi repates in GABAB receptor comparmentalisation., in *Society for Neuroscience*, Washington D.C.

Bilkei-Gorzo A, Racz I, Michel K and Zimmer A (2002) Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac1 gene. *J Neurosci* **22**:10046-10052.

Binet V, Goudet C, Brajon C, Le Corre L, Acher F, Pin JP and Prezeau L (2004) Molecular mechanisms of GABA(B) receptor activation: new insights from the mechanism of action of CGP7930, a positive allosteric modulator. *Biochem Soc Trans* **32**:871-872.

Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K and Bettler B (1999) Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *J Comp Neurol* **412**:1-16.

Bittiger H, Froestl W, Gentsch C, Jaekel J, Mickel S, Mondadori C, Olpe H and Schmutz M (1996) GABAB receptor antagonists: potential therapeutic applications, in *GABA: Receptors, Transporters and Metabolism* (NG TCaB ed) pp 297-305, Birkhaeuser Verlag, Basel.

Black J and Guilleminault C (2001) Medications for the treatment of narcolepsy. *Expert Opin Emerg Drugs* **6**:239-247.

Blampied NM and Kirk RC (1983) Defensive burying: effects of diazepam and oxprenolol measured in extinction. *Life Sci* **33**:695-699.

Blanchard DC, Griebel G and Blanchard RJ (2003) The Mouse Defense Test Battery: pharmacological and behavioral assays for anxiety and panic. *Eur J Pharmacol* **463**:97-116.

Blanchard RJ, Griebel G, Henrie JA and Blanchard DC (1997) Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neurosci Biobehav Rev* **21**:783-789.

Blanton MG, Shen JM and Kriegstein AR (1987) Evidence for the inhibitory neurotransmitter gamma-aminobutyric acid in aspiny and sparsely spiny nonpyramidal neurons of the turtle dorsal cortex. *J Comp Neurol* **259**:277-297.

Blaxter TJ, Carlen PL, Davies MF and Kujtan PW (1986) gamma-Aminobutyric acid hyperpolarizes rat hippocampal pyramidal cells through a calcium-dependent potassium conductance. *J Physiol* **373**:181-194.

Blendy JA and Maldonado R (1998) Genetic analysis of drug addiction: the role of cAMP response element binding protein. *J Mol Med* **76**:104-110.

Boistel J and Fatt P (1958) Membrane permeability change during inhibitory transmitter action in crustacean muscle. *J Physiol* **144**:176-191.

Bormann J (1988) Electrophysiology of GABAA and GABAB receptor subtypes. *Trends Neurosci* **11**:112-116.

Bormann J (2000) The 'ABC' of GABA receptors. *Trends Pharmacol Sci* **21**:16-19.

Borsini F, Giuliani S and Meli A (1986) Functional evidence for altered activity of GABAergic receptors following chronic desipramine treatment in rats. *J Pharm Pharmacol* **38**:934-935.

Borsini F, Giuliani S and Meli A (1988) Functional responses to baclofen and 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol (THIP) in rats repeatedly treated with desipramine. *Pharmacol Biochem Behav* **29**:189-191.

Borsini F and Meli A (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl)* **94**:147-160.

Borsini F, Podhorna J and Marazziti D (2002) Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl)* **163**:121-141.

Bourin M and Hascoet M (2003) The mouse light/dark box test. *Eur J Pharmacol* **463**:55-65.

Boutrel B, Franc B, Hen R, Hamon M and Adrien J (1999) Key role of 5-HT1B receptors in the regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. *J Neurosci* **19**:3204-3212.

Boutrel B, Monaca C, Hen R, Hamon M and Adrien J (2002) Involvement of 5-HT1A receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT1A knock-out mice. *J Neurosci* **22**:4686-4692.

Bowery NG (2005) GABA(B) receptor: a site of therapeutic benefit. *Curr Opin Pharmacol*.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI and Enna SJ (2002) International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* **54**:247-264.

Bowery NG, Doble A, Hill DR, Hudson AL, Turnbull MJ and Warrington R (1981) Structure/activity studies at a baclofen-sensitive, bicuculline-insensitive GABA receptor. *Adv Biochem Psychopharmacol* **29**:333-341.

Bowery NG, Hill DR and Hudson AL (1983) Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. *Br J Pharmacol* **78**:191-206.

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J and Turnbull M (1980) (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283**:92-94.

Bowery NG, Hudson AL and Price GW (1987) GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* **20**:365-383.

Brambilla P, Perez J, Barale F, Schettini G and Soares JC (2003) GABAergic dysfunction in mood disorders. *Mol Psychiatry* **8**:721-737, 715.

Brebner K, Ahn S and Phillips AG (2005) Attenuation of d-amphetamine self-administration by baclofen in the rat: behavioral and neurochemical correlates. *Psychopharmacology (Berl)* **177**:409-417.

Brebner K, Childress AR and Roberts DC (2002) A potential role for GABA(B) agonists in the treatment of psychostimulant addiction. *Alcohol Alcohol* **37**:478-484.

Brebner K, Phelan R and Roberts DC (2000) Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology (Berl)* **148**:314-321.

Bremner JD (2004) Brain imaging in anxiety disorders. *Expert Rev Neurother.* **4**:275-284.

Bremner JD, Innis RB, White T, Fujita M, Silbersweig D, Goddard AW, Staib L, Stern E, Cappiello A, Woods S, Baldwin R and Charney DS (2000a) SPECT [I-123]iomazenil measurement of the benzodiazepine receptor in panic disorder. *Biol Psychiatry* **47**:96-106.

Bremner JD, Krystal JH, Southwick SM and Charney DS (1996) Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse* **23**:28-38.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL and Charney DS (2000b) Hippocampal volume reduction in major depression. *Am J Psychiatry* **157**:115-118.

Breslow MF, Fankhauser MP, Potter RL, Meredith KE, Misiaszek J and Hope DG, Jr. (1989) Role of gamma-aminobutyric acid in antipanic drug efficacy. *Am J Psychiatry* **146**:353-356.

Broadbent J and Harless WE (1999) Differential effects of GABA(A) and GABA(B) agonists on sensitization to the locomotor stimulant effects of ethanol in DBA/2 J mice. *Psychopharmacology (Berl)* **141**:197-205.

Brody DL and Yue DT (2000) Relief of G-protein inhibition of calcium channels and short-term synaptic facilitation in cultured hippocampal neurons. *J Neurosci* **20**:889-898.

Brogden RN, Speight TM and Avery GS (1974) Baclofen: a preliminary report of its pharmacological properties and therapeutic efficacy in spasticity. *Drugs* **8**:1-14.

Buda-Levin A, Wojnicki FH and Corwin RL (2005) Baclofen reduces fat intake under binge-type conditions. *Physiol Behav* **86**:176-184.

Bueno CH, Zangrossi H, Jr., Nogueira RL, Soares VP and Viana MB (2005) Panicolytic-like effect induced by the stimulation of GABAA and GABAB receptors in the dorsal periaqueductal grey of rats. *Eur J Pharmacol* **516**:239-246.

Bunney WE, Jr. and Davis JM (1965) Norepinephrine in depressive reactions. A review. *Arch Gen Psychiatry* **13**:483-494.

Burman KJ, Ige AO, White JH, Marshall FH, Pangalos MN, Emson PC, Minson JB and Llewellyn-Smith IJ (2003) GABAB receptor subunits, R1 and R2, in brainstem catecholamine and serotonin neurons. *Brain Res* **970**:35-46.

Cadoni C and Di Chiara G (1999) Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. *Neuroscience* **90**:447-455.

Calo G, Guerrini R, Rizzi A, Salvadori S and Regoli D (2000) Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br J Pharmacol* **129**:1261-1283.

Calver AR, Davies CH and Pangalos M (2002) GABA(B) receptors: from monogamy to promiscuity. *Neurosignals* **11**:299-314.

Campbell UC, Lac ST and Carroll ME (1999) Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)* **143**:209-214.

Campbell V, Berrow N and Dolphin AC (1993) GABAB receptor modulation of Ca2+ currents in rat sensory neurones by the G protein G(0): antisense oligonucleotide studies. *J Physiol* **470**:1-11.

Carai MA, Colombo G, Brunetti G, Melis S, Serra S, Vacca G, Mastinu S, Pistuddi AM, Solinas C, Cignarella G, Minardi G and Gessa GL (2001) Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. *Eur J Pharmacol* **428**:315-321.

Carlezon WA, Jr., Thome J, Olson VG, Lane-Ladd SB, Brodkin ES, Hiroi N, Duman RS, Neve RL and Nestler EJ (1998) Regulation of cocaine reward by CREB. *Science* **282**:2272-2275.

Carr GD, Fibiger HC and Phillips AG (1989) Conditioned place preference as a measure of drug reward., in *The neuropharmacological basis of reward.* (Liebman JM and J. CS eds) pp 264-319, Clarendon Press, Oxford.

Carr GD and White NM (1983) Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sci* **33**:2551-2557.

Carter LP, Wu H, Chen W, Cruz CM, Lamb RJ, Koek W, Coop A and France CP (2004) Effects of gamma-hydroxybutyrate (GHB) on schedule-controlled responding in rats: role of GHB and GABAB receptors. *J Pharmacol Exp Ther* **308**:182-188.

Cash CD (1994) Gamma-hydroxybutyrate: an overview of the pros and cons for it being a neurotransmitter and/or a useful therapeutic agent. *Neurosci Biobehav Rev* **18**:291-304.

Chalmers DT, Lopez JF, Vazquez DM, Akil H and Watson SJ (1994) Regulation of hippocampal 5-HT1A receptor gene expression by dexamethasone. *Neuropsychopharmacology* **10**:215-222.

Chamberlain SR, Blackwell AD, Fineberg NA, Robbins TW and Sahakian BJ (2005) The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. *Neurosci Biobehav Rev* **29**:399-419.

Chaperon F and Thiebot MH (1996) Effects of dopaminergic D3-receptor-preferring ligands on the acquisition of place conditioning in rats. *Behav Pharmacol* **7**:105-109.

Charles KJ, Evans ML, Robbins MJ, Calver AR, Leslie RA and Pangalos MN (2001) Comparative immunohistochemical localisation of GABA(B1a), GABA(B1b) and GABA(B2) subunits in rat brain, spinal cord and dorsal root ganglion. *Neuroscience* **106**:447-467.

Charney DS, Heninger GR and Breier A (1984) Noradrenergic function in panic anxiety. Effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Arch Gen Psychiatry* **41**:751-763.

Charney DS and Manji HK (2004) Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Sci STKE* **2004**:re5.

Charney DS, Woods SW, Goodman WK and Heninger GR (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* **92**:14-24.

Cheeta S, Ruigt G, van Proosdij J and Willner P (1997) Changes in sleep architecture following chronic mild stress. *Biol Psychiatry* **41**:419-427.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF and Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry* **50**:260-265.

Chen J, Kelz MB, Hope BT, Nakabeppu Y and Nestler EJ (1997) Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J Neurosci* **17**:4933-4941.

Chen Y, Phillips K, Minton G and Sher E (2005) GABA(B) receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *Br J Pharmacol* **144**:926-932.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M and O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* **156**:11-18.

Chin RL, Sporer KA, Cullison B, Dyer JE and Wu TD (1998) Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med* **31**:716-722.

Christie MJ, Summers RJ, Stephenson JA, Cook CJ and Beart PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. *Neuroscience* **22**:425-439.

Chu DC, Albin RL, Young AB and Penney JB (1990) Distribution and kinetics of GABAB binding sites in rat central nervous system: a quantitative autoradiographic study. *Neuroscience* **34**:341-357.

Clark JA, Mezey E, Lam AS and Bonner TI (2000) Distribution of the GABA(B) receptor subunit gb2 in rat CNS. *Brain Res* **860**:41-52.

Coffman JA and Petty F (1985) Plasma GABA levels in chronic alcoholics. *Am J Psychiatry* **142**:1204-1205.

Colby CR, Whisler K, Steffen C, Nestler EJ and Self DW (2003) Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. *J Neurosci* **23**:2488-2493.

Cole RL, Konradi C, Douglass J and Hyman SE (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* **14**:813-823.

Connor TJ, Song C, Leonard BE, Anisman H and Merali Z (1999) Stressor-induced alterations in serotonergic activity in an animal model of depression. *Neuroreport* **10**:523-528.

Contarino A, Dellu F, Koob GF, Smith GW, Lee KF, Vale W and Gold LH (1999) Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. *Brain Res* **835**:1-9.

Conti AC, Cryan JF, Dalvi A, Lucki I and Blendy JA (2002) cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J Neurosci* **22**:3262-3268.

Cooper JR, Bloom FE and Roth RH (2002) *The Biochemical Basis of Neuropharmacology*. Oxford University Press, New York.

Coppen AJ (1968) Depressed states and indolealkylamines. *Adv Pharmacol* **6**:283-291.

Corbett R, Fielding S, Cornfeldt M and Dunn RW (1991) GABAmimetic agents display anxiolytic-like effects in the social interaction and elevated plus maze procedures. *Psychopharmacology (Berl)* **104**:312-316.

Corrigall WA and Coen KM (1989) Fixed-interval schedules for drug self-administration in the rat. *Psychopharmacology (Berl)* **99**:136-139.

Corrigall WA, Coen KM, Adamson KL, Chow BL and Zhang J (2000) Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology (Berl)* **149**:107-114.

Costa AC, Stasko MR, Stoffel M and Scott-McKean JJ (2005) G-protein-gated potassium (GIRK) channels containing the GIRK2 subunit are control hubs for pharmacologically induced hypothermic responses. *J Neurosci* **25**:7801-7804.

Costa E (1989) Allosteric modulatory centers of transmitter amino acid receptors. *Neuropsychopharmacology* **2**:167-174.

Cott J, Carlsson A, Engel J and Lindqvist M (1976) Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. *Naunyn Schmiedebergs Arch Pharmacol* **295**:203-209.

Couve A, Filippov AK, Connolly CN, Bettler B, Brown DA and Moss SJ (1998) Intracellular retention of recombinant GABAB receptors. *J Biol Chem* **273**:26361-26367.

Coyle JT (2004) The GABA-glutamate connection in schizophrenia: which is the proximate cause? *Biochem Pharmacol* **68**:1507-1514.

Crawley JN (2000) *Whats wrong with my mouse? Behavioral Phenotyping of Transgenic and Knockout Mice*. Wiley-Liss, New York.

Crawley JN and Davis LG (1982) Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. *Brain Res Bull* **8**:609-612.

Crunelli V and Leresche N (1991) A role for GABAB receptors in excitation and inhibition of thalamocortical cells. *Trends Neurosci* **14**:16-21.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA and Luscher C (2004) Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat Neurosci* **7**:153-159.

Cryan JF, Bruijnzeel AW, Skjei KL and Markou A (2003a) Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. *Psychopharmacology (Berl)*.

Cryan JF, Dalvi A, Jin SH, Hirsch BR, Lucki I and Thomas SA (2001) Use of dopamine-beta-hydroxylase-deficient mice to determine the role of norepinephrine in the mechanism of action of antidepressant drugs. *J Pharmacol Exp Ther* **298**:651-657.

Cryan JF and Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov* **4**:775-790.

Cryan JF, Hoyer D and Markou A (2003b) Withdrawal from chronic amphetamine induces Depressive-Like behavioral effects in rodents. *Biol Psychiatry* **54**:49-58.

Cryan JF and Kaupmann K (2005) Don't worry 'B' happy! a role for GABA(B) receptors in anxiety and depression. *Trends Pharmacol Sci* **26**:36-43.

Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, Bettler B, Kaupmann K and Spooren WP (2004) Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J Pharmacol Exp Ther* **310**:952-963.

Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ and Van Der Putten H (2003c) Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. *Eur J Neurosci* **17**:2409-2417.

Cryan JF and Leonard BE (2000) 5-HT1A and beyond: the role of serotonin and its receptors in depression and the antidepressant response. *Hum Psychopharmacol* **15**:113-135.

Cryan JF, Markou A and Lucki I (2002a) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **23**:238-245.

Cryan JF, McGrath C, Leonard BE and Norman TR (1998) Combining pindolol and paroxetine in an animal model of chronic antidepressant action--can early onset of action be detected? *Eur J Pharmacol* **352**:23-28.

Cryan JF, McGrath C, Leonard BE and Norman TR (1999) Onset of the effects of the 5-HT1A antagonist, WAY-100635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. *Pharmacol Biochem Behav* **63**:333-338.

Cryan JF and Mombereau C (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* **9**:326-357.

Cryan JF, Mombereau C and Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* **29**:571-625.

Cryan JF, Page ME and Lucki I (2002b) Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test. *Eur J Pharmacol* **436**:197-205.

Curran EJ, Akil H and Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. *Neurochem Res* **21**:1425-1435.

Dalia A and Wallace LJ (1995) Amphetamine induction of c-fos in the nucleus accumbens is not inhibited by glutamate antagonists. *Brain Res* **694**:299-307.

Dalvi A and Rodgers RJ (1996) GABAergic influences on plus-maze behaviour in mice. *Psychopharmacology (Berl)* **128**:380-397.

Dascal N (2001) Ion-channel regulation by G proteins. *Trends Endocrinol Metab* **12**:391-398.

Davis LL, Trivedi M, Choate A, Kramer GL and Petty F (1997) Growth hormone response to the GABAB agonist baclofen in major depressive disorder. *Psychoneuroendocrinology* **22**:129-140.

Davis M, Falls WA, Campeau S and Kim M (1993) Fear-potentiated startle: a neural and pharmacological analysis. *Behav Brain Res* **58**:175-198.

Dawson GR and Tricklebank MD (1995) Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends Pharmacol Sci* **16**:33-36.

de Kloet ER, Joels M and Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* **6**:463-475.

Dennis T, Beauchemin V and Lavoie N (1993) Differential effects of olfactory bulbectomy on GABAA and GABAB receptors in the rat brain. *Pharmacol Biochem Behav* **46**:77-82.

Detke MJ, Rickels M and Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)* **121**:66-72.

Di Ciano P and Everitt BJ (2003) The GABA(B) receptor agonist baclofen attenuates cocaine- and heroin-seeking behavior by rats. *Neuropsychopharmacology* **28**:510-518.

Dixon AK (1998) Ethological strategies for defence in animals and humans: their role in some psychiatric disorders. *Br J Med Psychol* **71 (Pt 4)**:417-445.

Dixon AK, Huber C and Lowe DA (1994) Clozapine promotes approach-oriented behavior in male mice. *J Clin Psychiatry* **55 Suppl B**:4-7.

Dobrovitsky V, Pimentel P, Duarte A, Froestl W, Stellar JR and Trzcinska M (2002) CGP 44532, a GABAB receptor agonist, is hedonically neutral and reduces cocaine-induced enhancement of reward. *Neuropharmacology* **42**:626-632.

Doherty MD and Gratton A (1999) Effects of medial prefrontal cortical injections of GABA receptor agonists and antagonists on the local and nucleus accumbens dopamine responses to stress. *Synapse* **32**:288-300.

Donny EC, Caggiula AR, Mielke MM, Jacobs KS, Rose C and Sved AF (1998) Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berl)* **136**:83-90.

Dowlatshahi D, MacQueen GM, Wang JF, Reiach JS and Young LT (1999) G Protein-coupled cyclic AMP signaling in postmortem brain of subjects with mood disorders: effects of diagnosis, suicide, and treatment at the time of death. *J Neurochem* **73**:1121-1126.

Dowlatshahi D, MacQueen GM, Wang JF and Young LT (1998) Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet* **352**:1754-1755.

Drake RG, Davis LL, Cates ME, Jewell ME, Ambrose SM and Lowe JS (2003) Baclofen treatment for chronic posttraumatic stress disorder. *Ann Pharmacother* **37**:1177-1181.

Drevets WC (2000) Neuroimaging studies of mood disorders. *Biol Psychiatry* **48**:813-829.

Ducottet C, Griebel G and Belzung C (2003) Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. *Prog Neuropsychopharmacol Biol Psychiatry* **27**:625-631.

Dulawa SC and Hen R (2005) Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev* **29**:771-783.

Dulawa SC, Holick KA, Gundersen B and Hen R (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* **29**:1321-1330.

Dunham N and Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm Ass*:208-209.

Dunn AL and Crnic LS (1993) Repeated injections of interferon-alpha A/D in Balb/c mice: behavioral effects. *Brain Behav Immun* **7**:104-111.

Dupuis DS, Relkovic D, Cryan JF and K. K (2004) MAPPING OF THE BINDING SITE OF THE GABAB POSITIVE MODULATOR GS39783 BY USE OF INTERSPECIES RAT/DROSOPHILA SUBUNIT COMBINATIONS, in *Society for Neuroscience*, San Diego.

Durkin MM, Gunwaldsen CA, Borowsky B, Jones KA and Branchek TA (1999) An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. *Brain Res Mol Brain Res* **71**:185-200.

Dutar P and Nicoll RA (1988) Pre- and postsynaptic GABAB receptors in the hippocampus have different pharmacological properties. *Neuron* **1**:585-591.

Eder M, Rammes G, Zieglgansberger W and Dodt HU (2001) GABA(A) and GABA(B) receptors on neocortical neurons are differentially distributed. *Eur J Neurosci* **13**:1065-1069.

El Hage W, Griebel G and Belzung C (2005) Long-term impaired memory following predatory stress in mice. *Physiol Behav*.

El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, Costentin J, Adrien J and Vaugeois JM (2003) Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. *Proc Natl Acad Sci U S A* **100**:6227-6232.

Epping-Jordan MP, Watkins SS, Koob GF and Markou A (1998) Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* **393**:76-79.

Epstein DH and Preston KL (2003) The reinstatement model and relapse prevention: a clinical perspective. *Psychopharmacology (Berl)* **168**:31-41.

Erhardt S, Mathe JM, Chergui K, Engberg G and Svensson TH (2002) GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. *Naunyn Schmiedebergs Arch Pharmacol* **365**:173-180.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N and Tobin AJ (1991) Two genes encode distinct glutamate decarboxylases. *Neuron* **7**:91-100.

Estape N and Steckler T (2002) Cholinergic blockade impairs performance in operant DNMTP in two inbred strains of mice. *Pharmacol Biochem Behav* **72**:319-334.

Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M and Martres MP (2000) Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci* **12**:2299-2310.

Fadda P, Scherma M, Fresu A, Collu M and Fratta W (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* **50**:1-6.

Feighner JP and Boyer WF (1989) Serotonin-1A anxiolytics: an overview. *Psychopathology* **22 Suppl 1**:21-26.

File SE and Andrews N (1993) Benzodiazepine withdrawal: behavioural pharmacology and neurochemical changes. *Biochem Soc Symp* **59**:97-106.

File SE and Hyde JR (1978) Can social interaction be used to measure anxiety? *Br J Pharmacol* **62**:19-24.

File SE, Zharkovsky A and Gulati K (1991) Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology* **30**:183-190.

File SE, Zharkovsky A and Hitchcott PK (1992) Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry* **16**:87-93.

Finn DA, Rutledge-Gorman MT and Crabbe JC (2003) Genetic animal models of anxiety. *Neurogenetics* **4**:109-135.

Flannery BA, Garbutt JC, Cody MW, Renn W, Grace K, Osborne M, Crosby K, Morreale M and Trivette A (2004) Baclofen for alcohol dependence: a preliminary open-label study. *Alcohol Clin Exp Res* **28**:1517-1523.

Florey E (1954) An inhibitory and an excitatory factor of mammalian central nervous system, and their action of a single sensory neuron. *Arch Int Physiol* **62**:33-53.

Flugge G (1995) Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. *J Neurosci* **15**:7132-7140.

Foltin RW (2005) Baclofen decreases feeding in non-human primates. *Pharmacol Biochem Behav*.

Forget B, Hamon M and Thiebot MH (2005) Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. *Psychopharmacology (Berl)*:1-13.

Frazer A (1997) Pharmacology of antidepressants. *J Clin Psychopharmacol* **17 Suppl 1**:2S-18S.

Freed WJ, Crump S and Jeste DV (1984) Genetic effects on PCP-induced stimulation in recombinant inbred strains of mice. *Pharmacol Biochem Behav* **21**:159-162.

Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D and Mohler H (1999) GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *Eur J Neurosci* **11**:761-768.

Frodl T, Meisenzahl E, Zetzsche T, Bottlender R, Born C, Groll C, Jager M, Leinsinger G, Hahn K and Moller HJ (2002) Enlargement of the amygdala in patients with a first episode of major depression. *Biol Psychiatry* **51**:708-714.

Frodl T, Meisenzahl EM, Zetzsche T, Born C, Jager M, Groll C, Bottlender R, Leinsinger G and Moller HJ (2003) Larger amygdala volumes in first depressive episode as compared to recurrent major depression and healthy control subjects. *Biol Psychiatry* **53**:338-344.

Froestl W, Bettler B, Bittiger H, Heid J, Kaupmann K, Mickel SJ and Strub D (1999) Ligands for the isolation of GABA(B) receptors [corrected]. *Neuropharmacology* **38**:1641-1646.

Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S, Mondadori CG and Pearlman R (2004) SGS742: the first GABA(B) receptor antagonist in clinical trials. *Biochem Pharmacol* **68**:1479-1487.

Froestl W and Mickel S (1997) Chemistry of GABAB modulators, in *The GABA Receptors* (Enna S and Bowery NG eds) pp pp 271-296, Humana

Press., Totowa, NJ.

Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J, Olpe HR and et al. (1995) Phosphinic acid analogues of GABA. 1. New potent and selective GABAB agonists. *J Med Chem* **38**:3297-3312.

Froger N, Palazzo E, Boni C, Hanoun N, Saurini F, Joubert C, Dutriez-Casteloot I, Enache M, Maccari S, Barden N, Cohen-Salmon C, Hamon M and Lanfumey L (2004) Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. *J Neurosci* **24**:2787-2796.

Frosini M, Valoti M and Sgaragli G (2004) Changes in rectal temperature and ECoG spectral power of sensorimotor cortex elicited in conscious rabbits by i.c.v. injection of GABA, GABA(A) and GABA(B) agonists and antagonists. *Br J Pharmacol* **141**:152-162.

Fudala PJ, Teoh KW and Iwamoto ET (1985) Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol Biochem Behav* **22**:237-241.

