Realia et naturalia

DISSERTATIONES
CHIMICAE
UNIVERSITATIS
TARTUENSIS
123

KARITA RAUDKIVI

Neurochemical studies on inter-individual differences in affect-related behaviour of the laboratory rat

TARTU ÜLIKOOL · UNIVERSITAS TARTUENSIS · 1632

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
123

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
123

KARITA RAUDKIVI

Neurochemical studies on inter-individual differences in affect-related behaviour of the laboratory rat

UNIVERSITY OF TARTU
PRESS
1632

Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia

Dissertation was accepted for the commencement of the degree of *Doctor philosophiae* in Chemistry at the University of Tartu on September 13th, 2012 by the Council of Institute of Chemistry, Faculty of Science and Technology, University of Tartu.

Supervisors: Prof. Jaanus Harro, MD, PhD
Department of Psychology, University of Tartu, Estonia

Prof. Ivo Leito, PhD
Institute of Chemistry, University of Tartu, Estonia

Opponent: Prof. Artur Swiergiel (dr. Hab), PhD
University of Gdansk, Poland

Commencement: November 19th 2012 at 9.30 in Tartu, Nooruse 1, room 121

ISSN 1406–0299
ISBN 978–9949–32–157–5 (print)
ISBN 978–9949–32–158–2 (pdf)

University of Tartu Press
www.tyk.ee
Order No. 527

"Different molecules or humans behave differently having different reactions or behaviors to changing situations."
John Hodgson

The Little Fun Book of Molecules/Humans: Molecules/Humans

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Kadri Kõiv, Rein Zobel, **Karita Raudkivi**, Toomas Kivastik, Jaanus Harro, 2011. The effect of denervation of the locus coeruleus projections with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on cocaine-induced locomotion and place preference in rats. Behavioural Brain Research, 216, 172–179.

II. Sadia Oreland, **Karita Raudkivi**, Lars Oreland, Jaanus Harro, Lotta Arborelius, Ingrid Nylander, 2011. Ethanol-induced effects on the dopamine and serotonin systems in adult Wistar rats are dependent on early-life experiences. Brain Research, 1405, 57–68.

III. Tanel Mällo, Kadri Kõiv, Indrek Koppel, **Karita Raudkivi**, Ain Uustare, Ago Rinken, Tõnis Timmusk, Jaanus Harro, 2008. Regulation of extracellular serotonin levels and brain-derived neurotrophic factor in rats with high are low exploratory activity. Brain Research, 1194, 110–117.

IV. **Karita Raudkivi,** Tanel Mällo, Jaanus Harro, 2012. Effect of chronic variable stress on corticosterone levels and hippocampal extracellular 5-HT in rats with persistent differences in positive affectivity. Acta Neuropsychiatrica, 24, 208–214.

V. **Karita Raudkivi**, Aet Alttoa, Ivo Leito, Jaanus Harro. Differences in extracellular glutamate levels in rats with high and low exploratory activity. Submitted to Pharmacological Reports for publication.

Contribution of the author:

Paper I: The author performed all the monoamine content measurements and participated in writing the respective parts of the manuscript.

Paper II: The author performed all the monoamine content measurements and analysis of the obtained data and participated in writing the respective parts of the manuscript.

Paper III: The author performed microdialysis and serotonin content measurements, analyzed the obtained data, participated in writing the respective parts of the manuscript.

Paper IV: The author performed microdialysis and serotonin content measurements, participated in analysing the statistical data and wrote the manuscript as the main author.

Paper V: The author planned experimental designs, carried all the behavioural experiments, microdialysis and analytical measurements, performed all the statistical analysis and wrote the manuscript as the main author.

ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine**,** serotonin
5-HTT	serotonin transporter
AFR	animal facility rearing
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
β-ME	β-mercaptoethanol
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CPP	conditioned place preference
CRF	corticotropin-releasing factor
CVS	chronic variable stress
DA	3-hydroxytyramine, dopamine
DG	dentate gyrus
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
DSP-4	*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine
EAAT2	excitatory amino acid (glutamate) transporter 2
EDTA	ethylenediaminetetraacetic acid
GABA	gamma-aminobutyric acid
HC-rats	rats with high levels of 50-kHz USVs, high chirping rats
HE-rats	rats with high levels of exploratory activity
HEF-rats	former rats with high levels of exploratory activity
HPA	hypothalamic-pituitary-adrenal axis
HPLC	high performance liquid chromatography high responder
HR	high responder
HVA	homovanillic acid
i.p.	intraperitoneal
kHz	kilohertz
LC	locus coeruleus
LC-rats	rats with low levels of 50-kHz USVs, low chirping rats
LE-rats	rats with low levels of exploratory activity
LR	low responder
MS15	15 min maternal separation
MS360	360 min maternal separation
NA	noradrenaline
NAcc	nucleus accumbens
NGF	nerve growth factor
NMDA	*N*-methyl-D-aspartic acid
NMN	normetanephrine
OPA	*o*-phtaldialdehyde
PCA	parachloroamphetamine
PDC	L-*trans*-pyrrolidine-2,4-dicarboxylic acid

PFC	prefrontal cortex
SEM	standard error of mean
USV	ultrasonic vocalization
VTA	ventral tegmental area

INTRODUCTION

Depression is a highly prevalent psychopathological condition, which affects millions of people worldwide and is predicted to increase. At the core of depression are negative affect and reduced ability to experience pleasure, and these features are expressed in passive coping strategies. The research of involvement of monoamine systems in depression-related disorders has contributed significantly to the knowledge of the pathophysiology and treatment of depression. Drugs that increase the synaptic availability of biogenic amines are in use to treat depression-related disorders, and the main targets in depression treatment are in serotonergic, dopaminergic and noradrenergic neurotransmission. Nevertheless, other neurotransmitters are believed to have a major role in the pathogenesis of depression.

Because many patients do not respond to the available drugs, and the first selected medicine often needs to be replaced, there is a need for more personalized medical approach.

Studies of the neurochemistry underlying development of different affective states are becoming increasingly important. Individuals are differently affected by the interaction of the surrounding environment and genetic factors. Stressful life events, especially in the vulnerable early age, may lead to changes in neuronal systems and thereby greater susceptibility to e.g., addiction and depression.

Animal models of affective disorders usually focus on states resembling fear, depressiveness, anxiety, neophobia and other expressions of negative affect but, in recent years, research has increasingly paid attention to positive affect. Using naturally occurring phenotypes in models for studying the inter-individual differences creates an opportunity to have a better understanding about behaviour, the affective states and nervous system functions, and inform modelling of human behaviour.

1. REVIEW OF LITERATURE

1.1. Neurochemistry of depression

Depression is a highly prevalent psychopathological condition that is characterized by persistently lowered mood, loss of pleasure, and reduced energy, accompanied by changes in body weight, appetite, sleep rhythm, agitation or inhibition, feeling of worthlessness and guilt, distractibility, concentration difficulties and suicidal ideation (APA, 1994; WHO, 1992). Adverse life events are the most important causal factor in the development of depression. Susceptibility to distress is affected by genetic factors and the surrounding environment, which all together lead to changes in the central nervous system (CNS) that serve as the substrate of affective disorders. The variety of factors leading to depressive states has led to suggestions to consider multiplicity in analysing the pathogenesis of depression. Obviously, the heterogeneity of depressive syndromes means that not all depressed individuals are suffering from identical alterations in their brain (Harro and Oreland, 2001).

Even though the symptoms of depression are well characterized, the underlying molecular mechanisms are largely unknown. The classic monoamine theory of depression suggests that a deficit in monoamine neurotransmitters in the synaptic cleft is the primary cause of depression (Coppen, 1967; Schildkraut, 1965). Much of the past 10–15 years of research on the neurochemistry of depression has led to considerable evidence implicating multiple systems, including all monoamines, particularly serotonin (5-HT), as well as several other neurotransmitter systems (Harro and Oreland, 2001; Sümegi, 2008).

Despite the differences between species in their neuroanatomy and neurochemical systems, the chemoarchitecture and connections of monoamine systems are largely comparable among mammals. This has frequently provided means for preclinical researchers to make successful predictions about human brain function (Harro and Oreland, 2001).

1.1.1. Neurochemistry of monoamines and their role in depression

Monoamine activity appears to be important in regulating many changes in neural function that underlie or enable shifts in behavioural functions, such as attention, motivation etc. Still, it is nonetheless possible to investigate experimentally specific responses and effects of monoamines in specific brain areas where their modulatory functions may influence most directly some of the key behavioural components of the dimensions that are dysregulated in depression and anxiety disorders. Knowledge on the modulatory influence of monoamines in regions associated with depression is essential to understanding how alterations in those functions can contribute to the efficacy of antidepressants in the treatment of depression (Morilak and Frazer, 2004). The treatment of depression with antidepressants is achieved via the increase of the synaptic

availability of monoamines, and therefore an enhancement of neurotransmission (Coppen, 1967; Morilak and Frazer, 2004).