Gallimberti L, Schifano F, Forza G, Miconi L and Ferrara SD (1994) Clinical efficacy of gamma-hydroxybutyric acid in treatment of opiate withdrawal. *Eur Arch Psychiatry Clin Neurosci* **244**:113-114.

Galvez T, Parmentier ML, Joly C, Malitschek B, Kaupmann K, Kuhn R, Bittiger H, Froestl W, Bettler B and Pin JP (1999) Mutagenesis and modeling of the GABAB receptor extracellular domain support a venus flytrap mechanism for ligand binding. *J Biol Chem* **274**:13362-13369.

Gambarana C, Scheggi S, Tagliamonte A, Tolu P and De Montis MG (2001) Animal models for the study of antidepressant activity. *Brain Res Brain Res Protoc* **7**:11-20.

Gardner EL, Schiffer WK, Horan BA, Highfield D, Dewey SL, Brodie JD and Ashby CR, Jr. (2002) Gamma-vinyl GABA, an irreversible inhibitor of GABA transaminase, alters the acquisition and expression of cocaine-induced sensitization in male rats. *Synapse* **46**:240-250.

Gartside SE, Umbers V, Hajos M and Sharp T (1995) Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* **115**:1064-1070.

Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Muller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jaton AL, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy JM, Luthi A, Kaupmann K and Bettler B (2004) Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *J Neurosci* **24**:6086-6097.

Gavioli EC, Marzola G, Guerrini R, Bertorelli R, Zucchini S, De Lima TC, Rae GA, Salvadori S, Regoli D and Calo G (2003) Blockade of nociceptin/orphanin FQ-NOP receptor signalling produces antidepressant-like effects: pharmacological and genetic evidences from the mouse forced swimming test. *Eur J Neurosci* **17**:1987-1990.

Gavioli EC, Vaughan CW, Marzola G, Guerrini R, Mitchell VA, Zucchini S, De Lima TC, Rae GA, Salvadori S, Regoli D and Calo G (2004) Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. *Naunyn Schmiedebergs Arch Pharmacol* **369**:547-553.

Geller I, Kulak JT, Jr. and Seifter J (1962) The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. *Psychopharmacologia* **3**:374-385.

Gerber U and Gahwiler BH (1994) GABAB and adenosine receptors mediate enhancement of the K+ current, IAHP, by reducing adenylyl cyclase activity in rat CA3 hippocampal neurons. *J Neurophysiol* **72**:2360-2367.

Geyer MA and Markou A (1995) Animal models of psychiatric disorders., in *Psychopharmacology: The Fourth Generation of Progress.* (Bloom FE and Kupfer DJ eds) pp 787-798, Raven Press.

Geyer MA and Markou A (2000) The role of preclinical models in the development of psychotropic drugs., in *Psychopharmacology: The Fifth Generation of Progress.* (Bloom FE and Kupfer DJ eds), Raven, New York.

Gilbert P and Allan S (1998) The role of defeat and entrapment (arrested flight) in depression: an exploration of an evolutionary view. *Psychol Med* **28**:585-598.

Gilliss B, Malanga CJ, Pieper JO and Carlezon WA, Jr. (2002) Cocaine and SKF-82958 potentiate brain stimulation reward in Swiss-Webster mice. *Psychopharmacology (Berl)* **163**:238-248.

Giorgetti M, Hotsenpiller G, Froestl W and Wolf ME (2002) In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. *Neuroscience* **109**:585-595.

Goddard AW, Mason GF, Almai A, Rothman DL, Behar KL, Petroff OA, Charney DS and Krystal JH (2001) Reductions in occipital cortex GABA levels in panic disorder detected with 1h-magnetic resonance spectroscopy. *Arch Gen Psychiatry* **58**:556-561.

Gold BI, Bowers MB, Jr., Roth RH and Sweeney DW (1980) GABA levels in CSF of patients with psychiatric disorders. *Am J Psychiatry* **137**:362-364.

Goldberg MR, Hollister AS and Robertson D (1983) Influence of yohimbine on blood pressure, autonomic reflexes, and plasma catecholamines in humans. *Hypertension* **5**:772-778.

Goldberg SR, Risner ME, Stolerman IP, Reavill C and Garcha HS (1989) Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. *Psychopharmacology (Berl)* **97**:295-302.

Gong W, Neill D and Justice JB, Jr. (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. *Brain Res* **754**:103-112.

Gong W, Neill DB and Justice JB, Jr. (1998) GABAergic modulation of ventral pallidal dopamine release studied by in vivo microdialysis in the freely moving rat. *Synapse* **29**:406-412.

Goodwin GM, De Souza RJ and Green AR (1985) Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. *Nature* **317**:531-533.

Gorman JM, Liebowitz MR, Fyer AJ, Goetz D, Campeas RB, Fyer MR, Davies SO and Klein DF (1987) An open trial of fluoxetine in the treatment of panic attacks. *J Clin Psychopharmacol* **7**:329-332.

Grabarits F, Lal H and Chessick RD (1966) Effects of cocaine on brain noradrenaline in relation to toxicity and convulsions in mice. *J Pharm Pharmacol* **18**:131-132.

Graeff FG, Guimaraes FS, De Andrade TG and Deakin JF (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav* **54**:129-141.

Gray JA, Goodwin GM, Heal DJ and Green AR (1987) Hypothermia induced by baclofen, a possible index of GABAB receptor function in mice, is enhanced by antidepressant drugs and ECS. *Br J Pharmacol* **92**:863-870.

Gray JA and Green AR (1987) Increased GABAB receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks. *Br J Pharmacol* **92**:357-362.

Graybiel AM, Moratalla R and Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc Natl Acad Sci U S A* **87**:6912-6916.

Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, Ballenger JC and Fyer AJ (1999) The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry* **60**:427-435.

Greengard P, Allen PB and Nairn AC (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* **23**:435-447.

Greif GJ, Sodickson DL, Bean BP, Neer EJ and Mende U (2000) Altered regulation of potassium and calcium channels by GABA(B) and adenosine receptors in hippocampal neurons from mice lacking Galpha(o). *J Neurophysiol* **83**:1010-1018.

Griebel G, Belzung C, Perrault G and Sanger DJ (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology (Berl)* **148**:164-170.

Griebel G, Simiand J, Serradeil-Le Gal C, Wagnon J, Pascal M, Scatton B, Maffrand JP and Soubrie P (2002a) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc Natl Acad Sci U S A* **99**:6370-6375.

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, Geslin M, Scatton B, Maffrand JP and Soubrie P (2002b) 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther* **301**:333-345.

Grippo AJ, Beltz TG and Johnson AK (2003) Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav* **78**:703-710.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S and Hen R (2002) Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**:396-400.

Guidotti A, Baraldi M, Leon A and Costa E (1980) Benzodiazepines: a tool to explore the biochemical and neurophysiological basis of anxiety. *Fed Proc* **39**:3039-3042.

Guitart X, Thompson MA, Mirante CK, Greenberg ME and Nestler EJ (1992) Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. *J Neurochem* **58**:1168-1171.

Guo TZ, Reid K, Davies MF, Nacif-Coelho C, Rabin BC, Gonzalez F and Maze M (1998) Chronic desipramine treatment desensitizes the rat to anesthetic and antinociceptive effects of the alpha2-adrenergic agonist dexmedetomidine. *Anesthesiology* **88**:1634-1642.

Haefely W, Kulcsar A and Mohler H (1975) Possible involvement of GABA in the central actions of benzodiazepines. *Psychopharmacol Bull* **11**:58-59.

Haller C, Casanova E, Muller M, Vacher CM, Vigot R, Doll T, Barbieri S, Gassmann M and Bettler B (2004) Floxed allele for conditional inactivation of the GABAB(1) gene. *Genesis* **40**:125-130.

Hansen HH, Sanchez C and Meier E (1997) Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? *J Pharmacol Exp Ther* **283**:1333-1341.

Harayama N, Shibuya I, Tanaka K, Kabashima N, Ueta Y and Yamashita H (1998) Inhibition of N- and P/Q-type calcium channels by postsynaptic GABAB receptor activation in rat supraoptic neurones. *J Physiol* **509 (Pt 2)**:371-383.

Harkin A, Houlihan DD and Kelly JP (2002) Reduction in preference for saccharin by repeated unpredictable stress in mice and its prevention by imipramine. *J Psychopharmacol* **16**:115-123.

Harrison AA, Gasparini F and Markou A (2002) Nicotine potentiation of brain stimulation reward reversed by DH beta E and SCH 23390, but not by eticlopride, LY 314582 or MPEP in rats. *Psychopharmacology (Berl)* **160**:56-66.

Harrison AA, Liem YT and Markou A (2001) Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. *Neuropsychopharmacology* **25**:55-71.

Harrison NL (1990) On the presynaptic action of baclofen at inhibitory synapses between cultured rat hippocampal neurones. *J Physiol* **422**:433-446.

Hascoet M and Bourin M (1998) A new approach to the light/dark test procedure in mice. *Pharmacol Biochem Behav* **60**:645-653.

Hashimoto T and Kuriyama K (1997) In vivo evidence that GABA(B) receptors are negatively coupled to adenylate cyclase in rat striatum. *J Neurochem* **69**:365-370.

Hausler A, Monnet G and Peter O (1993) Involvement of GABAB receptors in the regulation of the hypothalamo-pituitary-adrenocortical (HPA) axis in rats. *J Steroid Biochem Mol Biol* **46**:767-771.

Hawrot E, Xiao Y, Shi QL, Norman D, Kirkitadze M and Barlow PN (1998) Demonstration of a tandem pair of complement protein modules in GABA(B) receptor 1a. *FEBS Lett* **432**:103-108.

Hayashi T (1959) The inhibitory action of beta-hydroxy-gamma-aminobutyric acid upon the seizure following stimulation of the motor cortex of the dog. *J Physiol* **145**:570-578.

Heese K, Otten U, Mathivet P, Raiteri M, Marescaux C and Bernasconi R (2000) GABA(B) receptor antagonists elevate both mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats. *Neuropharmacology* **39**:449-462.

Heidbreder CA and Hagan JJ (2005) Novel pharmacotherapeutic approaches for the treatment of drug addiction and craving. *Curr Opin Pharmacol* **5**:107-118.

Hendry SH, Schwark HD, Jones EG and Yan J (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J Neurosci* **7**:1503-1519.

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T and Catterall WA (1996) Modulation of Ca2+ channels by G-protein beta gamma subunits. *Nature* **380**:258-262.

Hetherington HP, Pan JW, Chu WJ, Mason GF and Newcomer BR (1997) Biological and clinical MRS at ultra-high field. *NMR Biomed* **10**:360-371.

Higgins GA, Bradbury AJ, Jones BJ and Oakley NR (1988) Behavioural and biochemical consequences following activation of 5HT1-like and GABA receptors in the dorsal raphe nucleus of the rat. *Neuropharmacology* **27**:993-1001.

Hill DR and Bowery NG (1981) 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature* **290**:149-152.

Hinderer SR (1990) The supraspinal anxiolytic effect of baclofen for spasticity reduction. *Am J Phys Med Rehabil* **69**:254-258.

Hiroi N, Brown JR, Haile CN, Ye H, Greenberg ME and Nestler EJ (1997) FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc Natl Acad Sci U S A* **94**:10397-10402.

Hiroi N, Fienberg AA, Haile CN, Alburges M, Hanson GR, Greengard P and Nestler EJ (1999) Neuronal and behavioural abnormalities in striatal function in DARPP-32-mutant mice. *Eur J Neurosci* **11**:1114-1118.

Holmes A (2001) Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neurosci Biobehav Rev* **25**:261-273.

Holmes A, Heilig M, Rupniak NM, Steckler T and Griebel G (2003a) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci* **24**:580-588.

Holmes A, Parmigiani S, Ferrari PF, Palanza P and Rodgers RJ (2000) Behavioral profile of wild mice in the elevated plus-maze test for anxiety. *Physiol Behav* **71**:509-516.

Holmes A, Yang RJ and Crawley JN (2002) Evaluation of an anxiety-related phenotype in galanin overexpressing transgenic mice. *J Mol Neurosci* **18**:151-165.

Holmes A, Yang RJ, Lesch KP, Crawley JN and Murphy DL (2003b) Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology* **28**:2077-2088.

Holsboer F, Bender W, Benkert O, Klein HE and Schmauss M (1980) Diagnostic value of dexamethasone suppression test in depression. *Lancet* **2**:706.

Honig A, Bartlett JR, Bouras N and Bridges PK (1988) Amino acid levels in depression: a preliminary investigation. *J Psychiatr Res* **22**:159-164.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS and Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* **13**:1235-1244.

Hotsenpiller G and Wolf ME (2003) Baclofen attenuates conditioned locomotion to cues associated with cocaine administration and stabilizes extracellular glutamate levels in rat nucleus accumbens. *Neuroscience* **118**:123-134.

Hozumi S, Nakagawasai O, Tan-No K, Niijima F, Yamadera F, Murata A, Arai Y, Yasuhara H and Tadano T (2003) Characteristics of changes in cholinergic function and impairment of learning and memory-related behavior induced by olfactory bulbectomy. *Behav Brain Res* **138**:9-15.

Hu XT, Ford K and White FJ (2005) Repeated cocaine administration decreases calcineurin (PP2B) but enhances DARPP-32 modulation of sodium currents in rat nucleus accumbens neurons. *Neuropsychopharmacology* **30**:916-926.

Humeniuk RE, Ong J, Kerr DI and White JM (1995) Characterization of GABAB ligands in vivo. *Gen Pharmacol* **26**:417-424.

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. *Nature* **380**:255-258.

Imperato A, Mulas A and Di Chiara G (1987) The D-1 antagonist SCH 23390 stimulates while the D-1 agonist SKF 38393 fails to affect dopamine release in the dorsal caudate of freely moving rats. *Eur J Pharmacol* **142**:177-181.

Imperato A, Tanda G, Frau R and Di Chiara G (1988) Pharmacological profile of dopamine receptor agonists as studied by brain dialysis in behaving rats. *J Pharmacol Exp Ther* **245**:257-264.

Innis RB, Nestler EJ and Aghajanian GK (1988) Evidence for G protein mediation of serotonin- and GABAB-induced hyperpolarization of rat dorsal raphe neurons. *Brain Res* **459**:27-36.

Inoue T, Tsuchiya K and Koyama T (1994) Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* **49**:911-920.

Ito Y, Ishige K, Zaitsu E, Anzai K and Fukuda H (1995) gamma-Hydroxybutyric acid increases intracellular Ca2+ concentration and nuclear cyclic AMP-responsive element- and activator protein 1 DNA-binding activities through GABAB receptor in cultured cerebellar granule cells. *J Neurochem* **65**:75-83.

Izumi J, Washizuka M, Hayashi-Kuwabara Y, Yoshinaga K, Tanaka Y, Ikeda Y, Kiuchi Y and Oguchi K (1996) An attenuated alpha-1 potentiation of beta adrenoceptor-stimulated cyclic AMP formation after repeated saline injections in Fischer 344 strain rats. *Life Sci* **59**:33-42.

Jackson HC and Nutt DJ (1991) Inhibition of baclofen-induced hypothermia in mice by the novel GABAB antagonist CGP 35348. *Neuropharmacology* **30**:535-538.

Jacobs EH, Smit AB, de Vries TJ and Schoffelmeer AN (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci* **24**:566-573.

Jacobson LH and Cryan JF (2005) Differential sensitivity to the motor and hypothermic effects of the GABA B receptor agonist baclofen in various mouse strains. *Psychopharmacology (Berl)* **179**:688-699.

Jacobson LH, Kaupmann K, Bettler B and Cryan JF (2005) Differential influence of GABAB(1) subunit isoforms on baseline but not GABAB agonist-induced behavioural changes in mice., in *Society for Neuroscience*, Washington, D.C.

Jefferys D and Funder J (1996) Nitric oxide modulates retention of immobility in the forced swimming test in rats. *Eur J Pharmacol* **295**:131-135.

Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roever S, Kolczewski S, Adam G and Kilpatrick G (2000) A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: anxiolytic profile in the rat. *Proc Natl Acad Sci U S A* **97**:4938-4943.

Jesberger JA and Richardson JS (1988) Brain output dysregulation induced by olfactory bulbectomy: an approximation in the rat of major depressive disorder in humans? *Int J Neurosci* **38**:241-265.

Johnson SW and North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol* **450**:455-468.

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA and Gerald C (1998) GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* **396**:674-679.

Jones N, Duxon MS and King SM (2002a) Ethopharmacological analysis of the unstable elevated exposed plus maze, a novel model of extreme anxiety: predictive validity and sensitivity to anxiogenic agents. *Psychopharmacology (Berl)* **161**:314-323.

Jones N, King SM and Duxon MS (2002b) Further evidence for the predictive validity of the unstable elevated exposed plus-maze, a behavioural model of extreme anxiety in rats: differential effects of fluoxetine and chlordiazepoxide. *Behav Pharmacol* **13**:525-535.

Jongsma ME, Cremers TIF, Rea K, Bosker FJ, Hogg S and Westerink BHC (2004) POTENTIATION OF SSRI-INDUCED ELEVATION OF 5-NT BY CO-ADMINISTRATION OF GABA-B ANTAGONISTS, in *Society for Neuroscience*, San diego.

Kafkafi N, Pagis M, Lipkind D, Mayo CL, Bemjamini Y, Golani I and Elmer GI (2003) Darting behavior: a quantitative movement pattern designed for discrimination and replicability in mouse locomotor behavior. *Behav Brain Res* **142**:193-205.

Kalivas PW, Duffy P and Eberhardt H (1990) Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J Pharmacol Exp Ther* **253**:858-866.

Kalivas PW, Sorg BA and Hooks MS (1993) The pharmacology and neural circuitry of sensitization to psychostimulants. *Behav Pharmacol* **4**:315-334.

Kalivas PW and Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev* **16**:223-244.

Kalivas PW and Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* **162**:1403-1413.

Kano T, Suzuki Y, Shibuya M, Kiuchi K and Hagiwara M (1995) Cocaine-induced CREB phosphorylation and c-Fos expression are suppressed in Parkinsonism model mice. *Neuroreport* **6**:2197-2200.

Kaplan GB, Leite-Morris KA, Joshi M, Shoeb MH and Carey RJ (2003) Baclofen inhibits opiate-induced conditioned place preference and associated induction of Fos in cortical and limbic regions. *Brain Res* **987**:122-125.

Karlsson G, Pozza M and Olpe HR (1988) Phaclofen: a GABAB blocker reduces long-duration inhibition in the neocortex. *Eur J Pharmacol* **148**:485-486.

Karolyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, Seong E, Camper SA and Seasholtz AF (1999) Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. *Proc Natl Acad Sci U S A* **96**:11595-11600.

Kasture SB, Mandhane SN and Chopde CT (1996) Baclofen-induced catatonia: modification by serotonergic agents. *Neuropharmacology* **35**:595-598.

Kaufman DL, Houser CR and Tobin AJ (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J Neurochem* **56**:720-723.

Kaupmann K, Cryan J, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Bräuner-Osborne H, Waldmeier P and Bettler B (2003) Specific -Hydroxybutyrate (GHB) binding sites but loss of pharmacological effects of GHB in GABAB(1) deficient mice. *Eur J Neurosci*.

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386**:239-246.

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A and Bettler B (1998a) GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396**:683-687.

Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, Froestl W, Leonhard S, Pfaff T, Karschin A and Bettler B (1998b) Human gamma-aminobutyric acid type B

receptors are differentially expressed and regulate inwardly rectifying K+ channels. *Proc Natl Acad Sci U S A* **95**:14991-14996.

Kawahara Y, Kawahara H and Westerink BH (1999) Tonic regulation of the activity of noradrenergic neurons in the locus coeruleus of the conscious rat studied by dual-probe microdialysis. *Brain Res* **823**:42-48.

Ke Y, Cohen BM, Bang JY, Yang M and Renshaw PF (2000) Assessment of GABA concentration in human brain using two-dimensional proton magnetic resonance spectroscopy. *Psychiatry Res* **100**:169-178.

Kelly JP, Wrynn AS and Leonard BE (1997) The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* **74**:299-316.

Kelly PH, Seviour PW and Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res* **94**:507-522.

Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR and Nestler EJ (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* **401**:272-276.

Kelz MB and Nestler EJ (2000) deltaFosB: a molecular switch underlying long-term neural plasticity. *Curr Opin Neurol* **13**:715-720.

Kemmling AK, Rubio MC and Balerio GN (2002) Baclofen prevents morphine withdrawal irrespective of seasonal variation. *Behav Pharmacol* **13**:87-92.

Kenny PJ, Boutrel B, Gasparini F, Koob GF and Markou A (2005) Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)* **179**:247-254.

Kerr DI and Ong J (1995) GABAB receptors. *Pharmacol Ther* **67**:187-246.

Kerr DI, Ong J, Johnston GA, Abbenante J and Prager RH (1988) 2-Hydroxy-saclofen: an improved antagonist at central and peripheral GABAB receptors. *Neurosci Lett* **92**:92-96.

Kerr DI, Ong J, Prager RH, Gynther BD and Curtis DR (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res* **405**:150-154.

Kerr DI, Ong J, Puspawati NM and Prager RH (2002) Arylalkylamines are a novel class of positive allosteric modulators at GABA(B) receptors in rat neocortex. *Eur J Pharmacol* **451**:69-77.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE and Wang PS (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *Jama* **289**:3095-3105.

Ketelaars CE, Bollen EL, Rigter H and Bruinvels J (1988) GABA-B receptor activation and conflict behaviour. *Life Sci* **42**:933-942.

Ketelaars CE and Bruinvels J (1989) The anti-conflict effect of cyproheptadine is not mediated by its 5-hydroxytryptamine antagonistic property. *Life Sci* **44**:1743-1749.

Kilts CD (2001) Imaging the roles of the amygdala in drug addiction. *Psychopharmacol Bull* **35**:84-94.

Kim S, Lee S, Ryu S, Suk J and Park C (2002) Comparative analysis of the anxiety-related behaviors in four inbred mice. *Behav Processes* **60**:181-190.

King SM (1999a) Escape-related behaviours in an unstable elevated and exposed environment. I. A new behavioural model of extreme anxiety. *Behav Brain Res* **98**:113-126.

King SM (1999b) Escape-related behaviours in an unstable, elevated and exposed environment. II. Long-term sensitization after repetitive electrical stimulation of the rodent midbrain defence system. *Behav Brain Res* **98**:127-142.

Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G and Ordway GA (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. *J Neurosci* **17**:8451-8458.

Kniazeff J, Galvez T, Labesse G and Pin JP (2002) No ligand binding in the GB2 subunit of the GABA(B) receptor is required for activation and allosteric interaction between the subunits. *J Neurosci* **22**:7352-7361.

Koenig JI, Kirkpatrick B and Lee P (2002) Glucocorticoid hormones and early brain development in schizophrenia. *Neuropsychopharmacology* **27**:309-318.

Kokkinidis L, Zacharko RM and Anisman H (1986) Amphetamine withdrawal: a behavioral evaluation. *Life Sci* **38**:1617-1623.

Koob GF (1992) Neural mechanisms of drug reinforcement. *Ann N Y Acad Sci* **654**:171-191.

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. *Neuron* **16**:893-896.

Kornetsky C (2004) Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. *Neurosci Biobehav Rev* **27**:777-786.

Kram ML, Kramer GL, Steciuk M, Ronan PJ and Petty F (2000) Effects of learned helplessness on brain GABA receptors. *Neurosci Res* **38**:193-198.

Kravitz EA, Kuffler SW, Potter DD and Vangelder NM (1963) Gamma-Aminobutyric Acid and Other Blocking Compounds in Crustacea. Ii. Peripheral Nervous System. *J Neurophysiol* **26**:729-738.

Kreibich AS and Blendy JA (2004) cAMP response element-binding protein is required for stress but not cocaine-induced reinstatement. *J Neurosci* **24**:6686-6692.

Krnjevic K (1974) Chemical nature of synaptic transmission in vertebrates. *Physiol Rev* **54**:418-540.

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A and Mason GF (2002) Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry* **7 Suppl 1**:S71-80.

Kubota H, Katsurabayashi S, Moorhouse AJ, Murakami N, Koga H and Akaike N (2003) GABAB receptor transduction mechanisms, and cross-talk between protein kinases A and C, in GABAergic terminals synapsing onto neurons of the rat nucleus basalis of Meynert. *J Physiol* **551**:263-276.

Kuczenski R, Segal DS, Cho AK and Melega W (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci* **15**:1308-1317.

Kuffler SW and Edwards C (1958) Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J Neurophysiol* **21**:589-610.

Kuhn R (1957) [Treatment of depressive states with an iminodibenzyl derivative (G 22355).]. *Schweiz Med Wochenschr* **87**:1135-1140.

Kulik A, Nakadate K, Nyiri G, Notomi T, Malitschek B, Bettler B and Shigemoto R (2002) Distinct localization of GABA(B) receptors relative to synaptic sites in the rat cerebellum and ventrobasal thalamus. *Eur J Neurosci* **15**:291-307.

Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A and Kornau HC (1999) Role of heteromer formation in GABAB receptor function. *Science* **283**:74-77.

Kuroda Y, Watanabe Y, Albeck DS, Hastings NB and McEwen BS (1994) Effects of adrenalectomy and type I or type II glucocorticoid receptor activation on 5-HT1A and 5-HT2 receptor binding and 5-HT transporter mRNA expression in rat brain. *Brain Res* **648**:157-161.

Lacaille JC (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampal slices in vitro. *J Neurophysiol* **66**:1441-1454.

Lacey MG, Mercuri NB and North RA (1988) On the potassium conductance increase activated by GABAB and dopamine D2 receptors in rat substantia nigra neurones. *J Physiol* **401**:437-453.

Le Maitre E, Vilpoux C, Costentin J and Leroux-Nicollet I (2005) Opioid receptor-like 1 (NOP) receptors in the rat dorsal raphe nucleus: evidence for localization on serotoninergic neurons and functional adaptation after 5,7-dihydroxytryptamine lesion. *J Neurosci Res* **81**:488-496.

Le Poul E, Boni C, Hanoun N, Laporte AM, Laaris N, Chauveau J, Hamon M and Lanfumey L (2000) Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. *Neuropharmacology* **39**:110-122.

Lecci A, Borsini F, Volterra G and Meli A (1990) Pharmacological validation of a novel animal model of anticipatory anxiety in mice. *Psychopharmacology (Berl)* **101**:255-261.

Leitch MM, Ingram CD, Young AH, McQuade R and Gartside SE (2003) Flattening the corticosterone rhythm attenuates 5-HT1A autoreceptor function in the rat: relevance for depression. *Neuropsychopharmacology* **28**:119-125.

Leite-Morris KA, Fukudome EY, Shoeb MH and Kaplan GB (2004) GABA(B) receptor activation in the ventral tegmental area inhibits the acquisition and expression of opiate-induced motor sensitization. *J Pharmacol Exp Ther* **308**:667-678.

Leith NJ and Barrett RJ (1976) Amphetamine and the reward system: evidence for tolerance and post-drug depression. *Psychopharmacologia* **46**:19-25.

Lepicard EM, Joubert C, Hagneau I, Perez-Diaz F and Chapouthier G (2000) Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. *Pharmacol Biochem Behav* **67**:739-748.

Lepore M, Vorel SR, Lowinson J and Gardner EL (1995) Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. *Life Sci* **56**:2073-2080.

Leshner AI, Remler H, Biegon A and Samuel D (1979) Desmethylimipramine (DMI) counteracts learned helplessness in rats. *Psychopharmacology (Berl)* **66**:207-208.

Li SM, Yin LL, Ren YH, Pan LS and Zheng JW (2001) GABA(B) receptor agonist baclofen attenuates the development and expression of d-methamphetamine-induced place preference in rats. *Life Sci* **70**:349-356.

Lile JA, Stoops WW, Allen TS, Glaser PE, Hays LR and Rush CR (2004) Baclofen does not alter the reinforcing, subject-rated or cardiovascular effects of intranasal cocaine in humans. *Psychopharmacology (Berl)* **171**:441-449.

Lin XH, Hashimoto T, Kitamura N, Murakami N, Shirakawa O and Maeda K (2002) Decreased calcineurin and increased phosphothreonine-DARPP-32 in the striatum of rats behaviorally sensitized to methamphetamine. *Synapse* **44**:181-187.

Ling W, Shoptaw S and Majewska D (1998) Baclofen as a cocaine anti-craving medication: a preliminary clinical study. *Neuropsychopharmacology* **18**:403-404.

Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B and Mosbacher J (1999) Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* **38**:1667-1673.

Liu FC and Graybiel AM (1996) Spatiotemporal dynamics of CREB phosphorylation: transient versus sustained phosphorylation in the developing striatum. *Neuron* **17**:1133-1144.

Liu QS, Pu L and Poo MM (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature* **437**:1027-1031.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**:402-408.

Lloyd KG, Zivkovic B, Scatton B, Morselli PL and Bartholini G (1989) The gabaergic hypothesis of depression. *Prog Neuropsychopharmacol Biol Psychiatry* **13**:341-351.

Lobina C, Colombo G, Gessa GL and Carai MA (2005) Different sensitivity to the motor incoordinating effects of gamma-hydroxybutyric acid (GHB) and baclofen in GHB-sensitive and GHB-resistant rats. *Brain Res* **1033**:109-112.

Logan L, Seale TW, Cao W and Carney JM (1988) Effects of chronic amphetamine in BALB/cBy mice, a strain that is not stimulated by acute administration of amphetamine. *Pharmacol Biochem Behav* **31**:675-682.

Loomer HP, Saunders JC and Kline NS (1957) A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. *Psychiatr Res Rep Am Psychiatr Assoc* **135**:129-141.

Lopez JF, Chalmers DT, Little KY and Watson SJ (1998) A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry* **43**:547-573.

Losada ME (1991) Baclofen and noradrenergic function in the rat frontal cerebral cortex. *Eur J Pharmacol* **197**:93-98.

Lucki I (1997) The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* **8**:523-532.

Lucki I (2001) A prescription to resist proscriptions for murine models of depression. *Psychopharmacology (Berl)* **153**:395-398.

Lucki I, Dalvi A and Mayorga AJ (2001) Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)* **155**:315-322.

Lumia AR, Teicher MH, Salchli F, Ayers E and Possidente B (1992) Olfactory bulbectomy as a model for agitated hyposerotonergic depression. *Brain Res* **587**:181-185.

Luscher C, Jan LY, Stoffel M, Malenka RC and Nicoll RA (1997) G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **19**:687-695.

Lydiard RB (1998) The role of drug therapy in social phobia. *J Affect Disord* **50 Suppl 1**:S35-39.

Lyness WH, Friedle NM and Moore KE (1979) Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacol Biochem Behav* **11**:553-556.

Macey DJ, Froestl W, Koob GF and Markou A (2001) Both GABA(B) receptor agonist and antagonists decreased brain stimulation reward in the rat. *Neuropharmacology* **40**:676-685.

MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT and Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav Neurosci* **115**:1145-1153.

Maitre M (1997) The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol* **51**:337-361.

Maitre M, Hechler V, Vayer P, Gobaille S, Cash CD, Schmitt M and Bourguignon JJ (1990) A specific gamma-hydroxybutyrate receptor ligand possesses both antagonistic and anticonvulsant properties. *J Pharmacol Exp Ther* **255**:657-663.

Maldonado R, Blendy JA, Tzavara E, Gass P, Roques BP, Hanoune J and Schutz G (1996) Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. *Science* **273**:657-659.

Malitschek B, Schweizer C, Keir M, Heid J, Froestl W, Mosbacher J, Kuhn R, Henley J, Joly C, Pin JP, Kaupmann K and Bettler B (1999) The N-terminal domain of gamma-

aminobutyric Acid(B) receptors is sufficient to specify agonist and antagonist binding. *Mol Pharmacol* **56**:448-454.

Malizia AL, Cunningham VJ, Bell CJ, Liddle PF, Jones T and Nutt DJ (1998) Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. *Arch Gen Psychiatry* **55**:715-720.

Mann JJ (2005) The medical management of depression. *N Engl J Med* **353**:1819-1834.

Mannoury la Cour C, Hanoun N, Melfort M, Hen R, Lesch KP, Hamon M and Lanfumey L (2004) GABA(B) receptors in 5-HT transporter- and 5-HT1A receptor-knock-out mice: further evidence of a transduction pathway shared with 5-HT1A receptors. *J Neurochem* **89**:886-896.

Marchesi C, Chiodera P, De Ferri A, De Risio C, Dasso L, Menozzi P, Volpi R and Coiro V (1991) Reduction of GH response to the GABA-B agonist baclofen in patients with major depression. *Psychoneuroendocrinology* **16**:475-479.

Maren S (2005) Building and burying fear memories in the brain. *Neuroscientist* **11**:89-99.

Margeta-Mitrovic M, Jan YN and Jan LY (2000) A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron* **27**:97-106.

Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY and Basbaum AI (1999) Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. *J Comp Neurol* **405**:299-321.

Markou A and Koob GF (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology* **4**:17-26.

Markou A, Kosten TR and Koob GF (1998) Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology* **18**:135-174.

Markou A, Paterson NE and Semenova S (2004) Role of gamma-aminobutyric acid (GABA) and metabotropic glutamate receptors in nicotine reinforcement: potential pharmacotherapies for smoking cessation. *Ann N Y Acad Sci* **1025**:491-503.

Marshall FH, Jones KA, Kaupmann K and Bettler B (1999) GABAB receptors - the first 7TM heterodimers. *Trends Pharmacol Sci* **20**:396-399.

Martellotta MC, Cossu G, Fattore L, Gessa GL and Fratta W (1998a) Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice. *Eur Neuropsychopharmacol* **8**:293-296.

Martellotta MC, Cossu G, Fattore L, Gessa GL and Fratta W (1998b) Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. *Neuroscience* **85**:327-330.

Martin DL and Rimvall K (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J Neurochem* **60**:395-407.

Martin P, Beninger RJ, Hamon M and Puech AJ (1990a) Antidepressant-like action of 8-OH-DPAT, a 5-HT1A agonist, in the learned helplessness paradigm: evidence for a postsynaptic mechanism. *Behav Brain Res* **38**:135-144.

Martin P, Pichat P, Massol J, Soubrie P, Lloyd KG and Puech AJ (1989) Decreased GABA B receptors in helpless rats: reversal by tricyclic antidepressants. *Neuropsychobiology* **22**:220-224.

Martin P, Soubrie P and Puech AJ (1990b) Reversal of helpless behavior by serotonin uptake blockers in rats. *Psychopharmacology (Berl)* **101**:403-407.

Martin SC, Russek SJ and Farb DH (1999) Molecular identification of the human GABABR2: cell surface expression and coupling to adenylyl cyclase in the absence of GABABR1. *Mol Cell Neurosci* **13**:180-191.

Mathivet P, Bernasconi R, De Barry J, Marescaux C and Bittiger H (1997) Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. *Eur J Pharmacol* **321**:67-75.

Matsushima T, Tegner J, Hill RH and Grillner S (1993) GABAB receptor activation causes a depression of low- and high-voltage-activated Ca2+ currents, postinhibitory rebound, and postspike afterhyperpolarization in lamprey neurons. *J Neurophysiol* **70**:2606-2619.

Matthews K and Robbins TW (2003) Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. *Neurosci Biobehav Rev* **27**:45-55.

Mattson BJ, Bossert JM, Simmons DE, Nozaki N, Nagarkar D, Kreuter JD and Hope BT (2005) Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. *J Neurochem* **95**:1481-1494.

Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R and Lucki I (2001) Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. *J Pharmacol Exp Ther* **298**:1101-1107.

Mayorga AJ and Lucki I (2001) Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl)* **155**:110-112.

McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O and Nestler EJ (2004) DeltaFosB: a molecular switch for long-term adaptation in the brain. *Brain Res Mol Brain Res* **132**:146-154.

McCormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* **62**:1018-1027.

McFarland K and Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* **21**:8655-8663.

McKinney WTJ and Bunney WEJ (1969) Animal model of depression. I. Review of evidence: implications for research. *Arch. Gen. Psychiatry* **21**:240-248.

McLellan AT, Lewis DC, O'Brien CP and Kleber HD (2000) Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *Jama* **284**:1689-1695.

McManus DJ and Greenshaw AJ (1991) Differential effects of antidepressants on GABAB and beta-adrenergic receptors in rat cerebral cortex. *Biochem Pharmacol* **42**:1525-1528.

Meijer OC and de Kloet ER (1998) Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. *Crit Rev Neurobiol* **12**:1-20.

Menard J and Treit D (1999) Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev* **23**:591-613.

Menon-Johansson AS, Berrow N and Dolphin AC (1993) G(o) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. *Pflugers Arch* **425**:335-343.

Miczek KA, Weerts EM, Vivian JA and Barros HM (1995) Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. *Psychopharmacology (Berl)* **121**:38-56.

Millan MJ (2003) The neurobiology and control of anxious states. *Prog Neurobiol* **70**:83-244.

Miller LP and Walters JR (1979) Effects of depolarization on cofactor regulation of glutamic acid decarboxylase in substantia nigra synaptosomes. *J Neurochem* **33**:533-539.

Mintz IM and Bean BP (1993) GABAB receptor inhibition of P-type Ca2+ channels in central neurons. *Neuron* **10**:889-898.

Mitchell PJ and Redfern PH (1997) Potentiation of the time-dependent, antidepressant-induced changes in the agonistic behaviour of resident rats by the 5-HT1A receptor antagonist, WAY-100635. *Behav Pharmacol* **8**:585-606.

Mitsukawa K, Mombereau C, Lotscher E, Uzunov DP, van der Putten H, Flor PJ and Cryan JF (2005) Metabotropic Glutamate Receptor Subtype 7 Ablation Causes Dysregulation of the HPA Axis and Increases Hippocampal BDNF Protein Levels: Implications for Stress-Related Psychiatric Disorders. *Neuropsychopharmacology*.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H and Tabira T (2002) Chronic stress impairs rotarod performance in rats: implications for depressive state. *Pharmacol Biochem Behav* **71**:79-84.

Mizukami K, Ishikawa M, Hidaka S, Iwakiri M, Sasaki M and Iritani S (2002) Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:393-396.

Modesto-Lowe V and Fritz EM (2005) The opioidergic-alcohol link: implications for treatment. *CNS Drugs* **19**:693-707.

Moller HJ (2002) Anxiety associated with comorbid depression. *J Clin Psychiatry* **63 Suppl 14**:22-26.

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H and Cryan JF (2004) Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology* **29**:1050-1062.

Mongeau R, Blier P and de Montigny C (1997) The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Res Brain Res Rev* **23**:145-195.

Monteleone P, Steardo L, Tanzillo C and Maj M (1990) Chronic antidepressant drug treatment does not affect GH response to baclofen in depressed subjects. *J Neural Transm Gen Sect* **82**:147-152.

Moreau JL, Jenck F, Martin JR, Mortas P and Haefely WE (1992) Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. *Eur Neuropsychopharmacol* **2**:43-49.

Morishita R, Kato K and Asano T (1990) GABAB receptors couple to G proteins Go, Go* and Gi1 but not to Gi2. *FEBS Lett* **271**:231-235.

Moser PC and Sanger DJ (1999) 5-HT1A receptor antagonists neither potentiate nor inhibit the effects of fluoxetine and befloxatone in the forced swim test in rats. *Eur J Pharmacol* **372**:127-134.

Mucha RF, van der Kooy D, O'Shaughnessy M and Bucenieks P (1982) Drug reinforcement studied by the use of place conditioning in rat. *Brain Res* **243**:91-105.

Muller DL and Unterwald EM (2005) D1 dopamine receptors modulate deltaFosB induction in rat striatum after intermittent morphine administration. *J Pharmacol Exp Ther* **314**:148-154.

Murray CJ and Lopez AD (1997) Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* **349**:1498-1504.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M and Takashima T (1996a) Involvement of GABAB receptor systems in action of antidepressants. II: Baclofen attenuates the effect of desipramine whereas muscimol has no effect in learned helplessness paradigm in rats. *Brain Res* **728**:225-230.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M and Takashima T (1996b) Involvement of GABAB receptor systems in experimental depression: baclofen but not bicuculline exacerbates helplessness in rats. *Brain Res* **741**:240-245.

Nakagawa Y, Ishima T, Ishibashi Y, Yoshii T and Takashima T (1996c) Involvement of GABA(B) receptor systems in action of antidepressants: baclofen but not bicuculline attenuates the effects of antidepressants on the forced swim test in rats. *Brain Res* **709**:215-220.

Nakagawa Y, Sasaki A and Takashima T (1999) The GABA(B) receptor antagonist CGP36742 improves learned helplessness in rats. *Eur J Pharmacol* **381**:1-7.

Nakagawa Y and Takashima T (1997) The GABA(B) receptor antagonist CGP36742 attenuates the baclofen- and scopolamine-induced deficit in Morris water maze task in rats. *Brain Res* **766**:101-106.

Nastiti K, Benton D and Brain PF (1991) The effects of compounds acting at the benzodiazepine receptor complex on the ultrasonic calling of mouse pups. *Behav Pharmacol* **2**:121-128.

Nehring RB, Horikawa HP, El Far O, Kneussel M, Brandstatter JH, Stamm S, Wischmeyer E, Betz H and Karschin A (2000) The metabotropic GABAB receptor directly interacts with the activating transcription factor 4. *J Biol Chem* **275**:35185-35191.

Nemeroff CB (1989) Clinical significance of psychoneuroendocrinology in psychiatry: focus on the thyroid and adrenal. *J Clin Psychiatry* **50 Suppl**:13-20; discussion 21-12.

Nemeroff CB (2003) Anxiolytics: past, present, and future agents. *J Clin Psychiatry* **64 Suppl 3**:3-6.

Nestler EJ and Aghajanian GK (1997) Molecular and cellular basis of addiction. *Science* **278**:58-63.

Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L and Zalcman S (2002) Preclinical models: status of basic research in depression. *Biol Psychiatry* **52**:503-528.

Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, Eckelman W, Herscovitch P, Charney DS and Drevets WC (2004) Reduced serotonin type 1A receptor binding in panic disorder. *J Neurosci* **24**:589-591.

Newberry NR and Nicoll RA (1985) Comparison of the action of baclofen with gamma-aminobutyric acid on rat hippocampal pyramidal cells in vitro. *J Physiol* **360**:161-185.

Ng GY, Clark J, Coulombe N, Ethier N, Hebert TE, Sullivan R, Kargman S, Chateauneuf A, Tsukamoto N, McDonald T, Whiting P, Mezey E, Johnson MP, Liu Q, Kolakowski LF, Jr., Evans JF, Bonner TI and O'Neill GP (1999) Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. *J Biol Chem* **274**:7607-7610.

Ng TK and Yung KK (2001) Differential expression of GABA(B)R1 and GABA(B)R2 receptor immunoreactivity in neurochemically identified neurons of the rat neostriatum. *J Comp Neurol* **433**:458-470.

Nibuya M, Nestler EJ and Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* **16**:2365-2372.

Nic Dhonnchadha BA, Bourin M and Hascoet M (2003) Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. *Behav Brain Res* **140**:203-214.

Nicholson KL and Balster RL (2001) GHB: a new and novel drug of abuse. *Drug Alcohol Depend* **63**:1-22.

Nishi A, Snyder GL, Fienberg AA, Fisone G, Aperia A, Nairn AC and Greengard P (1999a) Requirement for DARPP-32 in mediating effect of dopamine D2 receptor activation. *Eur J Neurosci* **11**:2589-2592.

Nishi A, Snyder GL, Nairn AC and Greengard P (1999b) Role of calcineurin and protein phosphatase-2A in the regulation of DARPP-32 dephosphorylation in neostriatal neurons. *J Neurochem* **72**:2015-2021.

Nissbrandt H and Engberg G (1996) The GABAB-receptor antagonist, CGP 35348, antagonises gamma-hydroxybutyrate- and baclofen-induced alterations in locomotor activity and forebrain dopamine levels in mice. *J Neural Transm* **103**:1255-1263.

Noda Y, Yamada K, Furukawa H and Nabeshima T (1995) Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. *Br J Pharmacol* **116**:2531-2537.

Nonogaki K, Abdallah L, Goulding EH, Bonasera SJ and Tecott LH (2003) Hyperactivity and reduced energy cost of physical activity in serotonin 5-HT(2C) receptor mutant mice. *Diabetes* **52**:315-320.

Nonogaki K, Strack AM, Dallman MF and Tecott LH (1998) Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. *Nat Med* **4**:1152-1156.

Nowak G, Partyka A, Klodzinska A, Szewczyk B, Tatarczynska E, Wieronska JM, Librowski T, Czarnecki R, Chojnacka-Wojcik E and Pilc A (2004) Anxiolytic- and antidepressant-like activity of CGP36742 in rodent tests., in *FENS*.

Noyes R, Jr. (1985) Beta-adrenergic blocking drugs in anxiety and stress. *Psychiatr Clin North Am* **8**:119-132.

Nye HE, Hope BT, Kelz MB, Iadarola M and Nestler EJ (1995) Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J Pharmacol Exp Ther* **275**:1671-1680.

Nye HE and Nestler EJ (1996) Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. *Mol Pharmacol* **49**:636-645.

O'Flynn K and Dinan TG (1993) Baclofen-induced growth hormone release in major depression: relationship to dexamethasone suppression test result. *Am J Psychiatry* **150**:1728-1730.

O'Neill MF and Shaw G (1999) Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. *Psychopharmacology (Berl)* **145**:237-250.

Olds J and Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* **47**:419-427.

Olmstead MC and Burns LH (2005) Ultra-low-dose naltrexone suppresses rewarding effects of opiates and aversive effects of opiate withdrawal in rats. *Psychopharmacology (Berl)* **181**:576-581.

Olpe HR, Karlsson G, Pozza MF, Brugger F, Steinmann M, Van Riezen H, Fagg G, Hall RG, Froestl W and Bittiger H (1990) CGP 35348: a centrally active blocker of GABAB receptors. *Eur J Pharmacol* **187**:27-38.

Olpe HR, Koella WP, Wolf P and Haas HL (1977) The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. *Brain Res* **134**:577-580.

Olpe HR, Steinmann MW, Ferrat T, Pozza MF, Greiner K, Brugger F, Froestl W, Mickel SJ and Bittiger H (1993) The actions of orally active GABAB receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur J Pharmacol* **233**:179-186.

Olpe HR, Steinmann MW, Hall RG, Brugger F and Pozza MF (1988) GABAA and GABAB receptors in locus coeruleus: effects of blockers. *Eur J Pharmacol* **149**:183-185.

Olsen R and DeLorey T (1999) GABA and Glycine, in *Basic Neurochemistry* (Siegel ed) pp 335-346, Lippincott, Williams and Wilkins, Philadelphia.

Onali P, Mascia FM and Olianas MC (2003) Positive regulation of GABA(B) receptors dually coupled to cyclic AMP by the allosteric agent CGP7930. *Eur J Pharmacol* **471**:77-84.

Ordway GA, Schenk J, Stockmeier CA, May W and Klimek V (2003) Elevated agonist binding to alpha2-adrenoceptors in the locus coeruleus in major depression. *Biol Psychiatry* **53**:315-323.

Ordway GA, Smith KS and Haycock JW (1994) Elevated tyrosine hydroxylase in the locus coeruleus of suicide victims. *J Neurochem* **62**:680-685.

Otis TS, De Koninck Y and Mody I (1993) Characterization of synaptically elicited GABAB responses using patch-clamp recordings in rat hippocampal slices. *J Physiol* **463**:391-407.

Otmakhova NA, Gurevich EV, Katkov YA, Nesterova IV and Bobkova NV (1992) Dissociation of multiple behavioral effects between olfactory bulbectomized C57Bl/6J and DBA/2J mice. *Physiol Behav* **52**:441-448.

Otsuka M, Iversen LL, Hall ZW and Kravitz EA (1966) Release of gamma-aminobutyric acid from inhibitory nerves of lobster. *Proc Natl Acad Sci U S A* **56**:1110-1115.

Pagano A, Rovelli G, Mosbacher J, Lohmann T, Duthey B, Stauffer D, Ristig D, Schuler V, Meigel I, Lampert C, Stein T, Prezeau L, Blahos J, Pin J, Froestl W, Kuhn R, Heid J, Kaupmann K and Bettler B (2001) C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of GABA(b) receptors. *J Neurosci* **21**:1189-1202.

Panagis G, Kastellakis A, Spyraki C and Nomikos G (2000) Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl)* **149**:388-396.

Parks CL, Robinson PS, Sibille E, Shenk T and Toth M (1998) Increased anxiety of mice lacking the serotonin1A receptor. *Proc Natl Acad Sci U S A* **95**:10734-10739.

Patel S, Naeem S, Kesingland A, Froestl W, Capogna M, Urban L and Fox A (2001) The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. *Pain* **90**:217-226.

Paterson NE, Froestl W and Markou A (2004) The GABAB receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. *Psychopharmacology (Berl)* **172**:179-186.

Paterson NE, Froestl W and Markou A (2005) Repeated administration of the GABAB receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. *Neuropsychopharmacology* **30**:119-128.

Paterson NE and Markou A (2005) The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl)* **179**:255-261.

Peakman MC, Colby C, Perrotti LI, Tekumalla P, Carle T, Ulery P, Chao J, Duman C, Steffen C, Monteggia L, Allen MR, Stock JL, Duman RS, McNeish JD, Barrot M, Self DW, Nestler EJ and Schaeffer E (2003) Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain Res* **970**:73-86.

Peng CT, Ger J, Yang CC, Tsai WJ, Deng JF and Bullard MJ (1998) Prolonged severe withdrawal symptoms after acute-on-chronic baclofen overdose. *J Toxicol Clin Toxicol* **36**:359-363.

Petit-Demouliere B, Chenu F and Bourin M (2005) Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl)* **177**:245-255.

Pettit HO, Ettenberg A, Bloom FE and Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology (Berl)* **84**:167-173.

Petty F, Kramer GL, Dunnam D and Rush AJ (1990) Plasma GABA in mood disorders. *Psychopharmacol Bull* **26**:157-161.

Phillips AG, Blaha CD and Fibiger HC (1989) Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. *Neurosci Biobehav Rev* **13**:99-104.

Phillis BD, Ong J, White JM and Bonnielle C (2001) Modification of d-amphetamine-induced responses by baclofen in rats. *Psychopharmacology (Berl)* **153**:277-284.

Picciotto MR (1998) Common aspects of the action of nicotine and other drugs of abuse. *Drug Alcohol Depend* **51**:165-172.

Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R and Chiamulera C (1997) Common neural substrates for the addictive properties of nicotine and cocaine. *Science* **275**:83-86.

Pick CG, Peter Y, Terkel J, Gavish M and Weizman R (1996) Effect of the neuroactive steroid alpha-THDOC on staircase test behavior in mice. *Psychopharmacology (Berl)* **128**:61-66.

Pierce RC and Kumaresan V (2005) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev*.

Pilc A and Lloyd KG (1984) Chronic antidepressants and GABA "B" receptors: a GABA hypothesis of antidepressant drug action. *Life Sci* **35**:2149-2154.

Pirker W, Asenbaum S, Kasper S, Walter H, Angelberger P, Koch G, Pozzera A, Deecke L, Podreka I and Brücke T (1995) ß-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J. Neural. Transm. [Gen. Sect.]* **100**:247–250.

Pitler TA and Alger BE (1994) Differences between presynaptic and postsynaptic GABAB mechanisms in rat hippocampal pyramidal cells. *J Neurophysiol* **72**:2317-2327.

Pollard GT and Howard JL (1986) The staircase test: some evidence of nonspecificity for anxiolytics. *Psychopharmacology (Berl)* **89**:14-19.

Poncer JC, McKinney RA, Gahwiler BH and Thompson SM (1997) Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. *Neuron* **18**:463-472.

Pontieri FE, Tanda G, Orzi F and Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* **382**:255-257.

Popik P, Wrobel M and Bisaga A (2005) Reinstatement of Morphine-Conditioned Reward is Blocked by Memantine. *Neuropsychopharmacology*.

Porrino LJ, Esposito RU, Seeger TF, Crane AM, Pert A and Sokoloff L (1984) Metabolic mapping of the brain during rewarding self-stimulation. *Science* **224**:306-309.

Porsolt RD (2000) Animal models of depression: utility for transgenic research. *Rev Neurosci* **11**:53-58.