Most often the serotonergic system has been implicated in mechanisms of anxiety and affective disorders. Selective serotonin reuptake inhibitors increase the extracellular levels of the 5-HT by inhibiting its reuptake into the presynaptic cell, hence increasing the levels of 5-HT in the synaptic cleft available to bind to the postsynaptic receptor (Millan, 2003; Morilak and Frazer, 2004). The 5-HT transporter (5-HTT) function and 5-HT receptor levels are implicated in the pathophysiology of depression (Millan, 2003). For instance, different activity of 5-HT_{1B} autoreceptors, which regulate the release of 5-HT by inhibitory feedback, have been hypothesized to be supersensitive in depression and anxiety (Moret and Briley, 2000). In adult mice, elimination of the 5-HT_{1A} receptor has been shown to produce anxiety-type behaviour (Heisler et al., 1998). In general, the 5-HT_{1A} receptor function seems to be lower in emotionally less stable animals (Harro, 2010). As to the 5-HT transporter levels and function, opposite findings have been obtained when different models, or different brain regions within a model, are compared (Harro, 2010). The study of Malison et al. (1998) showed that the 5-HTT availability was reduced in the depressed patients. 5-HTT sites on platelets have been found to be lower in depression by most, but not all researchers (Briley et al., 1980; Maes and Meltzer, 1995; Nemeroff et al., 1994).

Aside the 5-HT, the noradrenaline (NA) system serves as a one of the targets in treatment of depression with antidepressants. NA deficiency, particularly in the pathways from the locus coeruleus (LC), the main source of NA-ergic projections, may affect working memory and cause psychomotor retardation. NA-ergic pathways are also involved in different emotional functions and believed to mediate stress response (Morilak and Frazer, 2004).

Studies on depressed patients indicate that the NA reuptake inhibitors require the availability of NA, but not 5-HT, for their beneficial effects, and the converse is true for the selective serotonin reuptake inhibitors (Delgado and Moreno, 2000). However, NA-ergic action plays an important effect in different antidepressants, as confirmed by the efficacy of dual action antidepressants such as the serotonin and noradrenaline reuptake inhibitors, and noradrenaline and dopamine (DA) reuptake inhibitors that enhance the NA-ergic transmission. Therefore, it seems that the cause of depression is more complex than just an alteration in the levels of the 5-HT and/or NA (Delgado and Moreno, 2000).

One model has proposed that primary defect emerges in the regulation of firing rates in brainstem monoaminergic neurons, which brings about a decrease in the tonic release of neurotransmitters in their projection areas, an increase in postsynaptic sensitivity, and concomitantly, exaggerated responses to acute increase in the presynaptic firing rate and transmitter release (Harro and Oreland, 2001). It is proposed that the initial defect involves, in particular, the NA-ergic innervation from the locus coeruleus. Dysregulation of the locus coeruleus projection activities may lead in turn to dysregulation of serotonergic and dopaminergic neurotransmission. Failure of the locus coeruleus function

could explain the basic impairments in the processing of novel information, intensive processing of irrational belief, and anxiety. Impairments in the 5-HT-ergic neurotransmission may contribute to the mood changes and reduction in the dopaminergic activity to loss of motivation and anhedonia.

Dopamine controls a variety of functions in the CNS, including locomotor activity, motivation and emotion, but probably the most acknowledged function of DA is its role in reward-related stimuli processing (Marsden, 2006). According to some theories, DA signals reward by mediating the learning signal that allows the system to predict better when rewards (e.g., food, sex, drugs) are likely to occur, and thereby contributes to the optimization of reward-seeking behaviours (Montague et al., 2004). Some of the symptoms of affective disorders that are improved by antidepressant drug treatment, for instance anhedonia, seem also related more to the DA-ergic function than 5-HT-ergic (Morilak and Frazer, 2004). Nevertheless, many antidepressants potently block the transporters mediating reuptake of 5-HT and/or NA, but not DA. The NA transporter may be the primary mechanism for DA reuptake in the frontal cortex (Morón et al., 2002) and drugs that block the NA transporter may also prevent the uptake of extracellular DA into NA-ergic terminals where the latter have much higher density (Pozzi et al., 1994). Preclinical studies have shown that noradrenaline uptake inhibitors elevate extracellular levels of DA in prefrontal cortex (PFC), but not in nucleus accumbens (NAcc) (Millan et al., 2000). By contrast, chronic treatment of rats with selective serotonin reuptake inhibitors did not elevate DA levels in PFC. On the other hand treatment with selective serotonin reuptake inhibitors and noradrenaline reuptake inhibitors enhances reward-related behavioural effects of DA in the NAcc, possibly through effects on mesolimbic DA neuronal activity (Bonhomme and Esposito, 1998).

1.1.2. Neurochemistry of glutamate system and its role in depression

Recent data from various studies have shown that amino acid neurotransmitters may also have a major role in the pathogenesis of depression (Gao and Bao, 2011; Skolnick et al., 2009). In particular the glutamate system has been implicated in depression, leading to increased efforts to understand its machinery (Maeng et al., 2008; Skolnick et al., 2009; Tokita et al., 2012).

Glutamate mediates a large part of the synaptic transmission in the CNS, is involved in the neurogenesis, and having a role in synaptic plasticity development is also important in cognitive functions like learning and memory (McEntee and Crook, 1993; McLennan et al., 1968). Upon stimulation of the nerve terminals, glutamate is released into the synaptic cleft and binds to glutamate receptors resulting in the propagation of an action potential. The regulation of glutamate in synaptic cleft is determined by frequent synaptic release and by localized glutamate uptake (Bergles et al., 1999; Clements et al., 1992). There are two groups of glutamate receptors – ionotropic [NMDA (*N*-

methyl D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate] and metabotropic receptors. Ionotropic receptors operate via ion channels which allow the flow of cations (K^+, Na^+, Ca^{2+}) in response to glutamate binding. Metabotropic receptors do not have ion channels, but mediate their functions via G-proteins, which then in turn trigger further second-messenger systems (Bleich et al., 2003). Five subtypes of glutamate transporters, EAAT1 to EAAT5, have been identified (Neuhauss et al., 2010). It is reported that glial glutamate transporter EAAT2 plays the largest role in regulation of extracellular glutamate levels (Danbolt et al., 1992; Tanaka et al., 1997; Shachnai et al., 2005), thus having the function to keep the glutamate concentration low in the extracellular space and to prevent excitotoxic brain damage (Danbolt, 2001). L-*trans*-pyrrolidine-2,4-dicarboxylate (PDC) that works via blocking the glial glutamate transporter EAAT2 (Danbolt, 2001) is considered to be the most efficient inhibitor (Bridges et al., 1991; Behrens et al., 2002; Del Arco et al., 2001).

Matsugami et al. (2006), using an animal model in which this glutamate transporter was genetically deleted, demonstrated that EAAT2 is necessary for brain development through regulation of extracellular glutamate concentration. Behrens et al. (2002) observed that transgenic mice expressing an N-terminal fragment of mutant huntingtin (R6/2) had higher striatal glutamate levels compared with controls after inhibition by PDC. Additionally, in the R6/2 mice the decrease of EAAT2 mRNA expression compared to wild type group was also observed, obviously resulting in reduction of transporter function. Several studies have shown that chronically administered antidepressants from different classes modulate the function of NMDA receptor and its antagonists (e.g. ketamine) have been shown to possess antidepressant activity in animal models of depression (Sanacora et al., 2012; Skolnick et al., 2009). In depressed patients, the treatment with ketamine elicited antidepressant effects (Zarate et al., 2006). Additionally, relieving effect of ketamine on postoperative depressive state and pain has been reported (Kudoh et al., 2002). Similarly, in animal studies, after stressful procedures (forced-swim test, learned helplessness), the antidepressant effects of ketamine-elicited appeared and the pretreatment with AMPA receptor agonist (NQOX) attenuated ketamine antidepressant-like behaviour (Maeng et al., 2008). Moreover, recent studies suggest that the antidepressant-like effects of NMDA antagonists may be dependent on enhancement of AMPA receptor activation (Maeng et al., 2008; Sanacora et al., 2012). Nevertheless, other studies question the universal need for AMPA receptor activation in relation to antidepressant properties of NMDA antagonists (Dybala et al., 2008; Popik et al., 2008).