Porsolt RD, Bertin A and Jalfre M (1978) "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. *Eur J Pharmacol* **51**:291-294.

Porsolt RD, Chermat R, Lenegre A, Avril I, Janvier S and Steru L (1987) Use of the automated tail suspension test for the primary screening of psychotropic agents. *Arch Int Pharmacodyn Ther* **288**:11-30.

Porsolt RD, Le Pichon M and Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**:730-732.

Porter RJ, Gallagher P, Watson S and Young AH (2004) Corticosteroid-serotonin interactions in depression: a review of the human evidence. *Psychopharmacology (Berl)* **173**:1-17.

Post RM, Ketter TA, Joffe RT and Kramlinger KL (1991) Lack of beneficial effects of l-baclofen in affective disorder. *Int Clin Psychopharmacol* **6**:197-207.

Potier B and Dutar P (1993) Presynaptic inhibitory effect of baclofen on hippocampal inhibitory synaptic transmission involves a pertussis toxin-sensitive G-protein. *Eur J Pharmacol* **231**:427-433.

Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, Soffin EM, Farmer CE, Lanneau C, Gray J, Schenck E, Warmerdam BS, Clapham C, Reavill C, Rogers DC, Stean T, Upton N, Humphreys K, Randall A, Geppert M, Davies CH and Pangalos MN (2001) Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Mol Cell Neurosci* **17**:1059-1070.

Queva C, Bremner-Danielsen M, Edlund A, Ekstrand AJ, Elg S, Erickson S, Johansson T, Lehmann A and Mattsson JP (2003) Effects of GABA agonists on body temperature regulation in GABAB(1)-/- mice. *Br J Pharmacol* **140**:315-322.

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D and Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* **95**:14476-14481.

Ranaldi R and Poeggel K (2002) Baclofen decreases methamphetamine self-administration in rats. *Neuroreport* **13**:1107-1110.

Reid I, Forbes N, Stewart C and Matthews K (1997) Chronic mild stress and depressive disorder: a useful new model? *Psychopharmacology (Berl)* **134**:365-367; discussion 371-367.

Ren X and Mody I (2003) Gamma-hydroxybutyrate reduces mitogen-activated protein kinase phosphorylation via GABA B receptor activation in mouse frontal cortex and hippocampus. *J Biol Chem* **278**:42006-42011.

Renard CE, Dailly E, David DJ, Hascoet M and Bourin M (2003) Monoamine metabolism changes following the mouse forced swimming test but not the tail suspension test. *Fundam Clin Pharmacol* **17**:449-455.

Ribeiro Do Couto B, Aguilar MA, Manzanedo C, Rodriguez-Arias M and Minarro J (2005) NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. *Brain Res Bull* **64**:493-503.

Risner ME and Goldberg SR (1983) A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive-ratio schedules of intravenous drug infusion. *J Pharmacol Exp Ther* **224**:319-326.

Roberts DC, Andrews MM and Vickers GJ (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* **15**:417-423.

Roberts DC and Brebner K (2000) GABA modulation of cocaine self-administration. *Ann N Y Acad Sci* **909**:145-158.

Roberts DC, Corcoran ME and Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav* **6**:615-620.

Roberts DC and Koob GF (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* **17**:901-904.

Roberts E (1986a) Failure of GABAergic inhibition: a key to local and global seizures. *Adv Neurol* **44**:319-341.

Roberts E (1986b) What do GABA neurons really do? They make possible variability generation in relation to demand. *Exp Neurol* **93**:279-290.

Roberts E and Frankel S (1950) gamma-Aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem* **187**:55-63.

Robinson TE and Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* **18**:247-291.

Rodgers RJ, Cao BJ, Dalvi A and Holmes A (1997) Animal models of anxiety: an ethological perspective. *Braz J Med Biol Res* **30**:289-304.

Romieu P, Meunier J, Garcia D, Zozime N, Martin-Fardon R, Bowen WD and Maurice T (2004) The sigma1 (sigma1) receptor activation is a key step for the reactivation of cocaine conditioned place preference by drug priming. *Psychopharmacology (Berl)* **175**:154-162.

Roth A, Hogan I and Farren C (1997) Naltrexone plus group therapy for the treatment of opiate-abusing health-care professionals. *J Subst Abuse Treat* **14**:19-22.

Rudolph U, Crestani F and Mohler H (2001) GABA(A) receptor subtypes: dissecting their pharmacological functions. *Trends Pharmacol Sci* **22**:188-194.

Russig H, Pezze MA, Nanz-Bahr NI, Pryce CR, Feldon J and Murphy CA (2003) Amphetamine withdrawal does not produce a depressive-like state in rats as measured by three behavioral tests. *Behav Pharmacol* **14**:1-18.

Sacchetti G, Bernini M, Gobbi M, Parini S, Pirona L, Mennini T and Samanin R (2001) Chronic treatment with desipramine facilitates its effect on extracellular noradrenaline in the rat hippocampus: studies on the role of presynaptic alpha2-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol* **363**:66-72.

Saint DA, Thomas T and Gage PW (1990) GABAB agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci Lett* **118**:9-13.

Salas R, Pieri F, Fung B, Dani JA and De Biasi M (2003) Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. *J Neurosci* **23**:6255-6263.

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH and Mason GF (2004) Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry* **61**:705-713.

Sanacora G, Mason GF, Rothman DL, Hyder F, Ciarcia JJ, Ostroff RB, Berman RM and Krystal JH (2003) Increased cortical GABA concentrations in depressed patients receiving ECT. *Am J Psychiatry* **160**:577-579.

Sanacora G, Mason GF, Rothman DL and Krystal JH (2002) Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. *Am J Psychiatry* **159**:663-665.

Sandoval MR and Palermo-Neto J (1985) Central nervous system supersensitivity and withdrawal from long-term treatment with barbital. *Neuropharmacology* **24**:1247-1251.

Sands SA, Reisman SA and Enna SJ (2004) Effect of antidepressants on GABA(B) receptor function and subunit expression in rat hippocampus. *Biochem Pharmacol* **68**:1489-1495.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C and Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**:805-809.

Sayin U, Sutula TP and Stafstrom CE (2004) Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. *Epilepsia* **45**:1539-1548.

Sbrenna S, Marti M, Morari M, Calo G, Guerrini R, Beani L and Bianchi C (2000) Modulation of 5-hydroxytryptamine efflux from rat cortical synaptosomes by opioids and nociceptin. *Br J Pharmacol* **130**:425-433.

Schildkraut JJ (1995) The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965. *J Neuropsychiatry Clin Neurosci* **7**:524-533; discussion 523-524.

Schlicker E, Classen K and Gothert M (1984) GABAB receptor-mediated inhibition of serotonin release in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **326**:99-105.

Schmidt M, Oitzl MS, Levine S and de Kloet ER (2002) The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. *Brain Res Dev Brain Res* **139**:39-49.

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA and et al. (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family. *Nature* **328**:221-227.

Schuler V, Luscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton AL, Vigouret J, Pozza M, Kelly PH, Mosbacher J, Froestl W, Kaslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H and Bettler

B (2001) Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron* **31**:47-58.

Scott RH and Dolphin AC (1986) Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen-mediated inhibition. *Neurosci Lett* **69**:59-64.

Scott RH, Wootton JF and Dolphin AC (1990) Modulation of neuronal T-type calcium channel currents by photoactivation of intracellular guanosine 5'-O(3-thio) triphosphate. *Neuroscience* **38**:285-294.

Seale TW and Carney JM (1991) Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. *J Addict Dis* **10**:141-162.

Seiden LS, Dahms JL and Shaughnessy RA (1985) Behavioral screen for antidepressants: the effects of drugs and electroconvulsive shock on performance under a differential-reinforcement-of-low-rate schedule. *Psychopharmacology (Berl)* **86**:55-60.

Seligman ME (1972) Learned helplessness. *Annu Rev Med* **23**:407-412.

Seligman ME, Rosellini RA and Kozak MJ (1975) Learned helplessness in the rat: time course, immunization, and reversibility. *J Comp Physiol Psychol* **88**:542-547.

Semenova S and Markou A (2003) Cocaine-seeking behavior after extended cocaine-free periods in rats: role of conditioned stimuli. *Psychopharmacology (Berl)* **168**:192-200.

Serrano JS, Minano FJ, Sancibrian M and Duran JA (1985) Involvement of bicuculline-insensitive receptors in the hypothermic effect of GABA and its agonists. *Gen Pharmacol* **16**:505-508.

Serrats J, Artigas F, Mengod G and Cortes R (2003) GABAB receptor mRNA in the raphe nuclei: co-expression with serotonin transporter and glutamic acid decarboxylase. *J Neurochem* **84**:743-752.

Shalev U, Grimm JW and Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev* **54**:1-42.

Shanks N and Anisman H (1993) Escape deficits induced by uncontrollable foot-shock in recombinant inbred strains of mice. *Pharmacol Biochem Behav* **46**:511-517.

Shaywitz AJ and Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* **68**:821-861.

Shekhar A, McCann UD, Meaney MJ, Blanchard DC, Davis M, Frey KA, Liberzon I, Overall KL, Shear MK, Tecott LH and Winsky L (2001) Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. *Psychopharmacology (Berl)* **157**:327-339.

Sheline YI, Wang PW, Gado MH, Csernansky JG and Vannier MW (1996) Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A* **93**:3908-3913.

Shen W and Slaughter MM (1999) Metabotropic GABA receptors facilitate L-type and inhibit N-type calcium channels in single salamander retinal neurons. *J Physiol* **516 (Pt 3)**:711-718.

Shen Y, Connor TJ, Nolan Y, Kelly JP and Leonard BE (1999) Differential effect of chronic antidepressant treatments on lipopolysaccharide-induced depressive-like behavioural symptoms in the rat. *Life Sci* **65**:1773-1786.

Shephard RA, Wedlock P and Wilson NE (1992) Direct evidence for mediation of an anticonflict effect of baclofen by GABAb receptors. *Pharmacol Biochem Behav* **41**:651-653.

Shepherd JK, Grewal SS, Fletcher A, Bill DJ and Dourish CT (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl)* **116**:56-64.

Sherman AD and Petty F (1982) Additivity of neurochemical changes in learned helplessness and imipramine. *Behav Neural Biol* **35**:344-353.

Shippenberg TS and Rea W (1997) Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* **57**:449-455.

Shirayama Y, Chen AC, Nakagawa S, Russell DS and Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* **22**:3251-3261.

Shoaib M, Swanner LS, Beyer CE, Goldberg SR and Schindler CW (1998) The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol* **9**:195-206.

Shoptaw S, Yang X, Rotheram-Fuller EJ, Hsieh YC, Kintaudi PC, Charuvastra VC and Ling W (2003) Randomized placebo-controlled trial of baclofen for cocaine dependence: preliminary effects for individuals with chronic patterns of cocaine use. *J Clin Psychiatry* **64**:1440-1448.

Sibille E and Hen R (2001) Serotonin(1A) receptors in mood disorders: a combined genetic and genomic approach. *Behav Pharmacol* **12**:429-438.

Sibille E, Pavlides C, Benke D and Toth M (2000) Genetic inactivation of the Serotonin(1A) receptor in mice results in downregulation of major GABA(A) receptor alpha subunits, reduction of GABA(A) receptor binding, and benzodiazepine-resistant anxiety. *J Neurosci* **20**:2758-2765.

Simiand J, Keane PE and Morre M (1984) The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. *Psychopharmacology (Berl)* **84**:48-53.

Simonds WF (1999) G protein regulation of adenylate cyclase. *Trends Pharmacol Sci* **20**:66-73.

Siniscalchi A, Rodi D, Beani L and Bianchi C (1999) Inhibitory effect of nociceptin on [3H]-5-HT release from rat cerebral cortex slices. *Br J Pharmacol* **128**:119-123.

Skolnick P, Hu RJ, Cook CM, Hurt SD, Trometer JD, Liu R, Huang Q and Cook JM (1997) [3H]RY 80: A high-affinity, selective ligand for gamma-aminobutyric acidA receptors containing alpha-5 subunits. *J Pharmacol Exp Ther* **283**:488-493.

Slattery DA, Desrayaud S and Cryan JF (2005a) GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *J Pharmacol Exp Ther* **312**:290-296.

Slattery DA, Markou A, Froestl W and Cryan JF (2005b) The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* **30**:2065-2072.

Sloviter RS, Ali-Akbarian L, Elliott RC, Bowery BJ and Bowery NG (1999) Localization of GABA(B) (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. *Neuropharmacology* **38**:1707-1721.

Smith MA, Makino S, Kvetnansky R and Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* **15**:1768-1777.

Smith MA, Yancey DL, Morgan D, Liu Y, Froestl W and Roberts DC (2004) Effects of positive allosteric modulators of the GABAB receptor on cocaine self-administration in rats. *Psychopharmacology (Berl)* **173**:105-111.

Snead OC, 3rd (1990) gamma-Hydroxybutyric acid-induced seizures bear no relation to core temperature. *Epilepsia* **31**:253-258.

Snead OC, 3rd (1994) The ontogeny of [3H]gamma-hydroxybutyrate and [3H]GABAB binding sites: relation to the development of experimental absence seizures. *Brain Res* **659**:147-156.

Snead OC, 3rd (2000) Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* **75**:1986-1996.

Soudijn W, van Wijngaarden I and AP IJ (2002) Allosteric modulation of G protein-coupled receptors. *Curr Opin Drug Discov Devel* **5**:749-755.

Spielewoy C and Markou A (2003) Withdrawal from chronic phencyclidine treatment induces long-lasting depression in brain reward function. *Neuropsychopharmacology* **28**:1106-1116.

Spooren W, Ballard T, Gasparini F, Amalric M, Mutel V and Schreiber R (2003) Insight into the function of Group I and Group II metabotropic glutamate (mGlu) receptors: behavioural characterization and implications for the treatment of CNS disorders. *Behav Pharmacol* **14**:257-277.

Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD and Gentsch C (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Ther* **295**:1267-1275.

Spyraki C, Fibiger HC and Phillips AG (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. *Brain Res* **253**:195-203.

Steffens DC, Byrum CE, McQuoid DR, Greenberg DL, Payne ME, Blitchington TF, MacFall JR and Krishnan KR (2000) Hippocampal volume in geriatric depression. *Biol Psychiatry* **48**:301-309.

Steketee JD and Beyer CE (2005) Injections of baclofen into the ventral medial prefrontal cortex block the initiation, but not the expression, of cocaine sensitization in rats. *Psychopharmacology (Berl)* **180**:352-358.

Steru L, Chermat R, Thierry B, Mico JA, Lenegre A, Steru M, Simon P and Porsolt RD (1987) The automated Tail Suspension Test: a computerized device which differentiates psychotropic drugs. *Prog Neuropsychopharmacol Biol Psychiatry* **11**:659-671.

Steru L, Chermat R, Thierry B and Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**:367-370.

Stewart J and Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* **4**:289-312.

Stewart RB and Grupp LA (1981) An investigation of the interaction between the reinforcing properties of food and ethanol using the place preference paradigm. *Prog Neuropsychopharmacol* **5**:609-613.

Stewart WF, Ricci JA, Chee E, Hahn SR and Morganstein D (2003) Cost of lost productive work time among US workers with depression. *Jama* **289**:3135-3144.

Stinus L, Cador M, Zorrilla EP and Koob GF (2005) Buprenorphine and a CRF1 antagonist block the acquisition of opiate withdrawal-induced conditioned place aversion in rats. *Neuropsychopharmacology* **30**:90-98.

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L and Rajkowska G (1998) Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J Neurosci* **18**:7394-7401.

Stolerman IP (1999) Inter-species consistency in the behavioural pharmacology of nicotine dependence. *Behav Pharmacol* **10**:559-580.

Stolerman IP and Jarvis MJ (1995) The scientific case that nicotine is addictive. *Psychopharmacology (Berl)* **117**:2-10; discussion 14-20.

Stork O, Ji FY, Kaneko K, Stork S, Yoshinobu Y, Moriya T, Shibata S and Obata K (2000) Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. *Brain Res* **865**:45-58.

Stork O, Yamanaka H, Stork S, Kume N and Obata K (2003) Altered conditioned fear behavior in glutamate decarboxylase 65 null mutant mice. *Genes Brain Behav* **2**:65-70.

Stretch R, Gerber GJ and Wood SM (1971) Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. *Can J Physiol Pharmacol* **49**:581-589.

Sullivan R, Chateauneuf A, Coulombe N, Kolakowski LF, Jr., Johnson MP, Hebert TE, Ethier N, Belley M, Metters K, Abramovitz M, O'Neill GP and Ng GY (2000) Coexpression of full-length gamma-aminobutyric acid(B) (GABA(B)) receptors with truncated receptors and metabotropic glutamate receptor 4 supports the GABA(B) heterodimer as the functional receptor. *J Pharmacol Exp Ther* **293**:460-467.

Swartzwelder HS, Tilson HA, McLamb RL and Wilson WA (1987) Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology (Berl)* **92**:398-401.

Takahashi T, Kajikawa Y and Tsujimoto T (1998) G-Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *J Neurosci* **18**:3138-3146.

Tanda G, Pontieri FE and Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* **276**:2048-2050.

Tang WJ and Gilman AG (1991) Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits. *Science* **254**:1500-1503.

Tao R, Ma Z and Auerbach SB (1996) Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. *Br J Pharmacol* **119**:1375-1384.

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ and Greenberg ME (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* **20**:709-726.

Tarantino LM, Gould TJ, Druhan JP and Bucan M (2000) Behavior and mutagenesis screens: the importance of baseline analysis of inbred strains. *Mamm Genome* **11**:555-564.

Tauscher J, Pirker W, de Zwaan M, Asenbaum S, Brucke T and Kasper S (1999) In vivo visualization of serotonin transporters in the human brain during fluoxetine treatment. *Eur Neuropsychopharmacol* **9**:177-179.

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM and Nestler EJ (1991) A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res* **548**:100-110.

Tessari M, Pilla M, Andreoli M, Hutcheson DM and Heidbreder CA (2004) Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking. *Eur J Pharmacol* **499**:121-133.

Teste JF, Pelsy-Johann I, Decelle T and Boulu RG (1993) Anti-immobility activity of different antidepressant drugs using the tail suspension test in normal or reserpinized mice. *Fundam Clin Pharmacol* **7**:219-226.

Thakker DR, Hoyer D and Cryan JF (2005) Interfering with the brain: Use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. *Pharmacol Ther*.

Thierry B, Steru L, Chermat R and Simon P (1984) Searching-waiting strategy: a candidate for an evolutionary model of depression? *Behav Neural Biol* **41**:180-189.

Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, Storm D and Duman RS (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J Neurosci* **20**:4030-4036.

Thompson SM and Gahwiler BH (1992) Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. *J Physiol* **451**:329-345.

Thuault SJ, Brown JT, Sheardown SA, Jourdain S, Fairfax B, Spencer JP, Restituito S, Nation JH, Topps S, Medhurst AD, Randall AD, Couve A, Moss SJ, Collingridge GL, Pangalos MN, Davies CH and Calver AR (2004) The GABA(B2) subunit is critical for the trafficking and function of native GABA(B) receptors. *Biochem Pharmacol* **68**:1655-1666.

Tiihonen J, Kuikka J, Rasanen P, Lepola U, Koponen H, Liuska A, Lehmusvaara A, Vainio P, Kononen M, Bergstrom K, Yu M, Kinnunen I, Akerman K and Karhu J (1997) Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: a fractal analysis. *Mol Psychiatry* **2**:463-471.

Treiman DM (2001) GABAergic mechanisms in epilepsy. *Epilepsia* **42 Suppl 3**:8-12.

Tremblay LK, Naranjo CA, Graham SJ, Herrmann N, Mayberg HS, Hevenor S and Busto UE (2005) Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. *Arch Gen Psychiatry* **62**:1228-1236.

Tsuji M, Nakagawa Y, Ishibashi Y, Yoshii T, Takashima T, Shimada M and Suzuki T (1996) Activation of ventral tegmental GABAB receptors inhibits morphine-induced place preference in rats. *Eur J Pharmacol* **313**:169-173.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* **56**:613-672.

Udenfriend S (1950) Identification of gamma-aminobutyric acid in brain by the isotope derivative method. *J Biol Chem* **187**:65-69.

Umezu T (1999) Effects of psychoactive drugs in the Vogel conflict test in mice. *Jpn J Pharmacol* **80**:111-118.

Urwyler S, Gjoni T, Koljatic J and Dupuis DS (2005) Mechanisms of allosteric modulation at GABAB receptors by CGP7930 and GS39783: effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology* **48**:343-353.

Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B and Kaupmann K (2001) Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* **60**:963-971.

Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M and Kaupmann K (2003) N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and Structurally Related Compounds: Novel Allosteric Enhancers of {gamma}-Aminobutyric AcidB Receptor Function. *J Pharmacol Exp Ther* **307**:322-330.

Vaccarino FJ, Pettit HO, Bloom FE and Koob GF (1985) Effects of intracerebroventricular administration of methyl naloxonium chloride on heroin self-administration in the rat. *Pharmacol Biochem Behav* **23**:495-498.

Valentino RJ and Commons KG (2005) Peptides that fine-tune the serotonin system. *Neuropeptides* **39**:1-8.

Van der Heyden JA, Zethof TJ and Olivier B (1997) Stress-induced hyperthermia in singly housed mice. *Physiol Behav* **62**:463-470.

van Gaalen MM, Stenzel-Poore M, Holsboer F and Steckler T (2003) Reduced attention in mice overproducing corticotropin-releasing hormone. *Behav Brain Res* **142**:69-79.

van Riezen H and Leonard BE (1990) Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol Ther* **47**:21-34.

Vaughan CW and Christie MJ (1996) Increase by the ORL1 receptor (opioid receptor-like1) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones. *Br J Pharmacol* **117**:1609-1611.

Velazquez-Moctezuma J and Diaz Ruiz O (1992) Neonatal treatment with clomipramine increased immobility in the forced swim test: an attribute of animal models of depression. *Pharmacol Biochem Behav* **42**:737-739.

Vogel G, Neill D, Kors D and Hagler M (1990) REM sleep abnormalities in a new animal model of endogenous depression. *Neurosci Biobehav Rev* **14**:77-83.

Vogel JR, Beer B and Clody DE (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* **21**:1-7.

Volkow ND (2005) What do we know about drug addiction? *Am J Psychiatry* **162**:1401-1402.

Volkow ND and Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cereb Cortex* **10**:318-325.

Volkow ND and Li TK (2004) Drug addiction: the neurobiology of behaviour gone awry. *Nat Rev Neurosci* **5**:963-970.

Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Angrist B, Gatley SJ, Logan J, Ding YS and Pappas N (1999) Association of methylphenidate-induced craving with changes in right striato-orbitofrontal metabolism in cocaine abusers: implications in addiction. *Am J Psychiatry* **156**:19-26.

Vollmayr B, Faust H, Lewicka S and Henn FA (2001) Brain-derived-neurotrophic-factor (BDNF) stress response in rats bred for learned helplessness. *Mol Psychiatry* **6**:471-474, 358.

Volpicelli JR, Alterman AI, Hayashida M and O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry* **49**:876-880.

Wahlsten D, Metten P and Crabbe JC (2003) A rating scale for wildness and ease of handling laboratory mice: results for 21 inbred strains tested in two laboratories. *Genes, Brain & Behavior* **2**:71-79.

Waldmeier PC (1991) The GABAB antagonist, CGP 35348, antagonizes the effects of baclofen, gamma-butyrolactone and HA 966 on rat striatal dopamine synthesis. *Naunyn Schmiedebergs Arch Pharmacol* **343**:173-178.

Walters CL and Blendy JA (2001) Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. *J Neurosci* **21**:9438-9444.

Watanabe A, Tohyama Y, Nguyen KQ, Hasegawa S, Debonnel G and Diksic M (2003) Regional brain serotonin synthesis is increased in the olfactory bulbectomy rat model of depression: an autoradiographic study. *J Neurochem* **85**:469-475.

Watanabe Y, Sakai RR, McEwen BS and Mendelson S (1993) Stress and antidepressant effects on hippocampal and cortical 5-HT1A and 5-HT2 receptors and transport sites for serotonin. *Brain Res* **615**:87-94.

Weizman R, Paz L, Backer MM, Amiri Z, Modai I and Pick CG (1999) Mouse strains differ in their sensitivity to alprazolam effect in the staircase test. *Brain Res* **839**:58-65.

Westerink BH, Kwint HF and deVries JB (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J Neurosci* **16**:2605-2611.

White FJ and Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. *Drug Alcohol Depend* **51**:141-153.

White JH, McIllhinney RA, Wise A, Ciruela F, Chan WY, Emson PC, Billinton A and Marshall FH (2000) The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc Natl Acad Sci U S A* **97**:13967-13972.

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM and Marshall FH (1998) Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* **396**:679-682.

White NM and McDonald RJ (1993) Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. *Behav Brain Res* **55**:269-281.

Williams JT, Colmers WF and Pan ZZ (1988) Voltage- and ligand-activated inwardly rectifying currents in dorsal raphe neurons in vitro. *J Neurosci* **8**:3499-3506.

Willner P (1983a) Dopamine and depression: a review of recent evidence. I. Empirical studies. *Brain Res* **287**:211-224.

Willner P (1983b) Dopamine and depression: a review of recent evidence. II. Theoretical approaches. *Brain Res* **287**:225-236.

Willner P (1983c) Dopamine and depression: a review of recent evidence. III. The effects of antidepressant treatments. *Brain Res* **287**:237-246.

Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* **134**:319-329.

Willner P and Mitchell PJ (2002) The validity of animal models of predisposition to depression. *Behav Pharmacol* **13**:169-188.

Willner P, Muscat R and Papp M (1992a) An animal model of anhedonia. *Clin Neuropharmacol* **15 Suppl 1 Pt A**:550A-551A.

Willner P, Muscat R and Papp M (1992b) Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* **16**:525-534.

Wirtshafter D and Sheppard AC (2001) Localization of GABA(B) receptors in midbrain monoamine containing neurons in the rat. *Brain Res Bull* **56**:1-5.

Wise RA, Bauco P, Carlezon WA, Jr. and Trojniar W (1992) Self-stimulation and drug reward mechanisms. *Ann N Y Acad Sci* **654**:192-198.

Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* **94**:469-492.