The olfactory bulbectomy is an established animal model of depression in rats. Ho et al. (2000) examined striatal glutamate levels in olfactory bulbectomized and sham-operated rats. In appeared that under (novelty) stress condition glutamate levels increased from the baseline value (up to 160%) in the case of olfactory bulbectomized rats. In learned helpless model, more helpless rats had a decrease of glutamate uptake in striatum and hippocampus

(Almeida et al., 2010). Additionally, as reduced expression of glutamate transporter of glia (EAAT2) has been observed in learned helplessness rats (Zink et al., 2010), it is suggested that glial cell abnormalities may at least partly account for the impairment in glutamate action (Kugaya and Sanacora, 2005). Altered levels of glutamate have been seen in cortico-limbic structures found to be dysregulated in depression (Sanacora et al., 2008). Acute stress is associated with increased glutamatergic neurotransmission in areas of the forebrain, and chronic treatment with antidepressant causes a reduction of glutamate release (Tokarski et al., 2008). Recent study by Musazzi et al. (2010) also showed that depolarization-dependent release of glutamate is selectively up-regulated by acute stress and that antidepressant treatment completely abolished this effect. There is evidence of various levels of interaction between glutamatergic and monoaminergic pathways and the antidepressant effects of the glutamatergic drugs can possibly be explained by a monoaminergic mechanism (Drago et al., 2011). For instance, interaction of glutamatergic and dopaminergic neurotransmission in the striatum is well known (Smith and Bolam, 1990). Mora et al. (2003) showed that increasing levels of glutamate lead to increasing levels of DA in striatum, and blockade of ionotropic glutamate receptors attenuates the increase of DA levels. In substantia nigra, the spontaneously hypertensive rats, considered as an attention-deficit/hyperactivity disorder animal model, are more active in the open field (Warton et al., 2009). Additionally, these rats showed higher glutamate-stimulated DA release, suggesting that altered glutamate regulation of DA neurons may contribute to novelty response at different levels. Nevertheless, there are no single-valued interactions between dopaminergic and glutaminergic transmission (David et al., 2005).

The 5-HT system involvement in depression is well demonstrated, but the 5-HT depletion alone does not decrease mood (Ruhé et al., 2007). This could be caused by a more complex biological liability, a part of which may be embedded in the glutamatergic-monoaminergic balance (Krystal et al., 2002; Skolnick et al., 2009). The downregulation of the adrenergic receptors or the enhancement of the serotonergic function is also associated with the administration of the glutamatergic drugs (Lejeune et al., 1994; Pallotta et al., 1998). Stressful experiences have been found to increase the release both of NA (Miyashita and Williams, 2004) and glutamate (McEwen et al., 1997) in hippocampus. Thus, the nature of the effects of glutamate-mediated transmission on the monoamine systems is dependent on the brain regions.

1.2. Stress as the cause/trigger of depression

Stressors are physical and psychological events that are the part of everyday life of humans and animals (McEwen, 2007). Different stressful life events (for example, loss of a close person) can either trigger or cause depressive episodes. Several clinical and epidemiological studies have presented that most episodes

of depression are preceded by stressful life events (Brown, 1998; Hammen, 2005; Monroe et al., 2007).

In animal models, different types of stressors are used dependent on which purpose the stressor should serve. The stressors are conditionally classified as systemic (involving an immediate physiologic threat e.g., low temperature or wet cage) and progressive (involving high-level cognitive processing e.g., novelty). Additionally the stressors can include or not various social stressful conditions (e.g., social defeat, maternal separation), and differ in duration - short (e.g., tail pinch) and longer impacts (e.g., overnight lighting) (Harro et al., 2001; Herman and Cullinan, 1997; Katz et al., 1981; Willner, 2005).

Aversive experiences give rise to behavioural attempts to cope with the stressors. When behavioural coping is possible, neurochemical systems are not overly taxed, and behavioural pathology will not occur (Anisman and Zacharko, 1982). A major element in determining the neurochemical changes is the ability of organism to cope with the aversive stimuli through behavioural means. Reciprocally, the effects of stressful experiences on affective state may be related to depletion of several neurotransmitters. If the effect of stressors is long-lasting, it might lead to changes in neuronal, hormonal and immune systems and thereby greater susceptibility to psychiatric disorders (McEwen, 2007).