Wise RA and Rompre PP (1989) Brain dopamine and reward. *Annu Rev Psychol* **40**:191-225.

Wong EH, Sonders MS, Amara SG, Tinholt PM, Piercey MF, Hoffmann WP, Hyslop DK, Franklin S, Porsolt RD, Bonsignori A, Carfagna N and McArthur RA (2000) Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol Psychiatry* **47**:818-829.

Wong ML and Licinio J (2001) Research and treatment approaches to depression. *Nat Rev Neurosci* **2**:343-351.

Wu LG and Saggau P (1997) Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* **20**:204-212.

Xi ZX and Stein EA (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J Pharmacol Exp Ther* **290**:1369-1374.

Yakimova K, Sann H, Schmid HA and Pierau FK (1996) Effects of GABA agonists and antagonists on temperature-sensitive neurones in the rat hypothalamus. *J Physiol* **494 (Pt 1)**:217-230.

Yirmiya R, Pollak Y, Barak O, Avitsur R, Ovadia H, Bette M, Weihe E and Weidenfeld J (2001) Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. *Neuropsychopharmacology* **24**:531-544.

Young ST, Porrino LJ and Iadarola MJ (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc Natl Acad Sci U S A* **88**:1291-1295.

Yun IA, Wakabayashi KT, Fields HL and Nicola SM (2004) The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J Neurosci* **24**:2923-2933.

Zachariou V, Benoit-Marand M, Allen PB, Ingrassia P, Fienberg AA, Gonon F, Greengard P and Picciotto MR (2002) Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors DARPP 32 or Inhibitor 1. *Biol Psychiatry* **51**:612-620.

Zachariou V, Sgambato-Faure V, Sasaki T, Svenningsson P, Berton O, Fienberg AA, Nairn AC, Greengard P and Nestler EJ (2005) Phosphorylation of DARPP-32 at Threonine-34 is Required for Cocaine Action. *Neuropsychopharmacology*.

Zamponi GW (2001) Determinants of G protein inhibition of presynaptic calcium channels. *Cell Biochem Biophys* **34**:79-94.

Zanko M and Beigon A (1983) Increased adrenergic receptor binding in human frontal cortex of suicide victims. **Abstract No. 210.5.**

Zarrindast M, Rostami P and Sadeghi-Hariri M (2001) GABA(A) but not GABA(B) receptor stimulation induces antianxiety profile in rats. *Pharmacol Biochem Behav* **69**:9-15.

Zezula J, Slany A and Sieghart W (1996) Interaction of allosteric ligands with GABAA receptors containing one, two, or three different subunits. *Eur J Pharmacol* **301**:207-214.

Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J and Xu M (2002) The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. *J Neurochem* **82**:1453-1464.

Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, Zhang J and Xu M (2004) Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J Neurosci* **24**:3344-3354.

Zhou D, Grecksch G, Becker A, Frank C, Pilz J and Huether G (1998) Serotonergic hyperinnervation of the frontal cortex in an animal model of depression, the bulbectomized rat. *J Neurosci Res* **54**:109-116.

Carr GD, Fibiger HC and Phillips AG (1989) Conditioned place preference as a measure of drug reward., in *The neuropharmacological basis of reward.* (Liebman JM and J. CS eds) pp 264-319, Clarendon Press, Oxford.

Carr GD and White NM (1983) Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sci* **33**:2551-2557.

Carter LP, Wu H, Chen W, Cruz CM, Lamb RJ, Koek W, Coop A and France CP (2004) Effects of gamma-hydroxybutyrate (GHB) on schedule-controlled responding in rats: role of GHB and GABAB receptors. *J Pharmacol Exp Ther* **308**:182-188.

Cash CD (1994) Gamma-hydroxybutyrate: an overview of the pros and cons for it being a neurotransmitter and/or a useful therapeutic agent. *Neurosci Biobehav Rev* **18**:291-304.

Chalmers DT, Lopez JF, Vazquez DM, Akil H and Watson SJ (1994) Regulation of hippocampal 5-HT1A receptor gene expression by dexamethasone. *Neuropsychopharmacology* **10**:215-222.

Chamberlain SR, Blackwell AD, Fineberg NA, Robbins TW and Sahakian BJ (2005) The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. *Neurosci Biobehav Rev* **29**:399-419.

Chaperon F and Thiebot MH (1996) Effects of dopaminergic D3-receptor-preferring ligands on the acquisition of place conditioning in rats. *Behav Pharmacol* **7**:105-109.

Charles KJ, Evans ML, Robbins MJ, Calver AR, Leslie RA and Pangalos MN (2001) Comparative immunohistochemical localisation of GABA(B1a), GABA(B1b) and GABA(B2) subunits in rat brain, spinal cord and dorsal root ganglion. *Neuroscience* **106**:447-467.

Charney DS, Heninger GR and Breier A (1984) Noradrenergic function in panic anxiety. Effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Arch Gen Psychiatry* **41**:751-763.

Charney DS and Manji HK (2004) Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Sci STKE* **2004**:re5.

Charney DS, Woods SW, Goodman WK and Heninger GR (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* **92**:14-24.

Cheeta S, Ruigt G, van Proosdij J and Willner P (1997) Changes in sleep architecture following chronic mild stress. *Biol Psychiatry* **41**:419-427.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF and Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry* **50**:260-265.

Chen J, Kelz MB, Hope BT, Nakabeppu Y and Nestler EJ (1997) Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J Neurosci* **17**:4933-4941.

Chen Y, Phillips K, Minton G and Sher E (2005) GABA(B) receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *Br J Pharmacol* **144**:926-932.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M and O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* **156**:11-18.

Chin RL, Sporer KA, Cullison B, Dyer JE and Wu TD (1998) Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med* **31**:716-722.

Christie MJ, Summers RJ, Stephenson JA, Cook CJ and Beart PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. *Neuroscience* **22**:425-439.

Chu DC, Albin RL, Young AB and Penney JB (1990) Distribution and kinetics of GABAB binding sites in rat central nervous system: a quantitative autoradiographic study. *Neuroscience* **34**:341-357.

Clark JA, Mezey E, Lam AS and Bonner TI (2000) Distribution of the GABA(B) receptor subunit gb2 in rat CNS. *Brain Res* **860**:41-52.

Coffman JA and Petty F (1985) Plasma GABA levels in chronic alcoholics. *Am J Psychiatry* **142**:1204-1205.

Colby CR, Whisler K, Steffen C, Nestler EJ and Self DW (2003) Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. *J Neurosci* **23**:2488-2493.

Cole RL, Konradi C, Douglass J and Hyman SE (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* **14**:813-823.

Connor TJ, Song C, Leonard BE, Anisman H and Merali Z (1999) Stressor-induced alterations in serotonergic activity in an animal model of depression. *Neuroreport* **10**:523-528.

Contarino A, Dellu F, Koob GF, Smith GW, Lee KF, Vale W and Gold LH (1999) Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. *Brain Res* **835**:1-9.

Conti AC, Cryan JF, Dalvi A, Lucki I and Blendy JA (2002) cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J Neurosci* **22**:3262-3268.

Cooper JR, Bloom FE and Roth RH (2002) *The Biochemical Basis of Neuropharmacology*. Oxford University Press, New York.

Coppen AJ (1968) Depressed states and indolealkylamines. *Adv Pharmacol* **6**:283-291.
Corbett R, Fielding S, Cornfeldt M and Dunn RW (1991) GABAmimetic agents display anxiolytic-like effects in the social interaction and elevated plus maze procedures. *Psychopharmacology (Berl)* **104**:312-316.
Corrigall WA and Coen KM (1989) Fixed-interval schedules for drug self-administration in the rat. *Psychopharmacology (Berl)* **99**:136-139.
Corrigall WA, Coen KM, Adamson KL, Chow BL and Zhang J (2000) Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology (Berl)* **149**:107-114.
Costa AC, Stasko MR, Stoffel M and Scott-McKean JJ (2005) G-protein-gated potassium (GIRK) channels containing the GIRK2 subunit are control hubs for pharmacologically induced hypothermic responses. *J Neurosci* **25**:7801-7804.
Costa E (1989) Allosteric modulatory centers of transmitter amino acid receptors. *Neuropsychopharmacology* **2**:167-174.
Cott J, Carlsson A, Engel J and Lindqvist M (1976) Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. *Naunyn Schmiedebergs Arch Pharmacol* **295**:203-209.
Couve A, Filippov AK, Connolly CN, Bettler B, Brown DA and Moss SJ (1998) Intracellular retention of recombinant GABAB receptors. *J Biol Chem* **273**:26361-26367.
Coyle JT (2004) The GABA-glutamate connection in schizophrenia: which is the proximate cause? *Biochem Pharmacol* **68**:1507-1514.
Crawley JN (2000) *Whats wrong with my mouse? Behavioral Phenotyping of Transgenic and Knockout Mice.* Wiley-Liss, New York.
Crawley JN and Davis LG (1982) Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. *Brain Res Bull* **8**:609-612.
Crunelli V and Leresche N (1991) A role for GABAB receptors in excitation and inhibition of thalamocortical cells. *Trends Neurosci* **14**:16-21.
Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA and Luscher C (2004) Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat Neurosci* **7**:153-159.
Cryan JF, Bruijnzeel AW, Skjei KL and Markou A (2003a) Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. *Psychopharmacology (Berl).*
Cryan JF, Dalvi A, Jin SH, Hirsch BR, Lucki I and Thomas SA (2001) Use of dopamine-beta-hydroxylase-deficient mice to determine the role of norepinephrine in the mechanism of action of antidepressant drugs. *J Pharmacol Exp Ther* **298**:651-657.
Cryan JF and Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov* **4**:775-790.
Cryan JF, Hoyer D and Markou A (2003b) Withdrawal from chronic amphetamine induces Depressive-Like behavioral effects in rodents. *Biol Psychiatry* **54**:49-58.
Cryan JF and Kaupmann K (2005) Don't worry 'B' happy! a role for GABA(B) receptors in anxiety and depression. *Trends Pharmacol Sci* **26**:36-43.
Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, Bettler B, Kaupmann K and Spooren WP (2004) Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J Pharmacol Exp Ther* **310**:952-963.
Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ and Van Der Putten H (2003c) Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. *Eur J Neurosci* **17**:2409-2417.

Cryan JF and Leonard BE (2000) 5-HT1A and beyond: the role of serotonin and its receptors in depression and the antidepressant response. *Hum Psychopharmacol* **15**:113-135.

Cryan JF, Markou A and Lucki I (2002a) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **23**:238-245.

Cryan JF, McGrath C, Leonard BE and Norman TR (1998) Combining pindolol and paroxetine in an animal model of chronic antidepressant action--can early onset of action be detected? *Eur J Pharmacol* **352**:23-28.

Cryan JF, McGrath C, Leonard BE and Norman TR (1999) Onset of the effects of the 5-HT1A antagonist, WAY-100635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. *Pharmacol Biochem Behav* **63**:333-338.

Cryan JF and Mombereau C (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* **9**:326-357.

Cryan JF, Mombereau C and Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* **29**:571-625.

Cryan JF, Page ME and Lucki I (2002b) Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test. *Eur J Pharmacol* **436**:197-205.

Curran EJ, Akil H and Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. *Neurochem Res* **21**:1425-1435.

Dalia A and Wallace LJ (1995) Amphetamine induction of c-fos in the nucleus accumbens is not inhibited by glutamate antagonists. *Brain Res* **694**:299-307.

Dalvi A and Rodgers RJ (1996) GABAergic influences on plus-maze behaviour in mice. *Psychopharmacology (Berl)* **128**:380-397.

Dascal N (2001) Ion-channel regulation by G proteins. *Trends Endocrinol Metab* **12**:391-398.

Davis LL, Trivedi M, Choate A, Kramer GL and Petty F (1997) Growth hormone response to the GABAB agonist baclofen in major depressive disorder. *Psychoneuroendocrinology* **22**:129-140.

Davis M, Falls WA, Campeau S and Kim M (1993) Fear-potentiated startle: a neural and pharmacological analysis. *Behav Brain Res* **58**:175-198.

Dawson GR and Tricklebank MD (1995) Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends Pharmacol Sci* **16**:33-36.

de Kloet ER, Joels M and Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* **6**:463-475.

Dennis T, Beauchemin V and Lavoie N (1993) Differential effects of olfactory bulbectomy on GABAA and GABAB receptors in the rat brain. *Pharmacol Biochem Behav* **46**:77-82.

Detke MJ, Rickels M and Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)* **121**:66-72.

Di Ciano P and Everitt BJ (2003) The GABA(B) receptor agonist baclofen attenuates cocaine- and heroin-seeking behavior by rats. *Neuropsychopharmacology* **28**:510-518.

Dixon AK (1998) Ethological strategies for defence in animals and humans: their role in some psychiatric disorders. *Br J Med Psychol* **71 (Pt 4)**:417-445.

Dixon AK, Huber C and Lowe DA (1994) Clozapine promotes approach-oriented behavior in male mice. *J Clin Psychiatry* **55 Suppl B**:4-7.

Dobrovitsky V, Pimentel P, Duarte A, Froestl W, Stellar JR and Trzcinska M (2002) CGP 44532, a GABAB receptor agonist, is hedonically neutral and reduces cocaine-induced enhancement of reward. *Neuropharmacology* **42**:626-632.

Doherty MD and Gratton A (1999) Effects of medial prefrontal cortical injections of GABA receptor agonists and antagonists on the local and nucleus accumbens dopamine responses to stress. *Synapse* **32**:288-300.

Donny EC, Caggiula AR, Mielke MM, Jacobs KS, Rose C and Sved AF (1998) Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berl)* **136**:83-90.

Dowlatshahi D, MacQueen GM, Wang JF, Reiach JS and Young LT (1999) G Protein-coupled cyclic AMP signaling in postmortem brain of subjects with mood disorders: effects of diagnosis, suicide, and treatment at the time of death. *J Neurochem* **73**:1121-1126.

Dowlatshahi D, MacQueen GM, Wang JF and Young LT (1998) Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet* **352**:1754-1755.

Drake RG, Davis LL, Cates ME, Jewell ME, Ambrose SM and Lowe JS (2003) Baclofen treatment for chronic posttraumatic stress disorder. *Ann Pharmacother* **37**:1177-1181.

Drevets WC (2000) Neuroimaging studies of mood disorders. *Biol Psychiatry* **48**:813-829.

Ducottet C, Griebel G and Belzung C (2003) Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. *Prog Neuropsychopharmacol Biol Psychiatry* **27**:625-631.

Dulawa SC and Hen R (2005) Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev* **29**:771-783.

Dulawa SC, Holick KA, Gundersen B and Hen R (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* **29**:1321-1330.

Dunham N and Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm Ass*:208-209.

Dunn AL and Crnic LS (1993) Repeated injections of interferon-alpha A/D in Balb/c mice: behavioral effects. *Brain Behav Immun* **7**:104-111.

Dupuis DS, Relkovic D, Cryan JF and K. K (2004) MAPPING OF THE BINDING SITE OF THE GABAB POSITIVE MODULATOR GS39783 BY USE OF INTERSPECIES RAT/DROSOPHILA SUBUNIT COMBINATIONS, in *Society for Neuroscience*, San Diego.

Durkin MM, Gunwaldsen CA, Borowsky B, Jones KA and Branchek TA (1999) An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. *Brain Res Mol Brain Res* **71**:185-200.

Dutar P and Nicoll RA (1988) Pre- and postsynaptic GABAB receptors in the hippocampus have different pharmacological properties. *Neuron* **1**:585-591.

Eder M, Rammes G, Zieglgansberger W and Dodt HU (2001) GABA(A) and GABA(B) receptors on neocortical neurons are differentially distributed. *Eur J Neurosci* **13**:1065-1069.

El Hage W, Griebel G and Belzung C (2005) Long-term impaired memory following predatory stress in mice. *Physiol Behav*.

El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, Costentin J, Adrien J and Vaugeois JM (2003) Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. *Proc Natl Acad Sci U S A* **100**:6227-6232.

Epping-Jordan MP, Watkins SS, Koob GF and Markou A (1998) Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* **393**:76-79.

Epstein DH and Preston KL (2003) The reinstatement model and relapse prevention: a clinical perspective. *Psychopharmacology (Berl)* **168**:31-41.

Erhardt S, Mathe JM, Chergui K, Engberg G and Svensson TH (2002) GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. *Naunyn Schmiedebergs Arch Pharmacol* **365**:173-180.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N and Tobin AJ (1991) Two genes encode distinct glutamate decarboxylases. *Neuron* **7**:91-100.

Estape N and Steckler T (2002) Cholinergic blockade impairs performance in operant DNMTP in two inbred strains of mice. *Pharmacol Biochem Behav* **72**:319-334.

Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M and Martres MP (2000) Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci* **12**:2299-2310.

Fadda P, Scherma M, Fresu A, Collu M and Fratta W (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* **50**:1-6.

Feighner JP and Boyer WF (1989) Serotonin-1A anxiolytics: an overview. *Psychopathology* **22 Suppl 1**:21-26.

File SE and Andrews N (1993) Benzodiazepine withdrawal: behavioural pharmacology and neurochemical changes. *Biochem Soc Symp* **59**:97-106.

File SE and Hyde JR (1978) Can social interaction be used to measure anxiety? *Br J Pharmacol* **62**:19-24.

File SE, Zharkovsky A and Gulati K (1991) Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology* **30**:183-190.

File SE, Zharkovsky A and Hitchcott PK (1992) Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry* **16**:87-93.

Finn DA, Rutledge-Gorman MT and Crabbe JC (2003) Genetic animal models of anxiety. *Neurogenetics* **4**:109-135.

Flannery BA, Garbutt JC, Cody MW, Renn W, Grace K, Osborne M, Crosby K, Morreale M and Trivette A (2004) Baclofen for alcohol dependence: a preliminary open-label study. *Alcohol Clin Exp Res* **28**:1517-1523.

Florey E (1954) An inhibitory and an excitatory factor of mammalian central nervous system, and their action of a single sensory neuron. *Arch Int Physiol* **62**:33-53.

Flugge G (1995) Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. *J Neurosci* **15**:7132-7140.

Foltin RW (2005) Baclofen decreases feeding in non-human primates. *Pharmacol Biochem Behav*.

Forget B, Hamon M and Thiebot MH (2005) Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. *Psychopharmacology (Berl)*:1-13.

Frazer A (1997) Pharmacology of antidepressants. *J Clin Psychopharmacol* **17 Suppl 1**:2S-18S.

Freed WJ, Crump S and Jeste DV (1984) Genetic effects on PCP-induced stimulation in recombinant inbred strains of mice. *Pharmacol Biochem Behav* **21**:159-162.

Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D and Mohler H (1999) GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *Eur J Neurosci* **11**:761-768.

Frodl T, Meisenzahl E, Zetzsche T, Bottlender R, Born C, Groll C, Jager M, Leinsinger G, Hahn K and Moller HJ (2002) Enlargement of the amygdala in patients with a first episode of major depression. *Biol Psychiatry* **51**:708-714.

Frodl T, Meisenzahl EM, Zetzsche T, Born C, Jager M, Groll C, Bottlender R, Leinsinger G and Moller HJ (2003) Larger amygdala volumes in first depressive episode as

compared to recurrent major depression and healthy control subjects. *Biol Psychiatry* **53**:338-344.

Froestl W, Bettler B, Bittiger H, Heid J, Kaupmann K, Mickel SJ and Strub D (1999) Ligands for the isolation of GABA(B) receptors [corrected]. *Neuropharmacology* **38**:1641-1646.

Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S, Mondadori CG and Pearlman R (2004) SGS742: the first GABA(B) receptor antagonist in clinical trials. *Biochem Pharmacol* **68**:1479-1487.

Froestl W and Mickel S (1997) Chemistry of GABAB modulators, in *The GABA Receptors* (Enna S and Bowery NG eds) pp pp 271-296, Humana

Press., Totowa, NJ.

Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J, Olpe HR and et al. (1995) Phosphinic acid analogues of GABA. 1. New potent and selective GABAB agonists. *J Med Chem* **38**:3297-3312.

Froger N, Palazzo E, Boni C, Hanoun N, Saurini F, Joubert C, Dutriez-Casteloot I, Enache M, Maccari S, Barden N, Cohen-Salmon C, Hamon M and Lanfumey L (2004) Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. *J Neurosci* **24**:2787-2796.

Frosini M, Valoti M and Sgaragli G (2004) Changes in rectal temperature and ECoG spectral power of sensorimotor cortex elicited in conscious rabbits by i.c.v. injection of GABA, GABA(A) and GABA(B) agonists and antagonists. *Br J Pharmacol* **141**:152-162.

Fudala PJ, Teoh KW and Iwamoto ET (1985) Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol Biochem Behav* **22**:237-241.

Gallimberti L, Schifano F, Forza G, Miconi L and Ferrara SD (1994) Clinical efficacy of gamma-hydroxybutyric acid in treatment of opiate withdrawal. *Eur Arch Psychiatry Clin Neurosci* **244**:113-114.

Galvez T, Parmentier ML, Joly C, Malitschek B, Kaupmann K, Kuhn R, Bittiger H, Froestl W, Bettler B and Pin JP (1999) Mutagenesis and modeling of the GABAB receptor extracellular domain support a venus flytrap mechanism for ligand binding. *J Biol Chem* **274**:13362-13369.

Gambarana C, Scheggi S, Tagliamonte A, Tolu P and De Montis MG (2001) Animal models for the study of antidepressant activity. *Brain Res Brain Res Protoc* **7**:11-20.

Gardner EL, Schiffer WK, Horan BA, Highfield D, Dewey SL, Brodie JD and Ashby CR, Jr. (2002) Gamma-vinyl GABA, an irreversible inhibitor of GABA transaminase, alters the acquisition and expression of cocaine-induced sensitization in male rats. *Synapse* **46**:240-250.

Gartside SE, Umbers V, Hajos M and Sharp T (1995) Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* **115**:1064-1070.

Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Muller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jaton AL, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy JM, Luthi A, Kaupmann K and Bettler B (2004) Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *J Neurosci* **24**:6086-6097.

Gavioli EC, Marzola G, Guerrini R, Bertorelli R, Zucchini S, De Lima TC, Rae GA, Salvadori S, Regoli D and Calo G (2003) Blockade of nociceptin/orphanin FQ-NOP receptor signalling produces antidepressant-like effects: pharmacological and genetic evidences from the mouse forced swimming test. *Eur J Neurosci* **17**:1987-1990.

Gavioli EC, Vaughan CW, Marzola G, Guerrini R, Mitchell VA, Zucchini S, De Lima TC, Rae GA, Salvadori S, Regoli D and Calo G (2004) Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. *Naunyn Schmiedebergs Arch Pharmacol* **369**:547-553.

Geller I, Kulak JT, Jr. and Seifter J (1962) The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. *Psychopharmacologia* **3**:374-385.

Gerber U and Gahwiler BH (1994) GABAB and adenosine receptors mediate enhancement of the K+ current, IAHP, by reducing adenylyl cyclase activity in rat CA3 hippocampal neurons. *J Neurophysiol* **72**:2360-2367.

Geyer MA and Markou A (1995) Animal models of psychiatric disorders., in *Psychopharmacology: The Fourth Generation of Progress.* (Bloom FE and Kupfer DJ eds) pp 787-798, Raven Press.

Geyer MA and Markou A (2000) The role of preclinical models in the development of psychotropic drugs., in *Psychopharmacology: The Fifth Generation of Progress.* (Bloom FE and Kupfer DJ eds), Raven, New York.

Gilbert P and Allan S (1998) The role of defeat and entrapment (arrested flight) in depression: an exploration of an evolutionary view. *Psychol Med* **28**:585-598.

Gilliss B, Malanga CJ, Pieper JO and Carlezon WA, Jr. (2002) Cocaine and SKF-82958 potentiate brain stimulation reward in Swiss-Webster mice. *Psychopharmacology (Berl)* **163**:238-248.

Giorgetti M, Hotsenpiller G, Froestl W and Wolf ME (2002) In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. *Neuroscience* **109**:585-595.

Goddard AW, Mason GF, Almai A, Rothman DL, Behar KL, Petroff OA, Charney DS and Krystal JH (2001) Reductions in occipital cortex GABA levels in panic disorder detected with 1h-magnetic resonance spectroscopy. *Arch Gen Psychiatry* **58**:556-561.

Gold BI, Bowers MB, Jr., Roth RH and Sweeney DW (1980) GABA levels in CSF of patients with psychiatric disorders. *Am J Psychiatry* **137**:362-364.

Goldberg MR, Hollister AS and Robertson D (1983) Influence of yohimbine on blood pressure, autonomic reflexes, and plasma catecholamines in humans. *Hypertension* **5**:772-778.

Goldberg SR, Risner ME, Stolerman IP, Reavill C and Garcha HS (1989) Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. *Psychopharmacology (Berl)* **97**:295-302.

Gong W, Neill D and Justice JB, Jr. (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. *Brain Res* **754**:103-112.

Gong W, Neill DB and Justice JB, Jr. (1998) GABAergic modulation of ventral pallidal dopamine release studied by in vivo microdialysis in the freely moving rat. *Synapse* **29**:406-412.

Goodwin GM, De Souza RJ and Green AR (1985) Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. *Nature* **317**:531-533.

Gorman JM, Liebowitz MR, Fyer AJ, Goetz D, Campeas RB, Fyer MR, Davies SO and Klein DF (1987) An open trial of fluoxetine in the treatment of panic attacks. *J Clin Psychopharmacol* **7**:329-332.

Grabarits F, Lal H and Chessick RD (1966) Effects of cocaine on brain noradrenaline in relation to toxicity and convulsions in mice. *J Pharm Pharmacol* **18**:131-132.

Graeff FG, Guimaraes FS, De Andrade TG and Deakin JF (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav* **54**:129-141.

Gray JA, Goodwin GM, Heal DJ and Green AR (1987) Hypothermia induced by baclofen, a possible index of GABAB receptor function in mice, is enhanced by antidepressant drugs and ECS. *Br J Pharmacol* **92**:863-870.

Gray JA and Green AR (1987) Increased GABAB receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks. *Br J Pharmacol* **92**:357-362.

Graybiel AM, Moratalla R and Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc Natl Acad Sci U S A* **87**:6912-6916.

Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, Ballenger JC and Fyer AJ (1999) The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry* **60**:427-435.