1.2.1. Neurochemical alterations after chronic stress

A wide variety of chronic stress procedures exists, so the following section will be restricted to studies with the chronic variable/mild stress paradigm that has become the most often used instrument in depression research. The main aim of chronic stress procedure developed originally by Katz et al. (1981) is to produce a depressed state by the variation and unpredictability of a number of stressors administered repeatedly over a long period of time (Muscat and Willner, 1992; Zurita et al., 1999). This concept rests on the assumption that stressors separately have no depression-like effect on behaviour, but act cumulatively all together (Muscat and Willner, 1992). The chronic variable stress (CVS) paradigm consistently brings out behavioural and physiological signs of anhedonia and emotional reactions such as anxiety and fear that resemble some of the symptoms of endogenous depression in humans (Bekris et al., 2005; Katz, 1982; Willner, 2005; Zurita et al., 1999). The animals are subjected to the stressors over a period of several weeks which results in changes in activity levels in tests used in depression and anxiety studies, which are reversible by antidepressant treatment (Katz, 1982; Willner, 2005).

As already mentioned, stressful situations might lead to greater vulnerability to different disorders, like depression, which still does not appear in all humans. Additionally, depression-related studies with rats and mice are consistent with this notion (Anisman and Zarharko, 1990; Nielsen et al., 2000), suggesting that there is a systematic inter-individual variability in the sensitivity to chronic stress. Studies with chronic stress have shown its various effects on behaviour, including a decrease in hedonic behaviour as measured by sucrose preference

tests; decreased sexual behaviour; reduction of active coping behaviour in the forced swimming test and increased freezing in case of fear stimuli (Grønli et al., 2005; Katz, 1982; Willner, 2005).

Chronic multi-stressor treatment induces changes in neurotransmitter levels (Chaouloff, 2000; Harro and Oreland, 2001). Many investigations suggest that anxiogenic stimuli elicit release of 5-HT (File et al., 2000), while chronic variable stress profoundly affects hippocampal 5-HT-ergic neurotransmission in this region, especially in the dentate gyrus (DG) (Joëls et al., 2004; McKittrick et al., 1995). Nevertheless, distinct 5-HT-ergic pathways can make different contributions (Millan, 2003; Morilak and Frazer, 2004). Stress related physiology also includes the hypothalamus as chronic unpredictable stress increased 5-HT-ergic activity in this brain area (Cox et al., 2011). Additionally, both 5-HT and DA neurotransmission are suggested to be related to the development of stress-induced anhedonia. Bekris et al. (2005) found that in PFC DA-ergic activity increased following chronic stress. In the same area, 5-HT-ergic activity was found to decrease, but increase in hippocampus. What may need to be considered is the non-linearity of connections between 5-HT-ergic activity and affective state of the animal: both increased and decreased 5-HT-ergic neurotransmission have been associated with negative emotionality within a paradigm (Harro and Oreland, 2001; Tõnissaar et al., 2004).

The stress response is mediated by an increased release of glucocorticoids via the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Arborelius et al., 1999; Kendler et al., 1999; Koob, 1999; Munck et al., 1984). The HPA system is the common pathway in the mediation of the stress response and changes in the balance of HPA axis are considered to be characteristic to depression (Holsboer, 2000). It is reported that in rats acute or chronic stress increases the levels of plasma corticosterone (Grippo et al., 2005; Strausbaugh et al., 1999), similar to the human cortisol response (Römer et al., 2009). Increased activity of corticotropin-releasing factor (CRF) system is considered to be a marker of depression because HPA axis hyperactivity normalizes following antidepressant treatment. The administration of CRF directly into the CNS to laboratory animals causes behavioural and physiological changes almost identical to those observed by stressful stimuli, including increased heart rate, suppression of exploratory behaviour in an unfamiliar environment, decreased food intake and decreased sexual behaviour (Koob et al., 1993; Owens and Nemeroff, 1991). Although clinical studies have not shown consistent changes in cerebrospinal fluid concentrations of CRF in patients with anxiety disorders, still, preclinical findings strongly indicate a role for CRF in the pathophysiology of certain mood disorders, probably through its effects on central NA-ergic systems (Sauvage and Steckler, 2001).