Greengard P, Allen PB and Nairn AC (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* **23**:435-447.

Greif GJ, Sodickson DL, Bean BP, Neer EJ and Mende U (2000) Altered regulation of potassium and calcium channels by GABA(B) and adenosine receptors in hippocampal neurons from mice lacking Galpha(o). *J Neurophysiol* **83**:1010-1018.

Griebel G, Belzung C, Perrault G and Sanger DJ (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology (Berl)* **148**:164-170.

Griebel G, Simiand J, Serradeil-Le Gal C, Wagnon J, Pascal M, Scatton B, Maffrand JP and Soubrie P (2002a) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc Natl Acad Sci U S A* **99**:6370-6375.

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, Geslin M, Scatton B, Maffrand JP and Soubrie P (2002b) 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther* **301**:333-345.

Grippo AJ, Beltz TG and Johnson AK (2003) Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav* **78**:703-710.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S and Hen R (2002) Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**:396-400.

Guidotti A, Baraldi M, Leon A and Costa E (1980) Benzodiazepines: a tool to explore the biochemical and neurophysiological basis of anxiety. *Fed Proc* **39**:3039-3042.

Guitart X, Thompson MA, Mirante CK, Greenberg ME and Nestler EJ (1992) Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. *J Neurochem* **58**:1168-1171.

Guo TZ, Reid K, Davies MF, Nacif-Coelho C, Rabin BC, Gonzalez F and Maze M (1998) Chronic desipramine treatment desensitizes the rat to anesthetic and antinociceptive effects of the alpha2-adrenergic agonist dexmedetomidine. *Anesthesiology* **88**:1634-1642.

Haefely W, Kulcsar A and Mohler H (1975) Possible involvement of GABA in the central actions of benzodiazepines. *Psychopharmacol Bull* **11**:58-59.

Haller C, Casanova E, Muller M, Vacher CM, Vigot R, Doll T, Barbieri S, Gassmann M and Bettler B (2004) Floxed allele for conditional inactivation of the GABAB(1) gene. *Genesis* **40**:125-130.

Hansen HH, Sanchez C and Meier E (1997) Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in

adult rats: a putative animal model of depression? *J Pharmacol Exp Ther* **283**:1333-1341.

Harayama N, Shibuya I, Tanaka K, Kabashima N, Ueta Y and Yamashita H (1998) Inhibition of N- and P/Q-type calcium channels by postsynaptic GABAB receptor activation in rat supraoptic neurones. *J Physiol* **509 (Pt 2)**:371-383.

Harkin A, Houlihan DD and Kelly JP (2002) Reduction in preference for saccharin by repeated unpredictable stress in mice and its prevention by imipramine. *J Psychopharmacol* **16**:115-123.

Harrison AA, Gasparini F and Markou A (2002) Nicotine potentiation of brain stimulation reward reversed by DH beta E and SCH 23390, but not by eticlopride, LY 314582 or MPEP in rats. *Psychopharmacology (Berl)* **160**:56-66.

Harrison AA, Liem YT and Markou A (2001) Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. *Neuropsychopharmacology* **25**:55-71.

Harrison NL (1990) On the presynaptic action of baclofen at inhibitory synapses between cultured rat hippocampal neurones. *J Physiol* **422**:433-446.

Hascoet M and Bourin M (1998) A new approach to the light/dark test procedure in mice. *Pharmacol Biochem Behav* **60**:645-653.

Hashimoto T and Kuriyama K (1997) In vivo evidence that GABA(B) receptors are negatively coupled to adenylate cyclase in rat striatum. *J Neurochem* **69**:365-370.

Hausler A, Monnet G and Peter O (1993) Involvement of GABAB receptors in the regulation of the hypothalamo-pituitary-adrenocortical (HPA) axis in rats. *J Steroid Biochem Mol Biol* **46**:767-771.

Hawrot E, Xiao Y, Shi QL, Norman D, Kirkitadze M and Barlow PN (1998) Demonstration of a tandem pair of complement protein modules in GABA(B) receptor 1a. *FEBS Lett* **432**:103-108.

Hayashi T (1959) The inhibitory action of beta-hydroxy-gamma-aminobutyric acid upon the seizure following stimulation of the motor cortex of the dog. *J Physiol* **145**:570-578.

Heese K, Otten U, Mathivet P, Raiteri M, Marescaux C and Bernasconi R (2000) GABA(B) receptor antagonists elevate both mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats. *Neuropharmacology* **39**:449-462.

Heidbreder CA and Hagan JJ (2005) Novel pharmacotherapeutic approaches for the treatment of drug addiction and craving. *Curr Opin Pharmacol* **5**:107-118.

Hendry SH, Schwark HD, Jones EG and Yan J (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J Neurosci* **7**:1503-1519.

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T and Catterall WA (1996) Modulation of Ca2+ channels by G-protein beta gamma subunits. *Nature* **380**:258-262.

Hetherington HP, Pan JW, Chu WJ, Mason GF and Newcomer BR (1997) Biological and clinical MRS at ultra-high field. *NMR Biomed* **10**:360-371.

Higgins GA, Bradbury AJ, Jones BJ and Oakley NR (1988) Behavioural and biochemical consequences following activation of 5HT1-like and GABA receptors in the dorsal raphe nucleus of the rat. *Neuropharmacology* **27**:993-1001.

Hill DR and Bowery NG (1981) 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature* **290**:149-152.

Hinderer SR (1990) The supraspinal anxiolytic effect of baclofen for spasticity reduction. *Am J Phys Med Rehabil* **69**:254-258.

Hiroi N, Brown JR, Haile CN, Ye H, Greenberg ME and Nestler EJ (1997) FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened

sensitivity to cocaine's psychomotor and rewarding effects. *Proc Natl Acad Sci U S A* **94**:10397-10402.

Hiroi N, Fienberg AA, Haile CN, Alburges M, Hanson GR, Greengard P and Nestler EJ (1999) Neuronal and behavioural abnormalities in striatal function in DARPP-32-mutant mice. *Eur J Neurosci* **11**:1114-1118.

Holmes A (2001) Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neurosci Biobehav Rev* **25**:261-273.

Holmes A, Heilig M, Rupniak NM, Steckler T and Griebel G (2003a) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci* **24**:580-588.

Holmes A, Parmigiani S, Ferrari PF, Palanza P and Rodgers RJ (2000) Behavioral profile of wild mice in the elevated plus-maze test for anxiety. *Physiol Behav* **71**:509-516.

Holmes A, Yang RJ and Crawley JN (2002) Evaluation of an anxiety-related phenotype in galanin overexpressing transgenic mice. *J Mol Neurosci* **18**:151-165.

Holmes A, Yang RJ, Lesch KP, Crawley JN and Murphy DL (2003b) Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology* **28**:2077-2088.

Holsboer F, Bender W, Benkert O, Klein HE and Schmauss M (1980) Diagnostic value of dexamethasone suppression test in depression. *Lancet* **2**:706.

Honig A, Bartlett JR, Bouras N and Bridges PK (1988) Amino acid levels in depression: a preliminary investigation. *J Psychiatr Res* **22**:159-164.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS and Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* **13**:1235-1244.

Hotsenpiller G and Wolf ME (2003) Baclofen attenuates conditioned locomotion to cues associated with cocaine administration and stabilizes extracellular glutamate levels in rat nucleus accumbens. *Neuroscience* **118**:123-134.

Hozumi S, Nakagawasai O, Tan-No K, Niijima F, Yamadera F, Murata A, Arai Y, Yasuhara H and Tadano T (2003) Characteristics of changes in cholinergic function and impairment of learning and memory-related behavior induced by olfactory bulbectomy. *Behav Brain Res* **138**:9-15.

Hu XT, Ford K and White FJ (2005) Repeated cocaine administration decreases calcineurin (PP2B) but enhances DARPP-32 modulation of sodium currents in rat nucleus accumbens neurons. *Neuropsychopharmacology* **30**:916-926.

Humeniuk RE, Ong J, Kerr DI and White JM (1995) Characterization of GABAB ligands in vivo. *Gen Pharmacol* **26**:417-424.

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. *Nature* **380**:255-258.

Imperato A, Mulas A and Di Chiara G (1987) The D-1 antagonist SCH 23390 stimulates while the D-1 agonist SKF 38393 fails to affect dopamine release in the dorsal caudate of freely moving rats. *Eur J Pharmacol* **142**:177-181.

Imperato A, Tanda G, Frau R and Di Chiara G (1988) Pharmacological profile of dopamine receptor agonists as studied by brain dialysis in behaving rats. *J Pharmacol Exp Ther* **245**:257-264.

Innis RB, Nestler EJ and Aghajanian GK (1988) Evidence for G protein mediation of serotonin- and GABAB-induced hyperpolarization of rat dorsal raphe neurons. *Brain Res* **459**:27-36.

Inoue T, Tsuchiya K and Koyama T (1994) Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* **49**:911-920.

Ito Y, Ishige K, Zaitsu E, Anzai K and Fukuda H (1995) gamma-Hydroxybutyric acid increases intracellular Ca2+ concentration and nuclear cyclic AMP-responsive element- and activator protein 1 DNA-binding activities through GABAB receptor in cultured cerebellar granule cells. *J Neurochem* **65**:75-83.

Izumi J, Washizuka M, Hayashi-Kuwabara Y, Yoshinaga K, Tanaka Y, Ikeda Y, Kiuchi Y and Oguchi K (1996) An attenuated alpha-1 potentiation of beta adrenoceptor-stimulated cyclic AMP formation after repeated saline injections in Fischer 344 strain rats. *Life Sci* **59**:33-42.

Jackson HC and Nutt DJ (1991) Inhibition of baclofen-induced hypothermia in mice by the novel GABAB antagonist CGP 35348. *Neuropharmacology* **30**:535-538.

Jacobs EH, Smit AB, de Vries TJ and Schoffelmeer AN (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci* **24**:566-573.

Jacobson LH and Cryan JF (2005) Differential sensitivity to the motor and hypothermic effects of the GABA B receptor agonist baclofen in various mouse strains. *Psychopharmacology (Berl)* **179**:688-699.

Jacobson LH, Kaupmann K, Bettler B and Cryan JF (2005) Differential influence of GABAB(1) subunit isoforms on baseline but not GABAB agonist-induced behavioural changes in mice., in *Society for Neuroscience*, Washington, D.C.

Jefferys D and Funder J (1996) Nitric oxide modulates retention of immobility in the forced swimming test in rats. *Eur J Pharmacol* **295**:131-135.

Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roever S, Kolczewski S, Adam G and Kilpatrick G (2000) A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: anxiolytic profile in the rat. *Proc Natl Acad Sci U S A* **97**:4938-4943.

Jesberger JA and Richardson JS (1988) Brain output dysregulation induced by olfactory bulbectomy: an approximation in the rat of major depressive disorder in humans? *Int J Neurosci* **38**:241-265.

Johnson SW and North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol* **450**:455-468.

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA and Gerald C (1998) GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* **396**:674-679.

Jones N, Duxon MS and King SM (2002a) Ethopharmacological analysis of the unstable elevated exposed plus maze, a novel model of extreme anxiety: predictive validity and sensitivity to anxiogenic agents. *Psychopharmacology (Berl)* **161**:314-323.

Jones N, King SM and Duxon MS (2002b) Further evidence for the predictive validity of the unstable elevated exposed plus-maze, a behavioural model of extreme anxiety in rats: differential effects of fluoxetine and chlordiazepoxide. *Behav Pharmacol* **13**:525-535.

Jongsma ME, Cremers TIF, Rea K, Bosker FJ, Hogg S and Westerink BHC (2004) POTENTIATION OF SSRI-INDUCED ELEVATION OF 5-NT BY CO-ADMINISTRATION OF GABA-B ANTAGONISTS, in *Society for Neuroscience*, San diego.

Kafkafi N, Pagis M, Lipkind D, Mayo CL, Bemjamini Y, Golani I and Elmer GI (2003) Darting behavior: a quantitative movement pattern designed for discrimination and replicability in mouse locomotor behavior. *Behav Brain Res* **142**:193-205.

Kalivas PW, Duffy P and Eberhardt H (1990) Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J Pharmacol Exp Ther* **253**:858-866.

Kalivas PW, Sorg BA and Hooks MS (1993) The pharmacology and neural circuitry of sensitization to psychostimulants. *Behav Pharmacol* **4**:315-334.

Kalivas PW and Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev* **16**:223-244.

Kalivas PW and Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* **162**:1403-1413.

Kano T, Suzuki Y, Shibuya M, Kiuchi K and Hagiwara M (1995) Cocaine-induced CREB phosphorylation and c-Fos expression are suppressed in Parkinsonism model mice. *Neuroreport* **6**:2197-2200.

Kaplan GB, Leite-Morris KA, Joshi M, Shoeb MH and Carey RJ (2003) Baclofen inhibits opiate-induced conditioned place preference and associated induction of Fos in cortical and limbic regions. *Brain Res* **987**:122-125.

Karlsson G, Pozza M and Olpe HR (1988) Phaclofen: a GABAB blocker reduces long-duration inhibition in the neocortex. *Eur J Pharmacol* **148**:485-486.

Karolyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, Seong E, Camper SA and Seasholtz AF (1999) Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. *Proc Natl Acad Sci U S A* **96**:11595-11600.

Kasture SB, Mandhane SN and Chopde CT (1996) Baclofen-induced catatonia: modification by serotonergic agents. *Neuropharmacology* **35**:595-598.

Kaufman DL, Houser CR and Tobin AJ (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J Neurochem* **56**:720-723.

Kaupmann K, Cryan J, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Bräuner-Osborne H, Waldmeier P and Bettler B (2003) Specific -Hydroxybutyrate (GHB) binding sites but loss of pharmacological effects of GHB in GABAB(1) deficient mice. *Eur J Neurosci*.

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386**:239-246.

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A and Bettler B (1998a) GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396**:683-687.

Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, Froestl W, Leonhard S, Pfaff T, Karschin A and Bettler B (1998b) Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K+ channels. *Proc Natl Acad Sci U S A* **95**:14991-14996.

Kawahara Y, Kawahara H and Westerink BH (1999) Tonic regulation of the activity of noradrenergic neurons in the locus coeruleus of the conscious rat studied by dual-probe microdialysis. *Brain Res* **823**:42-48.

Ke Y, Cohen BM, Bang JY, Yang M and Renshaw PF (2000) Assessment of GABA concentration in human brain using two-dimensional proton magnetic resonance spectroscopy. *Psychiatry Res* **100**:169-178.

Kelly JP, Wrynn AS and Leonard BE (1997) The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* **74**:299-316.

Kelly PH, Seviour PW and Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res* **94**:507-522.

Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman

RS, Picciotto MR and Nestler EJ (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* **401**:272-276.

Kelz MB and Nestler EJ (2000) deltaFosB: a molecular switch underlying long-term neural plasticity. *Curr Opin Neurol* **13**:715-720.

Kemmling AK, Rubio MC and Balerio GN (2002) Baclofen prevents morphine withdrawal irrespective of seasonal variation. *Behav Pharmacol* **13**:87-92.

Kenny PJ, Boutrel B, Gasparini F, Koob GF and Markou A (2005) Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)* **179**:247-254.

Kerr DI and Ong J (1995) GABAB receptors. *Pharmacol Ther* **67**:187-246.

Kerr DI, Ong J, Johnston GA, Abbenante J and Prager RH (1988) 2-Hydroxy-saclofen: an improved antagonist at central and peripheral GABAB receptors. *Neurosci Lett* **92**:92-96.

Kerr DI, Ong J, Prager RH, Gynther BD and Curtis DR (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res* **405**:150-154.

Kerr DI, Ong J, Puspawati NM and Prager RH (2002) Arylalkylamines are a novel class of positive allosteric modulators at GABA(B) receptors in rat neocortex. *Eur J Pharmacol* **451**:69-77.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE and Wang PS (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *Jama* **289**:3095-3105.

Ketelaars CE, Bollen EL, Rigter H and Bruinvels J (1988) GABA-B receptor activation and conflict behaviour. *Life Sci* **42**:933-942.

Ketelaars CE and Bruinvels J (1989) The anti-conflict effect of cyproheptadine is not mediated by its 5-hydroxytryptamine antagonistic property. *Life Sci* **44**:1743-1749.

Kilts CD (2001) Imaging the roles of the amygdala in drug addiction. *Psychopharmacol Bull* **35**:84-94.

Kim S, Lee S, Ryu S, Suk J and Park C (2002) Comparative analysis of the anxiety-related behaviors in four inbred mice. *Behav Processes* **60**:181-190.

King SM (1999a) Escape-related behaviours in an unstable elevated and exposed environment. I. A new behavioural model of extreme anxiety. *Behav Brain Res* **98**:113-126.

King SM (1999b) Escape-related behaviours in an unstable, elevated and exposed environment. II. Long-term sensitization after repetitive electrical stimulation of the rodent midbrain defence system. *Behav Brain Res* **98**:127-142.

Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G and Ordway GA (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. *J Neurosci* **17**:8451-8458.

Kniazeff J, Galvez T, Labesse G and Pin JP (2002) No ligand binding in the GB2 subunit of the GABA(B) receptor is required for activation and allosteric interaction between the subunits. *J Neurosci* **22**:7352-7361.

Koenig JI, Kirkpatrick B and Lee P (2002) Glucocorticoid hormones and early brain development in schizophrenia. *Neuropsychopharmacology* **27**:309-318.

Kokkinidis L, Zacharko RM and Anisman H (1986) Amphetamine withdrawal: a behavioral evaluation. *Life Sci* **38**:1617-1623.

Koob GF (1992) Neural mechanisms of drug reinforcement. *Ann N Y Acad Sci* **654**:171-191.

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. *Neuron* **16**:893-896.

Kornetsky C (2004) Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. *Neurosci Biobehav Rev* **27**:777-786.

Kram ML, Kramer GL, Steciuk M, Ronan PJ and Petty F (2000) Effects of learned helplessness on brain GABA receptors. *Neurosci Res* **38**:193-198.

Kravitz EA, Kuffler SW, Potter DD and Vangelder NM (1963) Gamma-Aminobutyric Acid and Other Blocking Compounds in Crustacea. Ii. Peripheral Nervous System. *J Neurophysiol* **26**:729-738.

Kreibich AS and Blendy JA (2004) cAMP response element-binding protein is required for stress but not cocaine-induced reinstatement. *J Neurosci* **24**:6686-6692.

Krnjevic K (1974) Chemical nature of synaptic transmission in vertebrates. *Physiol Rev* **54**:418-540.

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A and Mason GF (2002) Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry* **7 Suppl 1**:S71-80.

Kubota H, Katsurabayashi S, Moorhouse AJ, Murakami N, Koga H and Akaike N (2003) GABAB receptor transduction mechanisms, and cross-talk between protein kinases A and C, in GABAergic terminals synapsing onto neurons of the rat nucleus basalis of Meynert. *J Physiol* **551**:263-276.

Kuczenski R, Segal DS, Cho AK and Melega W (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci* **15**:1308-1317.

Kuffler SW and Edwards C (1958) Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J Neurophysiol* **21**:589-610.

Kuhn R (1957) [Treatment of depressive states with an iminodibenzyl derivative (G 22355).]. *Schweiz Med Wochenschr* **87**:1135-1140.

Kulik A, Nakadate K, Nyiri G, Notomi T, Malitschek B, Bettler B and Shigemoto R (2002) Distinct localization of GABA(B) receptors relative to synaptic sites in the rat cerebellum and ventrobasal thalamus. *Eur J Neurosci* **15**:291-307.

Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A and Kornau HC (1999) Role of heteromer formation in GABAB receptor function. *Science* **283**:74-77.

Kuroda Y, Watanabe Y, Albeck DS, Hastings NB and McEwen BS (1994) Effects of adrenalectomy and type I or type II glucocorticoid receptor activation on 5-HT1A and 5-HT2 receptor binding and 5-HT transporter mRNA expression in rat brain. *Brain Res* **648**:157-161.

Lacaille JC (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampal slices in vitro. *J Neurophysiol* **66**:1441-1454.

Lacey MG, Mercuri NB and North RA (1988) On the potassium conductance increase activated by GABAB and dopamine D2 receptors in rat substantia nigra neurones. *J Physiol* **401**:437-453.

Le Maitre E, Vilpoux C, Costentin J and Leroux-Nicollet I (2005) Opioid receptor-like 1 (NOP) receptors in the rat dorsal raphe nucleus: evidence for localization on serotoninergic neurons and functional adaptation after 5,7-dihydroxytryptamine lesion. *J Neurosci Res* **81**:488-496.

Le Poul E, Boni C, Hanoun N, Laporte AM, Laaris N, Chauveau J, Hamon M and Lanfumey L (2000) Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. *Neuropharmacology* **39**:110-122.

Lecci A, Borsini F, Volterra G and Meli A (1990) Pharmacological validation of a novel animal model of anticipatory anxiety in mice. *Psychopharmacology (Berl)* **101**:255-261.

Leitch MM, Ingram CD, Young AH, McQuade R and Gartside SE (2003) Flattening the corticosterone rhythm attenuates 5-HT1A autoreceptor function in the rat: relevance for depression. *Neuropsychopharmacology* **28**:119-125.

Leite-Morris KA, Fukudome EY, Shoeb MH and Kaplan GB (2004) GABA(B) receptor activation in the ventral tegmental area inhibits the acquisition and expression of opiate-induced motor sensitization. *J Pharmacol Exp Ther* **308**:667-678.

Leith NJ and Barrett RJ (1976) Amphetamine and the reward system: evidence for tolerance and post-drug depression. *Psychopharmacologia* **46**:19-25.

Lepicard EM, Joubert C, Hagneau I, Perez-Diaz F and Chapouthier G (2000) Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. *Pharmacol Biochem Behav* **67**:739-748.

Lepore M, Vorel SR, Lowinson J and Gardner EL (1995) Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. *Life Sci* **56**:2073-2080.

Leshner AI, Remler H, Biegon A and Samuel D (1979) Desmethylimipramine (DMI) counteracts learned helplessness in rats. *Psychopharmacology (Berl)* **66**:207-208.

Li SM, Yin LL, Ren YH, Pan LS and Zheng JW (2001) GABA(B) receptor agonist baclofen attenuates the development and expression of d-methamphetamine-induced place preference in rats. *Life Sci* **70**:349-356.

Lile JA, Stoops WW, Allen TS, Glaser PE, Hays LR and Rush CR (2004) Baclofen does not alter the reinforcing, subject-rated or cardiovascular effects of intranasal cocaine in humans. *Psychopharmacology (Berl)* **171**:441-449.

Lin XH, Hashimoto T, Kitamura N, Murakami N, Shirakawa O and Maeda K (2002) Decreased calcineurin and increased phosphothreonine-DARPP-32 in the striatum of rats behaviorally sensitized to methamphetamine. *Synapse* **44**:181-187.

Ling W, Shoptaw S and Majewska D (1998) Baclofen as a cocaine anti-craving medication: a preliminary clinical study. *Neuropsychopharmacology* **18**:403-404.

Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B and Mosbacher J (1999) Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* **38**:1667-1673.

Liu FC and Graybiel AM (1996) Spatiotemporal dynamics of CREB phosphorylation: transient versus sustained phosphorylation in the developing striatum. *Neuron* **17**:1133-1144.

Liu QS, Pu L and Poo MM (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature* **437**:1027-1031.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**:402-408.

Lloyd KG, Zivkovic B, Scatton B, Morselli PL and Bartholini G (1989) The gabaergic hypothesis of depression. *Prog Neuropsychopharmacol Biol Psychiatry* **13**:341-351.

Lobina C, Colombo G, Gessa GL and Carai MA (2005) Different sensitivity to the motor incoordinating effects of gamma-hydroxybutyric acid (GHB) and baclofen in GHB-sensitive and GHB-resistant rats. *Brain Res* **1033**:109-112.

Logan L, Seale TW, Cao W and Carney JM (1988) Effects of chronic amphetamine in BALB/cBy mice, a strain that is not stimulated by acute administration of amphetamine. *Pharmacol Biochem Behav* **31**:675-682.

Loomer HP, Saunders JC and Kline NS (1957) A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. *Psychiatr Res Rep Am Psychiatr Assoc* **135**:129-141.

Lopez JF, Chalmers DT, Little KY and Watson SJ (1998) A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry* **43**:547-573.

Losada ME (1991) Baclofen and noradrenergic function in the rat frontal cerebral cortex. *Eur J Pharmacol* **197**:93-98.

Lucki I (1997) The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* **8**:523-532.

Lucki I (2001) A prescription to resist proscriptions for murine models of depression. *Psychopharmacology (Berl)* **153**:395-398.

Lucki I, Dalvi A and Mayorga AJ (2001) Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)* **155**:315-322.

Lumia AR, Teicher MH, Salchli F, Ayers E and Possidente B (1992) Olfactory bulbectomy as a model for agitated hyposerotonergic depression. *Brain Res* **587**:181-185.

Luscher C, Jan LY, Stoffel M, Malenka RC and Nicoll RA (1997) G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **19**:687-695.

Lydiard RB (1998) The role of drug therapy in social phobia. *J Affect Disord* **50 Suppl 1**:S35-39.

Lyness WH, Friedle NM and Moore KE (1979) Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacol Biochem Behav* **11**:553-556.

Macey DJ, Froestl W, Koob GF and Markou A (2001) Both GABA(B) receptor agonist and antagonists decreased brain stimulation reward in the rat. *Neuropharmacology* **40**:676-685.

MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT and Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav Neurosci* **115**:1145-1153.

Maitre M (1997) The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol* **51**:337-361.

Maitre M, Hechler V, Vayer P, Gobaille S, Cash CD, Schmitt M and Bourguignon JJ (1990) A specific gamma-hydroxybutyrate receptor ligand possesses both antagonistic and anticonvulsant properties. *J Pharmacol Exp Ther* **255**:657-663.

Maldonado R, Blendy JA, Tzavara E, Gass P, Roques BP, Hanoune J and Schutz G (1996) Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. *Science* **273**:657-659.

Malitschek B, Schweizer C, Keir M, Heid J, Froestl W, Mosbacher J, Kuhn R, Henley J, Joly C, Pin JP, Kaupmann K and Bettler B (1999) The N-terminal domain of gamma-aminobutyric Acid(B) receptors is sufficient to specify agonist and antagonist binding. *Mol Pharmacol* **56**:448-454.

Malizia AL, Cunningham VJ, Bell CJ, Liddle PF, Jones T and Nutt DJ (1998) Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. *Arch Gen Psychiatry* **55**:715-720.

Mann JJ (2005) The medical management of depression. *N Engl J Med* **353**:1819-1834.

Mannoury la Cour C, Hanoun N, Melfort M, Hen R, Lesch KP, Hamon M and Lanfumey L (2004) GABA(B) receptors in 5-HT transporter- and 5-HT1A receptor-knock-out mice: further evidence of a transduction pathway shared with 5-HT1A receptors. *J Neurochem* **89**:886-896.

Marchesi C, Chiodera P, De Ferri A, De Risio C, Dasso L, Menozzi P, Volpi R and Coiro V (1991) Reduction of GH response to the GABA-B agonist baclofen in patients with major depression. *Psychoneuroendocrinology* **16**:475-479.

Maren S (2005) Building and burying fear memories in the brain. *Neuroscientist* **11**:89-99.

Margeta-Mitrovic M, Jan YN and Jan LY (2000) A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron* **27**:97-106.

Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY and Basbaum AI (1999) Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. *J Comp Neurol* **405**:299-321.

Markou A and Koob GF (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology* **4**:17-26.

Markou A, Kosten TR and Koob GF (1998) Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology* **18**:135-174.

Markou A, Paterson NE and Semenova S (2004) Role of gamma-aminobutyric acid (GABA) and metabotropic glutamate receptors in nicotine reinforcement: potential pharmacotherapies for smoking cessation. *Ann N Y Acad Sci* **1025**:491-503.

Marshall FH, Jones KA, Kaupmann K and Bettler B (1999) GABAB receptors - the first 7TM heterodimers. *Trends Pharmacol Sci* **20**:396-399.

Martellotta MC, Cossu G, Fattore L, Gessa GL and Fratta W (1998a) Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice. *Eur Neuropsychopharmacol* **8**:293-296.

Martellotta MC, Cossu G, Fattore L, Gessa GL and Fratta W (1998b) Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. *Neuroscience* **85**:327-330.

Martin DL and Rimvall K (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J Neurochem* **60**:395-407.

Martin P, Beninger RJ, Hamon M and Puech AJ (1990a) Antidepressant-like action of 8-OH-DPAT, a 5-HT1A agonist, in the learned helplessness paradigm: evidence for a postsynaptic mechanism. *Behav Brain Res* **38**:135-144.

Martin P, Pichat P, Massol J, Soubrie P, Lloyd KG and Puech AJ (1989) Decreased GABA B receptors in helpless rats: reversal by tricyclic antidepressants. *Neuropsychobiology* **22**:220-224.

Martin P, Soubrie P and Puech AJ (1990b) Reversal of helpless behavior by serotonin uptake blockers in rats. *Psychopharmacology (Berl)* **101**:403-407.

Martin SC, Russek SJ and Farb DH (1999) Molecular identification of the human GABABR2: cell surface expression and coupling to adenylyl cyclase in the absence of GABABR1. *Mol Cell Neurosci* **13**:180-191.

Mathivet P, Bernasconi R, De Barry J, Marescaux C and Bittiger H (1997) Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. *Eur J Pharmacol* **321**:67-75.

Matsushima T, Tegner J, Hill RH and Grillner S (1993) GABAB receptor activation causes a depression of low- and high-voltage-activated Ca2+ currents, postinhibitory rebound, and postspike afterhyperpolarization in lamprey neurons. *J Neurophysiol* **70**:2606-2619.

Matthews K and Robbins TW (2003) Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. *Neurosci Biobehav Rev* **27**:45-55.

Mattson BJ, Bossert JM, Simmons DE, Nozaki N, Nagarkar D, Kreuter JD and Hope BT (2005) Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. *J Neurochem* **95**:1481-1494.

Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R and Lucki I (2001) Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. *J Pharmacol Exp Ther* **298**:1101-1107.

Mayorga AJ and Lucki I (2001) Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl)* **155**:110-112.

McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O and Nestler EJ (2004) DeltaFosB: a molecular switch for long-term adaptation in the brain. *Brain Res Mol Brain Res* **132**:146-154.

McCormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* **62**:1018-1027.

McFarland K and Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* **21**:8655-8663.

McKinney WTJ and Bunney WEJ (1969) Animal model of depression. I. Review of evidence: implications for research. *Arch. Gen. Psychiatry* **21**:240-248.

McLellan AT, Lewis DC, O'Brien CP and Kleber HD (2000) Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *Jama* **284**:1689-1695.

McManus DJ and Greenshaw AJ (1991) Differential effects of antidepressants on GABAB and beta-adrenergic receptors in rat cerebral cortex. *Biochem Pharmacol* **42**:1525-1528.

Meijer OC and de Kloet ER (1998) Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. *Crit Rev Neurobiol* **12**:1-20.

Menard J and Treit D (1999) Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev* **23**:591-613.

Menon-Johansson AS, Berrow N and Dolphin AC (1993) G(o) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. *Pflugers Arch* **425**:335-343.

Miczek KA, Weerts EM, Vivian JA and Barros HM (1995) Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. *Psychopharmacology (Berl)* **121**:38-56.

Millan MJ (2003) The neurobiology and control of anxious states. *Prog Neurobiol* **70**:83-244.

Miller LP and Walters JR (1979) Effects of depolarization on cofactor regulation of glutamic acid decarboxylase in substantia nigra synaptosomes. *J Neurochem* **33**:533-539.

Mintz IM and Bean BP (1993) GABAB receptor inhibition of P-type Ca2+ channels in central neurons. *Neuron* **10**:889-898.

Mitchell PJ and Redfern PH (1997) Potentiation of the time-dependent, antidepressant-induced changes in the agonistic behaviour of resident rats by the 5-HT1A receptor antagonist, WAY-100635. *Behav Pharmacol* **8**:585-606.

Mitsukawa K, Mombereau C, Lotscher E, Uzunov DP, van der Putten H, Flor PJ and Cryan JF (2005) Metabotropic Glutamate Receptor Subtype 7 Ablation Causes Dysregulation of the HPA Axis and Increases Hippocampal BDNF Protein Levels: Implications for Stress-Related Psychiatric Disorders. *Neuropsychopharmacology*.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H and Tabira T (2002) Chronic stress impairs rotarod performance in rats: implications for depressive state. *Pharmacol Biochem Behav* **71**:79-84.

Mizukami K, Ishikawa M, Hidaka S, Iwakiri M, Sasaki M and Iritani S (2002) Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:393-396.

Modesto-Lowe V and Fritz EM (2005) The opioidergic-alcohol link: implications for treatment. *CNS Drugs* **19**:693-707.

Moller HJ (2002) Anxiety associated with comorbid depression. *J Clin Psychiatry* **63 Suppl 14**:22-26.

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H and Cryan JF (2004) Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology* **29**:1050-1062.

Mongeau R, Blier P and de Montigny C (1997) The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Res Brain Res Rev* **23**:145-195.

Monteleone P, Steardo L, Tanzillo C and Maj M (1990) Chronic antidepressant drug treatment does not affect GH response to baclofen in depressed subjects. *J Neural Transm Gen Sect* **82**:147-152.

Moreau JL, Jenck F, Martin JR, Mortas P and Haefely WE (1992) Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. *Eur Neuropsychopharmacol* **2**:43-49.

Morishita R, Kato K and Asano T (1990) GABAB receptors couple to G proteins Go, Go* and Gi1 but not to Gi2. *FEBS Lett* **271**:231-235.

Moser PC and Sanger DJ (1999) 5-HT1A receptor antagonists neither potentiate nor inhibit the effects of fluoxetine and befloxatone in the forced swim test in rats. *Eur J Pharmacol* **372**:127-134.

Mucha RF, van der Kooy D, O'Shaughnessy M and Bucenieks P (1982) Drug reinforcement studied by the use of place conditioning in rat. *Brain Res* **243**:91-105.

Muller DL and Unterwald EM (2005) D1 dopamine receptors modulate deltaFosB induction in rat striatum after intermittent morphine administration. *J Pharmacol Exp Ther* **314**:148-154.

Murray CJ and Lopez AD (1997) Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* **349**:1498-1504.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M and Takashima T (1996a) Involvement of GABAB receptor systems in action of antidepressants. II: Baclofen attenuates the effect of desipramine whereas muscimol has no effect in learned helplessness paradigm in rats. *Brain Res* **728**:225-230.

Nakagawa Y, Ishima T, Ishibashi Y, Tsuji M and Takashima T (1996b) Involvement of GABAB receptor systems in experimental depression: baclofen but not bicuculline exacerbates helplessness in rats. *Brain Res* **741**:240-245.

Nakagawa Y, Ishima T, Ishibashi Y, Yoshii T and Takashima T (1996c) Involvement of GABA(B) receptor systems in action of antidepressants: baclofen but not bicuculline attenuates the effects of antidepressants on the forced swim test in rats. *Brain Res* **709**:215-220.

Nakagawa Y, Sasaki A and Takashima T (1999) The GABA(B) receptor antagonist CGP36742 improves learned helplessness in rats. *Eur J Pharmacol* **381**:1-7.

Nakagawa Y and Takashima T (1997) The GABA(B) receptor antagonist CGP36742 attenuates the baclofen- and scopolamine-induced deficit in Morris water maze task in rats. *Brain Res* **766**:101-106.

Nastiti K, Benton D and Brain PF (1991) The effects of compounds acting at the benzodiazepine receptor complex on the ultrasonic calling of mouse pups. *Behav Pharmacol* **2**:121-128.

Nehring RB, Horikawa HP, El Far O, Kneussel M, Brandstatter JH, Stamm S, Wischmeyer E, Betz H and Karschin A (2000) The metabotropic GABAB receptor directly interacts with the activating transcription factor 4. *J Biol Chem* **275**:35185-35191.

Nemeroff CB (1989) Clinical significance of psychoneuroendocrinology in psychiatry: focus on the thyroid and adrenal. *J Clin Psychiatry* **50 Suppl**:13-20; discussion 21-12.

Nemeroff CB (2003) Anxiolytics: past, present, and future agents. *J Clin Psychiatry* **64 Suppl 3**:3-6.

Nestler EJ and Aghajanian GK (1997) Molecular and cellular basis of addiction. *Science* **278**:58-63.

Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L and Zalcman S (2002) Preclinical models: status of basic research in depression. *Biol Psychiatry* **52**:503-528.

Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, Eckelman W, Herscovitch P, Charney DS and Drevets WC (2004) Reduced serotonin type 1A receptor binding in panic disorder. *J Neurosci* **24**:589-591.

Newberry NR and Nicoll RA (1985) Comparison of the action of baclofen with gamma-aminobutyric acid on rat hippocampal pyramidal cells in vitro. *J Physiol* **360**:161-185.

Ng GY, Clark J, Coulombe N, Ethier N, Hebert TE, Sullivan R, Kargman S, Chateauneuf A, Tsukamoto N, McDonald T, Whiting P, Mezey E, Johnson MP, Liu Q, Kolakowski LF, Jr., Evans JF, Bonner TI and O'Neill GP (1999) Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. *J Biol Chem* **274**:7607-7610.

Ng TK and Yung KK (2001) Differential expression of GABA(B)R1 and GABA(B)R2 receptor immunoreactivity in neurochemically identified neurons of the rat neostriatum. *J Comp Neurol* **433**:458-470.

Nibuya M, Nestler EJ and Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* **16**:2365-2372.

Nic Dhonnchadha BA, Bourin M and Hascoet M (2003) Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. *Behav Brain Res* **140**:203-214.

Nicholson KL and Balster RL (2001) GHB: a new and novel drug of abuse. *Drug Alcohol Depend* **63**:1-22.

Nishi A, Snyder GL, Fienberg AA, Fisone G, Aperia A, Nairn AC and Greengard P (1999a) Requirement for DARPP-32 in mediating effect of dopamine D2 receptor activation. *Eur J Neurosci* **11**:2589-2592.

Nishi A, Snyder GL, Nairn AC and Greengard P (1999b) Role of calcineurin and protein phosphatase-2A in the regulation of DARPP-32 dephosphorylation in neostriatal neurons. *J Neurochem* **72**:2015-2021.

Nissbrandt H and Engberg G (1996) The GABAB-receptor antagonist, CGP 35348, antagonises gamma-hydroxybutyrate- and baclofen-induced alterations in locomotor activity and forebrain dopamine levels in mice. *J Neural Transm* **103**:1255-1263.

Noda Y, Yamada K, Furukawa H and Nabeshima T (1995) Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. *Br J Pharmacol* **116**:2531-2537.

Nonogaki K, Abdallah L, Goulding EH, Bonasera SJ and Tecott LH (2003) Hyperactivity and reduced energy cost of physical activity in serotonin 5-HT(2C) receptor mutant mice. *Diabetes* **52**:315-320.

Nonogaki K, Strack AM, Dallman MF and Tecott LH (1998) Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. *Nat Med* **4**:1152-1156.

Nowak G, Partyka A, Klodzinska A, Szewczyk B, Tatarczynska E, Wieronska JM, Librowski T, Czarnecki R, Chojnacka-Wojcik E and Pilc A (2004) Anxiolytic- and antidepressant-like activity of CGP36742 in rodent tests., in *FENS*.

Noyes R, Jr. (1985) Beta-adrenergic blocking drugs in anxiety and stress. *Psychiatr Clin North Am* **8**:119-132.

Nye HE, Hope BT, Kelz MB, Iadarola M and Nestler EJ (1995) Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J Pharmacol Exp Ther* **275**:1671-1680.

Nye HE and Nestler EJ (1996) Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. *Mol Pharmacol* **49**:636-645.

O'Flynn K and Dinan TG (1993) Baclofen-induced growth hormone release in major depression: relationship to dexamethasone suppression test result. *Am J Psychiatry* **150**:1728-1730.

O'Neill MF and Shaw G (1999) Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. *Psychopharmacology (Berl)* **145**:237-250.

Olds J and Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* **47**:419-427.

Olmstead MC and Burns LH (2005) Ultra-low-dose naltrexone suppresses rewarding effects of opiates and aversive effects of opiate withdrawal in rats. *Psychopharmacology (Berl)* **181**:576-581.

Olpe HR, Karlsson G, Pozza MF, Brugger F, Steinmann M, Van Riezen H, Fagg G, Hall RG, Froestl W and Bittiger H (1990) CGP 35348: a centrally active blocker of GABAB receptors. *Eur J Pharmacol* **187**:27-38.

Olpe HR, Koella WP, Wolf P and Haas HL (1977) The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. *Brain Res* **134**:577-580.

Olpe HR, Steinmann MW, Ferrat T, Pozza MF, Greiner K, Brugger F, Froestl W, Mickel SJ and Bittiger H (1993) The actions of orally active GABAB receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur J Pharmacol* **233**:179-186.

Olpe HR, Steinmann MW, Hall RG, Brugger F and Pozza MF (1988) GABAA and GABAB receptors in locus coeruleus: effects of blockers. *Eur J Pharmacol* **149**:183-185.

Olsen R and DeLorey T (1999) GABA and Glycine, in *Basic Neurochemistry* (Siegel ed) pp 335-346, Lippincott, Williams and Wilkins, Philadelphia.

Onali P, Mascia FM and Olianas MC (2003) Positive regulation of GABA(B) receptors dually coupled to cyclic AMP by the allosteric agent CGP7930. *Eur J Pharmacol* **471**:77-84.

Ordway GA, Schenk J, Stockmeier CA, May W and Klimek V (2003) Elevated agonist binding to alpha2-adrenoceptors in the locus coeruleus in major depression. *Biol Psychiatry* **53**:315-323.

Ordway GA, Smith KS and Haycock JW (1994) Elevated tyrosine hydroxylase in the locus coeruleus of suicide victims. *J Neurochem* **62**:680-685.

Otis TS, De Koninck Y and Mody I (1993) Characterization of synaptically elicited GABAB responses using patch-clamp recordings in rat hippocampal slices. *J Physiol* **463**:391-407.

Otmakhova NA, Gurevich EV, Katkov YA, Nesterova IV and Bobkova NV (1992) Dissociation of multiple behavioral effects between olfactory bulbectomized C57Bl/6J and DBA/2J mice. *Physiol Behav* **52**:441-448.

Otsuka M, Iversen LL, Hall ZW and Kravitz EA (1966) Release of gamma-aminobutyric acid from inhibitory nerves of lobster. *Proc Natl Acad Sci U S A* **56**:1110-1115.

Pagano A, Rovelli G, Mosbacher J, Lohmann T, Duthey B, Stauffer D, Ristig D, Schuler V, Meigel I, Lampert C, Stein T, Prezeau L, Blahos J, Pin J, Froestl W, Kuhn R, Heid J, Kaupmann K and Bettler B (2001) C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of GABA(b) receptors. *J Neurosci* **21**:1189-1202.

Panagis G, Kastellakis A, Spyraki C and Nomikos G (2000) Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl)* **149**:388-396.

Parks CL, Robinson PS, Sibille E, Shenk T and Toth M (1998) Increased anxiety of mice lacking the serotonin1A receptor. *Proc Natl Acad Sci U S A* **95**:10734-10739.

Patel S, Naeem S, Kesingland A, Froestl W, Capogna M, Urban L and Fox A (2001) The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. *Pain* **90**:217-226.

Paterson NE, Froestl W and Markou A (2004) The GABAB receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. *Psychopharmacology (Berl)* **172**:179-186.

Paterson NE, Froestl W and Markou A (2005) Repeated administration of the GABAB receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. *Neuropsychopharmacology* **30**:119-128.

Paterson NE and Markou A (2005) The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl)* **179**:255-261.

Peakman MC, Colby C, Perrotti LI, Tekumalla P, Carle T, Ulery P, Chao J, Duman C, Steffen C, Monteggia L, Allen MR, Stock JL, Duman RS, McNeish JD, Barrot M, Self DW, Nestler EJ and Schaeffer E (2003) Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain Res* **970**:73-86.

Peng CT, Ger J, Yang CC, Tsai WJ, Deng JF and Bullard MJ (1998) Prolonged severe withdrawal symptoms after acute-on-chronic baclofen overdose. *J Toxicol Clin Toxicol* **36**:359-363.

Petit-Demouliere B, Chenu F and Bourin M (2005) Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl)* **177**:245-255.

Pettit HO, Ettenberg A, Bloom FE and Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology (Berl)* **84**:167-173.

Petty F, Kramer GL, Dunnam D and Rush AJ (1990) Plasma GABA in mood disorders. *Psychopharmacol Bull* **26**:157-161.

Phillips AG, Blaha CD and Fibiger HC (1989) Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. *Neurosci Biobehav Rev* **13**:99-104.

Phillis BD, Ong J, White JM and Bonnielle C (2001) Modification of d-amphetamine-induced responses by baclofen in rats. *Psychopharmacology (Berl)* **153**:277-284.

Picciotto MR (1998) Common aspects of the action of nicotine and other drugs of abuse. *Drug Alcohol Depend* **51**:165-172.

Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R and Chiamulera C (1997) Common neural substrates for the addictive properties of nicotine and cocaine. *Science* **275**:83-86.

Pick CG, Peter Y, Terkel J, Gavish M and Weizman R (1996) Effect of the neuroactive steroid alpha-THDOC on staircase test behavior in mice. *Psychopharmacology (Berl)* **128**:61-66.

Pierce RC and Kumaresan V (2005) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev*.

Pilc A and Lloyd KG (1984) Chronic antidepressants and GABA "B" receptors: a GABA hypothesis of antidepressant drug action. *Life Sci* **35**:2149-2154.

Pirker W, Asenbaum S, Kasper S, Walter H, Angelberger P, Koch G, Pozzera A, Deecke L, Podreka I and Brücke T (1995) ß-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J. Neural. Transm. [Gen. Sect.]* **100**:247–250.

Pitler TA and Alger BE (1994) Differences between presynaptic and postsynaptic GABAB mechanisms in rat hippocampal pyramidal cells. *J Neurophysiol* **72**:2317-2327.

Pollard GT and Howard JL (1986) The staircase test: some evidence of nonspecificity for anxiolytics. *Psychopharmacology (Berl)* **89**:14-19.

Poncer JC, McKinney RA, Gahwiler BH and Thompson SM (1997) Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. *Neuron* **18**:463-472.

Pontieri FE, Tanda G, Orzi F and Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* **382**:255-257.

Popik P, Wrobel M and Bisaga A (2005) Reinstatement of Morphine-Conditioned Reward is Blocked by Memantine. *Neuropsychopharmacology*.

Porrino LJ, Esposito RU, Seeger TF, Crane AM, Pert A and Sokoloff L (1984) Metabolic mapping of the brain during rewarding self-stimulation. *Science* **224**:306-309.

Porsolt RD (2000) Animal models of depression: utility for transgenic research. *Rev Neurosci* **11**:53-58.

Porsolt RD, Bertin A and Jalfre M (1978) "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. *Eur J Pharmacol* **51**:291-294.

Porsolt RD, Chermat R, Lenegre A, Avril I, Janvier S and Steru L (1987) Use of the automated tail suspension test for the primary screening of psychotropic agents. *Arch Int Pharmacodyn Ther* **288**:11-30.

Porsolt RD, Le Pichon M and Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**:730-732.

Porter RJ, Gallagher P, Watson S and Young AH (2004) Corticosteroid-serotonin interactions in depression: a review of the human evidence. *Psychopharmacology (Berl)* **173**:1-17.

Post RM, Ketter TA, Joffe RT and Kramlinger KL (1991) Lack of beneficial effects of l-baclofen in affective disorder. *Int Clin Psychopharmacol* **6**:197-207.

Potier B and Dutar P (1993) Presynaptic inhibitory effect of baclofen on hippocampal inhibitory synaptic transmission involves a pertussis toxin-sensitive G-protein. *Eur J Pharmacol* **231**:427-433.

Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, Soffin EM, Farmer CE, Lanneau C, Gray J, Schenck E, Warmerdam BS, Clapham C, Reavill C, Rogers DC, Stean T, Upton N, Humphreys K, Randall A, Geppert M, Davies CH and Pangalos MN (2001) Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Mol Cell Neurosci* **17**:1059-1070.

Queva C, Bremner-Danielsen M, Edlund A, Ekstrand AJ, Elg S, Erickson S, Johansson T, Lehmann A and Mattsson JP (2003) Effects of GABA agonists on body temperature regulation in GABAB(1)-/- mice. *Br J Pharmacol* **140**:315-322.

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D and Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* **95**:14476-14481.

Ranaldi R and Poeggel K (2002) Baclofen decreases methamphetamine self-administration in rats. *Neuroreport* **13**:1107-1110.

Reid I, Forbes N, Stewart C and Matthews K (1997) Chronic mild stress and depressive disorder: a useful new model? *Psychopharmacology (Berl)* **134**:365-367; discussion 371-367.

Ren X and Mody I (2003) Gamma-hydroxybutyrate reduces mitogen-activated protein kinase phosphorylation via GABA B receptor activation in mouse frontal cortex and hippocampus. *J Biol Chem* **278**:42006-42011.

Renard CE, Dailly E, David DJ, Hascoet M and Bourin M (2003) Monoamine metabolism changes following the mouse forced swimming test but not the tail suspension test. *Fundam Clin Pharmacol* **17**:449-455.

Ribeiro Do Couto B, Aguilar MA, Manzanedo C, Rodriguez-Arias M and Minarro J (2005) NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. *Brain Res Bull* **64**:493-503.

Risner ME and Goldberg SR (1983) A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive-ratio schedules of intravenous drug infusion. *J Pharmacol Exp Ther* **224**:319-326.

Roberts DC, Andrews MM and Vickers GJ (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* **15**:417-423.

Roberts DC and Brebner K (2000) GABA modulation of cocaine self-administration. *Ann N Y Acad Sci* **909**:145-158.

Roberts DC, Corcoran ME and Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav* **6**:615-620.

Roberts DC and Koob GF (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* **17**:901-904.

Roberts E (1986a) Failure of GABAergic inhibition: a key to local and global seizures. *Adv Neurol* **44**:319-341.

Roberts E (1986b) What do GABA neurons really do? They make possible variability generation in relation to demand. *Exp Neurol* **93**:279-290.

Roberts E and Frankel S (1950) gamma-Aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem* **187**:55-63.

Robinson TE and Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* **18**:247-291.

Rodgers RJ, Cao BJ, Dalvi A and Holmes A (1997) Animal models of anxiety: an ethological perspective. *Braz J Med Biol Res* **30**:289-304.

Romieu P, Meunier J, Garcia D, Zozime N, Martin-Fardon R, Bowen WD and Maurice T (2004) The sigma1 (sigma1) receptor activation is a key step for the reactivation of cocaine conditioned place preference by drug priming. *Psychopharmacology (Berl)* **175**:154-162.

Roth A, Hogan I and Farren C (1997) Naltrexone plus group therapy for the treatment of opiate-abusing health-care professionals. *J Subst Abuse Treat* **14**:19-22.

Rudolph U, Crestani F and Mohler H (2001) GABA(A) receptor subtypes: dissecting their pharmacological functions. *Trends Pharmacol Sci* **22**:188-194.

Russig H, Pezze MA, Nanz-Bahr NI, Pryce CR, Feldon J and Murphy CA (2003) Amphetamine withdrawal does not produce a depressive-like state in rats as measured by three behavioral tests. *Behav Pharmacol* **14**:1-18.

Sacchetti G, Bernini M, Gobbi M, Parini S, Pirona L, Mennini T and Samanin R (2001) Chronic treatment with desipramine facilitates its effect on extracellular noradrenaline in the rat hippocampus: studies on the role of presynaptic alpha2-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol* **363**:66-72.

Saint DA, Thomas T and Gage PW (1990) GABAB agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci Lett* **118**:9-13.

Salas R, Pieri F, Fung B, Dani JA and De Biasi M (2003) Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. *J Neurosci* **23**:6255-6263.

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH and Mason GF (2004) Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry* **61**:705-713.

Sanacora G, Mason GF, Rothman DL, Hyder F, Ciarcia JJ, Ostroff RB, Berman RM and Krystal JH (2003) Increased cortical GABA concentrations in depressed patients receiving ECT. *Am J Psychiatry* **160**:577-579.