Various non-aminergic mechanisms, like neurotrophins, have been also implicated in depression (Castrén et al., 2007). Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 and neurotrophin-4 are called neurotrophins, a family of secreted growth that promote to survival, maintenance of specific neuronal populations and regulate activity-dependent

synaptic plasticity (Bibel and Barde, 2000; Castrén et al., 2007). BDNF deficiency is believed to lead to a number of pathologies, including depression and anxiety-related behaviour (Binder and Scharfman, 2004; Martinowich and Lu, 2008). In depressed patients lowered BDNF levels have been reported in hippocampus and prefrontal cortex, the brain areas which have received special attention in animal research of depression and antidepressants (Dranovsky and Hen, 2006). Similarly, in animals, lower BDNF levels were obtained after chronic stress (Berton et al., 2006; Lee et al., 2010). BDNF signalling plays an important role in mechanisms of antidepressant action, as treatment with antidepressant drugs restores normal BDNF content (Hashimoto, 2010). Antidepressant drug treatment is efficient in normalizing many of the behavioural and neurochemical variations induced by chronic multiple stressors (Bekris et al., 2005; Bondi et al., 2007; Willner, 2005).

1.2.2. Neurochemical alterations after early life stress

Early life stress has long been thought to contribute to the pathophysiology of affective disorders (Holmes et al., 2005). Studies with early life stress have indicated major changes in neuroplasticity, which refer to the ability of neurons to alter some functional property in response to alternations in input (Gillick and Zirpel, 2012; Shaw et al., 1994). Early social interactions, including attachment to the mother, are known to be essential, not only for establishment of social behaviour but also for normal physiological development (Hofer, 1994). Manipulations during the three first postnatal weeks thus alter these developmental processes.

The rodent maternal separation (MS) model is a commonly used animal experimental model in studies of the impact of the early environmental on physiological and behavioural functions later in life (Holmes et al., 2005). A number of studies have shown that separation of rat pups from their mothers during the early postnatal period produces permanent increases in anxiety-related behaviour when the offspring are tested as adults (Huot et al., 2001; Pryce et al., 2001a, 2001b). Different maternal separation protocols are in use, but initial maternal separation model originates from studies by Otto Weininger (1954), who showed that short handling of the pups, for instance stroking their back for ten minutes, caused positive effects in adulthood. Later on, there have been studies including several modifications with different light cycles, temperatures, either individual or litter-wise separations with various durations (Lehmann and Feldon, 2000; Levine, 1957; Pryce and Feldon, 2003; Roman and Nylander, 2005; Zimmerberg and Sageser, 2011). Common maternal separation protocols consists of short maternal separation, where pups are separated for 3–15 min, and prolonged maternal separation lasting 180–360 min (Lehmann and Feldon, 2000; Moffett et al., 2007; Roman and Nylander, 2005; Zimmerberg and Sageser, 2011). Three different groups - the non-handled, handled but not separated, those reared as normal animal facility group - are used as a control group (Jaworski et al., 2005).

Maternal separation causes behavioural changes in rats: more activity in the open plus-maze, higher anxiety in elevated plus-maze and exaggerated acute stress response (Kalinichev et al., 2002; Lukas et al., 2011; Sanders and Anticevic, 2007; Zimmerberg and Sageser, 2011). A large number of studies have presented neurobiological differences in animals subjected to maternal separation. Decreased levels of DA were observed in frontal cortex, NAcc and amygdala (Arborelius and Eklund, 2007; Ploj et al., 2003a) decreased levels of 5-HT in NAcc and amygdala (Arborelius and Eklund, 2007; Vicentic et al., 2006) and decreased levels of NA in frontal cortex (Arborelius and Eklund, 2007; Liu et al., 2000; Matthews et al., 2001). Additionally, there are maternal separation-induced effects in the glutamatergic system (Pickering et al., 2006). Maternal separation also includes effects on stress reactivity. The HPA axis is an essential component for individuals in capacity to cope with stress and its activation leads to elevated release of corticosterone, the levels of which are used as indicator of stress (de Kloet et al., 1999). It is reported that maternally separated rats had high HPA axis reactivity to acute stress and decreased glucocorticoid receptor levels (Aisa et al., 2007, 2008).