Sanacora G, Mason GF, Rothman DL and Krystal JH (2002) Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. *Am J Psychiatry* **159**:663-665.

Sandoval MR and Palermo-Neto J (1985) Central nervous system supersensitivity and withdrawal from long-term treatment with barbital. *Neuropharmacology* **24**:1247-1251.

Sands SA, Reisman SA and Enna SJ (2004) Effect of antidepressants on GABA(B) receptor function and subunit expression in rat hippocampus. *Biochem Pharmacol* **68**:1489-1495.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C and Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**:805-809.

Sayin U, Sutula TP and Stafstrom CE (2004) Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. *Epilepsia* **45**:1539-1548.

Sbrenna S, Marti M, Morari M, Calo G, Guerrini R, Beani L and Bianchi C (2000) Modulation of 5-hydroxytryptamine efflux from rat cortical synaptosomes by opioids and nociceptin. *Br J Pharmacol* **130**:425-433.

Schildkraut JJ (1995) The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965. *J Neuropsychiatry Clin Neurosci* **7**:524-533; discussion 523-524.

Schlicker E, Classen K and Gothert M (1984) GABAB receptor-mediated inhibition of serotonin release in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **326**:99-105.

Schmidt M, Oitzl MS, Levine S and de Kloet ER (2002) The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. *Brain Res Dev Brain Res* **139**:39-49.

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA and et al. (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family. *Nature* **328**:221-227.

Schuler V, Luscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton AL, Vigouret J, Pozza M, Kelly PH, Mosbacher J, Froestl W, Kaslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H and Bettler B (2001) Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron* **31**:47-58.

Scott RH and Dolphin AC (1986) Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen-mediated inhibition. *Neurosci Lett* **69**:59-64.

Scott RH, Wootton JF and Dolphin AC (1990) Modulation of neuronal T-type calcium channel currents by photoactivation of intracellular guanosine 5'-O(3-thio) triphosphate. *Neuroscience* **38**:285-294.

Seale TW and Carney JM (1991) Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. *J Addict Dis* **10**:141-162.

Seiden LS, Dahms JL and Shaughnessy RA (1985) Behavioral screen for antidepressants: the effects of drugs and electroconvulsive shock on performance under a differential-reinforcement-of-low-rate schedule. *Psychopharmacology (Berl)* **86**:55-60.

Seligman ME (1972) Learned helplessness. *Annu Rev Med* **23**:407-412.

Seligman ME, Rosellini RA and Kozak MJ (1975) Learned helplessness in the rat: time course, immunization, and reversibility. *J Comp Physiol Psychol* **88**:542-547.

Semenova S and Markou A (2003) Cocaine-seeking behavior after extended cocaine-free periods in rats: role of conditioned stimuli. *Psychopharmacology (Berl)* **168**:192-200.

Serrano JS, Minano FJ, Sancibrian M and Duran JA (1985) Involvement of bicuculline-insensitive receptors in the hypothermic effect of GABA and its agonists. *Gen Pharmacol* **16**:505-508.

Serrats J, Artigas F, Mengod G and Cortes R (2003) GABAB receptor mRNA in the raphe nuclei: co-expression with serotonin transporter and glutamic acid decarboxylase. *J Neurochem* **84**:743-752.

Shalev U, Grimm JW and Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev* **54**:1-42.

Shanks N and Anisman H (1993) Escape deficits induced by uncontrollable foot-shock in recombinant inbred strains of mice. *Pharmacol Biochem Behav* **46**:511-517.

Shaywitz AJ and Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* **68**:821-861.

Shekhar A, McCann UD, Meaney MJ, Blanchard DC, Davis M, Frey KA, Liberzon I, Overall KL, Shear MK, Tecott LH and Winsky L (2001) Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. *Psychopharmacology (Berl)* **157**:327-339.

Sheline YI, Wang PW, Gado MH, Csernansky JG and Vannier MW (1996) Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A* **93**:3908-3913.

Shen W and Slaughter MM (1999) Metabotropic GABA receptors facilitate L-type and inhibit N-type calcium channels in single salamander retinal neurons. *J Physiol* **516 (Pt 3)**:711-718.

Shen Y, Connor TJ, Nolan Y, Kelly JP and Leonard BE (1999) Differential effect of chronic antidepressant treatments on lipopolysaccharide-induced depressive-like behavioural symptoms in the rat. *Life Sci* **65**:1773-1786.

Shephard RA, Wedlock P and Wilson NE (1992) Direct evidence for mediation of an anticonflict effect of baclofen by GABAb receptors. *Pharmacol Biochem Behav* **41**:651-653.

Shepherd JK, Grewal SS, Fletcher A, Bill DJ and Dourish CT (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl)* **116**:56-64.

Sherman AD and Petty F (1982) Additivity of neurochemical changes in learned helplessness and imipramine. *Behav Neural Biol* **35**:344-353.

Shippenberg TS and Rea W (1997) Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* **57**:449-455.

Shirayama Y, Chen AC, Nakagawa S, Russell DS and Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* **22**:3251-3261.

Shoaib M, Swanner LS, Beyer CE, Goldberg SR and Schindler CW (1998) The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol* **9**:195-206.

Shoptaw S, Yang X, Rotheram-Fuller EJ, Hsieh YC, Kintaudi PC, Charuvastra VC and Ling W (2003) Randomized placebo-controlled trial of baclofen for cocaine dependence: preliminary effects for individuals with chronic patterns of cocaine use. *J Clin Psychiatry* **64**:1440-1448.

Sibille E and Hen R (2001) Serotonin(1A) receptors in mood disorders: a combined genetic and genomic approach. *Behav Pharmacol* **12**:429-438.

Sibille E, Pavlides C, Benke D and Toth M (2000) Genetic inactivation of the Serotonin(1A) receptor in mice results in downregulation of major GABA(A) receptor alpha

subunits, reduction of GABA(A) receptor binding, and benzodiazepine-resistant anxiety. *J Neurosci* **20**:2758-2765.

Simiand J, Keane PE and Morre M (1984) The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. *Psychopharmacology (Berl)* **84**:48-53.

Simonds WF (1999) G protein regulation of adenylate cyclase. *Trends Pharmacol Sci* **20**:66-73.

Siniscalchi A, Rodi D, Beani L and Bianchi C (1999) Inhibitory effect of nociceptin on [3H]-5-HT release from rat cerebral cortex slices. *Br J Pharmacol* **128**:119-123.

Skolnick P, Hu RJ, Cook CM, Hurt SD, Trometer JD, Liu R, Huang Q and Cook JM (1997) [3H]RY 80: A high-affinity, selective ligand for gamma-aminobutyric acidA receptors containing alpha-5 subunits. *J Pharmacol Exp Ther* **283**:488-493.

Slattery DA, Desrayaud S and Cryan JF (2005a) GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *J Pharmacol Exp Ther* **312**:290-296.

Slattery DA, Markou A, Froestl W and Cryan JF (2005b) The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* **30**:2065-2072.

Sloviter RS, Ali-Akbarian L, Elliott RC, Bowery BJ and Bowery NG (1999) Localization of GABA(B) (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. *Neuropharmacology* **38**:1707-1721.

Smith MA, Makino S, Kvetnansky R and Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* **15**:1768-1777.

Smith MA, Yancey DL, Morgan D, Liu Y, Froestl W and Roberts DC (2004) Effects of positive allosteric modulators of the GABAB receptor on cocaine self-administration in rats. *Psychopharmacology (Berl)* **173**:105-111.

Snead OC, 3rd (1990) gamma-Hydroxybutyric acid-induced seizures bear no relation to core temperature. *Epilepsia* **31**:253-258.

Snead OC, 3rd (1994) The ontogeny of [3H]gamma-hydroxybutyrate and [3H]GABAB binding sites: relation to the development of experimental absence seizures. *Brain Res* **659**:147-156.

Snead OC, 3rd (2000) Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* **75**:1986-1996.

Soudijn W, van Wijngaarden I and AP IJ (2002) Allosteric modulation of G protein-coupled receptors. *Curr Opin Drug Discov Devel* **5**:749-755.

Spielewoy C and Markou A (2003) Withdrawal from chronic phencyclidine treatment induces long-lasting depression in brain reward function. *Neuropsychopharmacology* **28**:1106-1116.

Spooren W, Ballard T, Gasparini F, Amalric M, Mutel V and Schreiber R (2003) Insight into the function of Group I and Group II metabotropic glutamate (mGlu) receptors: behavioural characterization and implications for the treatment of CNS disorders. *Behav Pharmacol* **14**:257-277.

Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD and Gentsch C (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Ther* **295**:1267-1275.

Spyraki C, Fibiger HC and Phillips AG (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. *Brain Res* **253**:195-203.

Steffens DC, Byrum CE, McQuoid DR, Greenberg DL, Payne ME, Blitchington TF, MacFall JR and Krishnan KR (2000) Hippocampal volume in geriatric depression. *Biol Psychiatry* **48**:301-309.

Steketee JD and Beyer CE (2005) Injections of baclofen into the ventral medial prefrontal cortex block the initiation, but not the expression, of cocaine sensitization in rats. *Psychopharmacology (Berl)* **180**:352-358.

Steru L, Chermat R, Thierry B, Mico JA, Lenegre A, Steru M, Simon P and Porsolt RD (1987) The automated Tail Suspension Test: a computerized device which differentiates psychotropic drugs. *Prog Neuropsychopharmacol Biol Psychiatry* **11**:659-671.

Steru L, Chermat R, Thierry B and Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**:367-370.

Stewart J and Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* **4**:289-312.

Stewart RB and Grupp LA (1981) An investigation of the interaction between the reinforcing properties of food and ethanol using the place preference paradigm. *Prog Neuropsychopharmacol* **5**:609-613.

Stewart WF, Ricci JA, Chee E, Hahn SR and Morganstein D (2003) Cost of lost productive work time among US workers with depression. *Jama* **289**:3135-3144.

Stinus L, Cador M, Zorrilla EP and Koob GF (2005) Buprenorphine and a CRF1 antagonist block the acquisition of opiate withdrawal-induced conditioned place aversion in rats. *Neuropsychopharmacology* **30**:90-98.

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L and Rajkowska G (1998) Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J Neurosci* **18**:7394-7401.

Stolerman IP (1999) Inter-species consistency in the behavioural pharmacology of nicotine dependence. *Behav Pharmacol* **10**:559-580.

Stolerman IP and Jarvis MJ (1995) The scientific case that nicotine is addictive. *Psychopharmacology (Berl)* **117**:2-10; discussion 14-20.

Stork O, Ji FY, Kaneko K, Stork S, Yoshinobu Y, Moriya T, Shibata S and Obata K (2000) Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. *Brain Res* **865**:45-58.

Stork O, Yamanaka H, Stork S, Kume N and Obata K (2003) Altered conditioned fear behavior in glutamate decarboxylase 65 null mutant mice. *Genes Brain Behav* **2**:65-70.

Stretch R, Gerber GJ and Wood SM (1971) Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. *Can J Physiol Pharmacol* **49**:581-589.

Sullivan R, Chateauneuf A, Coulombe N, Kolakowski LF, Jr., Johnson MP, Hebert TE, Ethier N, Belley M, Metters K, Abramovitz M, O'Neill GP and Ng GY (2000) Coexpression of full-length gamma-aminobutyric acid(B) (GABA(B)) receptors with truncated receptors and metabotropic glutamate receptor 4 supports the GABA(B) heterodimer as the functional receptor. *J Pharmacol Exp Ther* **293**:460-467.

Swartzwelder HS, Tilson HA, McLamb RL and Wilson WA (1987) Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology (Berl)* **92**:398-401.

Takahashi T, Kajikawa Y and Tsujimoto T (1998) G-Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *J Neurosci* **18**:3138-3146.

Tanda G, Pontieri FE and Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* **276**:2048-2050.

Tang WJ and Gilman AG (1991) Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits. *Science* **254**:1500-1503.

Tao R, Ma Z and Auerbach SB (1996) Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. *Br J Pharmacol* **119**:1375-1384.

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ and Greenberg ME (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* **20**:709-726.

Tarantino LM, Gould TJ, Druhan JP and Bucan M (2000) Behavior and mutagenesis screens: the importance of baseline analysis of inbred strains. *Mamm Genome* **11**:555-564.

Tauscher J, Pirker W, de Zwaan M, Asenbaum S, Brucke T and Kasper S (1999) In vivo visualization of serotonin transporters in the human brain during fluoxetine treatment. *Eur Neuropsychopharmacol* **9**:177-179.

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM and Nestler EJ (1991) A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res* **548**:100-110.

Tessari M, Pilla M, Andreoli M, Hutcheson DM and Heidbreder CA (2004) Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking. *Eur J Pharmacol* **499**:121-133.

Teste JF, Pelsy-Johann I, Decelle T and Boulu RG (1993) Anti-immobility activity of different antidepressant drugs using the tail suspension test in normal or reserpinized mice. *Fundam Clin Pharmacol* **7**:219-226.

Thakker DR, Hoyer D and Cryan JF (2005) Interfering with the brain: Use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. *Pharmacol Ther*.

Thierry B, Steru L, Chermat R and Simon P (1984) Searching-waiting strategy: a candidate for an evolutionary model of depression? *Behav Neural Biol* **41**:180-189.

Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, Storm D and Duman RS (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J Neurosci* **20**:4030-4036.

Thompson SM and Gahwiler BH (1992) Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. *J Physiol* **451**:329-345.

Thuault SJ, Brown JT, Sheardown SA, Jourdain S, Fairfax B, Spencer JP, Restituito S, Nation JH, Topps S, Medhurst AD, Randall AD, Couve A, Moss SJ, Collingridge GL, Pangalos MN, Davies CH and Calver AR (2004) The GABA(B2) subunit is critical for the trafficking and function of native GABA(B) receptors. *Biochem Pharmacol* **68**:1655-1666.

Tiihonen J, Kuikka J, Rasanen P, Lepola U, Koponen H, Liuska A, Lehmusvaara A, Vainio P, Kononen M, Bergstrom K, Yu M, Kinnunen I, Akerman K and Karhu J (1997) Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: a fractal analysis. *Mol Psychiatry* **2**:463-471.

Treiman DM (2001) GABAergic mechanisms in epilepsy. *Epilepsia* **42 Suppl 3**:8-12.

Tremblay LK, Naranjo CA, Graham SJ, Herrmann N, Mayberg HS, Hevenor S and Busto UE (2005) Functional neuroanatomical substrates of altered reward processing in major

depressive disorder revealed by a dopaminergic probe. *Arch Gen Psychiatry* **62**:1228-1236.

Tsuji M, Nakagawa Y, Ishibashi Y, Yoshii T, Takashima T, Shimada M and Suzuki T (1996) Activation of ventral tegmental GABAB receptors inhibits morphine-induced place preference in rats. *Eur J Pharmacol* **313**:169-173.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* **56**:613-672.

Udenfriend S (1950) Identification of gamma-aminobutyric acid in brain by the isotope derivative method. *J Biol Chem* **187**:65-69.

Umezu T (1999) Effects of psychoactive drugs in the Vogel conflict test in mice. *Jpn J Pharmacol* **80**:111-118.

Urwyler S, Gjoni T, Koljatic J and Dupuis DS (2005) Mechanisms of allosteric modulation at GABAB receptors by CGP7930 and GS39783: effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology* **48**:343-353.

Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B and Kaupmann K (2001) Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* **60**:963-971.

Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M and Kaupmann K (2003) N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and Structurally Related Compounds: Novel Allosteric Enhancers of {gamma}-Aminobutyric AcidB Receptor Function. *J Pharmacol Exp Ther* **307**:322-330.

Vaccarino FJ, Pettit HO, Bloom FE and Koob GF (1985) Effects of intracerebroventricular administration of methyl naloxonium chloride on heroin self-administration in the rat. *Pharmacol Biochem Behav* **23**:495-498.

Valentino RJ and Commons KG (2005) Peptides that fine-tune the serotonin system. *Neuropeptides* **39**:1-8.

Van der Heyden JA, Zethof TJ and Olivier B (1997) Stress-induced hyperthermia in singly housed mice. *Physiol Behav* **62**:463-470.

van Gaalen MM, Stenzel-Poore M, Holsboer F and Steckler T (2003) Reduced attention in mice overproducing corticotropin-releasing hormone. *Behav Brain Res* **142**:69-79.

van Riezen H and Leonard BE (1990) Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol Ther* **47**:21-34.

Vaughan CW and Christie MJ (1996) Increase by the ORL1 receptor (opioid receptor-like1) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones. *Br J Pharmacol* **117**:1609-1611.

Velazquez-Moctezuma J and Diaz Ruiz O (1992) Neonatal treatment with clomipramine increased immobility in the forced swim test: an attribute of animal models of depression. *Pharmacol Biochem Behav* **42**:737-739.

Vogel G, Neill D, Kors D and Hagler M (1990) REM sleep abnormalities in a new animal model of endogenous depression. *Neurosci Biobehav Rev* **14**:77-83.

Vogel JR, Beer B and Clody DE (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* **21**:1-7.

Volkow ND (2005) What do we know about drug addiction? *Am J Psychiatry* **162**:1401-1402.

Volkow ND and Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cereb Cortex* **10**:318-325.

Volkow ND and Li TK (2004) Drug addiction: the neurobiology of behaviour gone awry. *Nat Rev Neurosci* **5**:963-970.

Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Angrist B, Gatley SJ, Logan J, Ding YS and Pappas N (1999) Association of methylphenidate-induced craving with changes in right striato-orbitofrontal metabolism in cocaine abusers: implications in addiction. *Am J Psychiatry* **156**:19-26.

Vollmayr B, Faust H, Lewicka S and Henn FA (2001) Brain-derived-neurotrophic-factor (BDNF) stress response in rats bred for learned helplessness. *Mol Psychiatry* **6**:471-474, 358.

Volpicelli JR, Alterman AI, Hayashida M and O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry* **49**:876-880.

Wahlsten D, Metten P and Crabbe JC (2003) A rating scale for wildness and ease of handling laboratory mice: results for 21 inbred strains tested in two laboratories. *Genes, Brain & Behavior* **2**:71-79.

Waldmeier PC (1991) The GABAB antagonist, CGP 35348, antagonizes the effects of baclofen, gamma-butyrolactone and HA 966 on rat striatal dopamine synthesis. *Naunyn Schmiedebergs Arch Pharmacol* **343**:173-178.

Walters CL and Blendy JA (2001) Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. *J Neurosci* **21**:9438-9444.

Watanabe A, Tohyama Y, Nguyen KQ, Hasegawa S, Debonnel G and Diksic M (2003) Regional brain serotonin synthesis is increased in the olfactory bulbectomy rat model of depression: an autoradiographic study. *J Neurochem* **85**:469-475.

Watanabe Y, Sakai RR, McEwen BS and Mendelson S (1993) Stress and antidepressant effects on hippocampal and cortical 5-HT1A and 5-HT2 receptors and transport sites for serotonin. *Brain Res* **615**:87-94.

Weizman R, Paz L, Backer MM, Amiri Z, Modai I and Pick CG (1999) Mouse strains differ in their sensitivity to alprazolam effect in the staircase test. *Brain Res* **839**:58-65.

Westerink BH, Kwint HF and deVries JB (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J Neurosci* **16**:2605-2611.

White FJ and Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. *Drug Alcohol Depend* **51**:141-153.

White JH, McIllhinney RA, Wise A, Ciruela F, Chan WY, Emson PC, Billinton A and Marshall FH (2000) The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc Natl Acad Sci U S A* **97**:13967-13972.

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM and Marshall FH (1998) Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* **396**:679-682.

White NM and McDonald RJ (1993) Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. *Behav Brain Res* **55**:269-281.

Williams JT, Colmers WF and Pan ZZ (1988) Voltage- and ligand-activated inwardly rectifying currents in dorsal raphe neurons in vitro. *J Neurosci* **8**:3499-3506.

Willner P (1983a) Dopamine and depression: a review of recent evidence. I. Empirical studies. *Brain Res* **287**:211-224.

Willner P (1983b) Dopamine and depression: a review of recent evidence. II. Theoretical approaches. *Brain Res* **287**:225-236.

Willner P (1983c) Dopamine and depression: a review of recent evidence. III. The effects of antidepressant treatments. *Brain Res* **287**:237-246.

Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* **134**:319-329.

Willner P and Mitchell PJ (2002) The validity of animal models of predisposition to depression. *Behav Pharmacol* **13**:169-188.

Willner P, Muscat R and Papp M (1992a) An animal model of anhedonia. *Clin Neuropharmacol* **15 Suppl 1 Pt A**:550A-551A.

Willner P, Muscat R and Papp M (1992b) Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* **16**:525-534.

Wirtshafter D and Sheppard AC (2001) Localization of GABA(B) receptors in midbrain monoamine containing neurons in the rat. *Brain Res Bull* **56**:1-5.

Wise RA, Bauco P, Carlezon WA, Jr. and Trojniar W (1992) Self-stimulation and drug reward mechanisms. *Ann N Y Acad Sci* **654**:192-198.

Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* **94**:469-492.

Wise RA and Rompre PP (1989) Brain dopamine and reward. *Annu Rev Psychol* **40**:191-225.

Wong EH, Sonders MS, Amara SG, Tinholt PM, Piercey MF, Hoffmann WP, Hyslop DK, Franklin S, Porsolt RD, Bonsignori A, Carfagna N and McArthur RA (2000) Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol Psychiatry* **47**:818-829.

Wong ML and Licinio J (2001) Research and treatment approaches to depression. *Nat Rev Neurosci* **2**:343-351.

Wu LG and Saggau P (1997) Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* **20**:204-212.

Xi ZX and Stein EA (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J Pharmacol Exp Ther* **290**:1369-1374.

Yakimova K, Sann H, Schmid HA and Pierau FK (1996) Effects of GABA agonists and antagonists on temperature-sensitive neurones in the rat hypothalamus. *J Physiol* **494 (Pt 1)**:217-230.

Yirmiya R, Pollak Y, Barak O, Avitsur R, Ovadia H, Bette M, Weihe E and Weidenfeld J (2001) Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. *Neuropsychopharmacology* **24**:531-544.

Young ST, Porrino LJ and Iadarola MJ (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc Natl Acad Sci U S A* **88**:1291-1295.

Yun IA, Wakabayashi KT, Fields HL and Nicola SM (2004) The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J Neurosci* **24**:2923-2933.

Zachariou V, Benoit-Marand M, Allen PB, Ingrassia P, Fienberg AA, Gonon F, Greengard P and Picciotto MR (2002) Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors DARPP 32 or Inhibitor 1. *Biol Psychiatry* **51**:612-620.

Zachariou V, Sgambato-Faure V, Sasaki T, Svenningsson P, Berton O, Fienberg AA, Nairn AC, Greengard P and Nestler EJ (2005) Phosphorylation of DARPP-32 at Threonine-34 is Required for Cocaine Action. *Neuropsychopharmacology*.

Zamponi GW (2001) Determinants of G protein inhibition of presynaptic calcium channels. *Cell Biochem Biophys* **34**:79-94.

Zanko M and Beigon A (1983) Increased adrenergic receptor binding in human frontal cortex of suicide victims. **Abstract No. 210.5.**

Zarrindast M, Rostami P and Sadeghi-Hariri M (2001) GABA(A) but not GABA(B) receptor stimulation induces antianxiety profile in rats. *Pharmacol Biochem Behav* **69**:9-15.

Zezula J, Slany A and Sieghart W (1996) Interaction of allosteric ligands with GABAA receptors containing one, two, or three different subunits. *Eur J Pharmacol* **301**:207-214.

Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J and Xu M (2002) The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. *J Neurochem* **82**:1453-1464.

Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, Zhang J and Xu M (2004) Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J Neurosci* **24**:3344-3354.

Zhou D, Grecksch G, Becker A, Frank C, Pilz J and Huether G (1998) Serotonergic hyperinnervation of the frontal cortex in an animal model of depression, the bulbectomized rat. *J Neurosci Res* **54**:109-116.

ETUDE DU ROLE DES RECEPTEURS $GABA_B$ DANS L'ANXIETE, LA DEPRESSION ET L'ADDICTION : APPROCHE PHARMACOLOGIQUE ET GENETIQUE.

Notre travail visait à caractériser le rôle des récepteurs $GABA_B$ dans l'anxiété, la dépression et l'addiction, prenant avantage du récent développement de souris $GABA_B$ knockout et d'un modulateur positif allostérique du récepteur $GABA_B$ (GS39783). Dans un premier temps, nous avons observé que l'ablation du gène codant pour le récepteur $GABA_B$ induisait un effet anxiogène. Inversement, la stimulation pharmacologique de ces récepteurs produisait une réponse anxiolytique. De plus, nous avons mis en évidence que l'inactivation génétique de ces récepteurs et leur blocage pharmacologique induisait un effet antidépresseur. Finalement, nous avons observé que le GS39783 s'opposait à la fois aux comportements et aux adaptations moléculaires associés à l'administration de psychostimulants. Ainsi, ces études nous permettent de conclure que les récepteurs $GABA_B$ pourraient représenter une cible dans le développement de nouvelles stratégies thérapeutique dans le traitement de ces trois pathologies.

Taking advantage of the recent development of $GABA_B$ positive modulator GS39783 and mice lacking $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunits, the studies in the present thesis are focus on addressing a broad hypothesis that GABAB receptors play a key role in the manifestation of psychiatric disorders, such as anxiety, depression and addiction. In a first time, we demonstrated that targetted deletion of either $GABA_B$ receptor subunit induced strong anxiety. Conversely, we also observed that $GABA_B$ activation, via administration of GS39783, induced anxiolytic-like effect in several paradigms. Secondly, we also demonstrated that genetic inactivation of either $GABA_B$ receptor subunit induced antidepressant-like effects. In confirmation of the genetic data, acute and chronic blockade of $GABA_B$ receptor decreased immobility in the forced swim test. Finnaly, we also demonstrated that pharmacological activation of $GABA_B$ receptor counteracted both molecular and behavioural adaptations elicited by a single administration of cocaine or nicotine. Altogether, our data support the role of $GABA_B$ receptor in anxiety, depression and
addiction and that $GABA_B$ receptor might be considered as one of the most promising therapeuthic targets for treating these disorders.

ROLE OF $GABA_B$ RECEPTORS IN ANXIETY, DEPRESSION AND ADDICTION: PHARMACOLOGICAL AND GENETIC APPROACHES.