The early environmental impact on voluntary ethanol consumption has been studied using a protocol with daily repeated litter-wise maternal separation for 15 min (MS15) and 360 min (MS360) (Gustafsson et al., 2007; Ploj et al., 2003a, 2003b; Roman and Nylander, 2005). Based on the results from these studies it was suggested that MS15 represents a protective and MS360 a risk environment. The MS15-rats have shown the low ethanol consumption and prefer low concentrations of ethanol in adulthood. The MS360-rats are characterized by higher propensity for high ethanol preference and consumption (Ploj et al., 2003a; Roman et al., 2003, 2005).

1.3. Inter-individual differences in behaviour of rats: implications for depression and addiction research

Inter-individual differences in behaviour of animals have been used in research of behavioural phenotype-based models. Phenotypes result from the genes as well as the influence of environmental factors and are defined as the composite of observable characteristics or traits of organisms. This “trait” is defined as a pattern of a specific behaviour, which differs between individuals, but which is relatively constant within subjects over time and situations (Amelang et al., 2006). If these traits are stable in their expression, they create an opportunity to model human traits and different conditions by studying the underlying neurobiological mechanisms. This approach has helped to clarify mechanisms behind neurochemical and physiological measurements and thereby offers strategy for psychobiological research, including depression, anxiety and addiction research (Harro, 2010; Pawlak et al., 2008).

1.3.1. Inter-individual differences in exploratory behaviour

Different animal models in research of human psychopathology have been used for many years and of these probably the most studied are the models of depression. At the core of depression are negative affect and reduced ability to experience pleasure, and these features are expressed in passive coping strategies, including low exploratory activity in novel environments (Harro, 2010).

Novelty-related behaviour, usually called exploratory behaviour, is evoked by novel stimuli and it consists of behavioural acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment (Crusio and van Abeelen, 1986; File and Hyde, 1978). Exploratory behaviour in animals is essential for survival due to the enhanced possibilities it provides to find food, water, mating partner, shelter etc (Berlyne, 1950). In situation, where animals are in a novel environment, their behaviour is influenced by two conflicting motivations to explore the unknown, the potentially dangerous environment, or to stay in secure and familiar surroundings. Thus, behavioural activity of an animal in a novel context is always a function of its curiosity and neophobia (Belzung, 1999; Harro, 1993).

There are various approaches to measure the exploratory behaviour in rats (Harro, 1993, for review). In general, they are divided in two groups: 'free' tests, where animals have a choice of environments differing in the degree of novelty, and 'forced' tests, where locomotor response is assessed in animals placed into novel inescapable environments. The classical open-field test is probably the most widely used (Prut and Belzung, 2003), representing the forced access to novelty. However, it has been pointed out that this might be stressful as corticosterone levels in rats increase in response to an exposure to an open field (Marin et al., 2007), but not to free-choice novelty (Misslin et al., 1982).

Probably the most studied aspects of inter-individual differences of behaviour in animals are related with to exploratory behaviour and anxiety (reviewed in Pawlak et al., 2008). The paradigm most influential in studies of inter-individual differences overall has been the low/high responder (LR/HR) model that is based on locomotor/exploratory activity in a novel environment: rats (Sprague-Dawley) were classified based on median split of their two-hour locomotor activity in a novel inescapable circular corridor (Piazza et al., 1989, 1991). Hereafter, the low and high responder phenotypes have been used often by a number of research groups, measuring activity in an inescapable novel environment, that can vary from a small novel photocell-equipped plexiglas cage to a large open field (Chefer et al., 2003; Hooks et al., 1991, 1994a; Verheij et al., 2009). Recently, bred lines of low and high responders were introduced (Stead et al., 2006). While in the original LR/HR studies the focus was put on prediction of individual levels of drug-seeking behaviour, the phenotype has a general significance to novelty and exploration: the HR-rats prefer more novel and modified environments in case of free choice between familiar and novel, and also exhibition lower levels of anxiety compared to LR-

rats (Dellu et al., 1993; Kabbaj et al., 2000; Piazza and Le Moal, 1997; Verheij et al., 2009).

As another alternative to the LR/HR paradigm and the plus-maze selection studies (e.g. Harro et al., 1990; Landgraf et al., 2007; Schwarting and Pawlak 2004), the exploration box test (Harro et al., 1995; Otter et al., 1997) was developed to measure novelty-related behaviour in rats. During testing, the rat has a free choice either to stay in the dark home-cage-like chamber or to explore a large open area that has novel objects in it (Otter et al., 1997). This test yields a rather bimodal distribution based on the sum of exploratory activity, and thus separates the animals tested into groups with persistently high neophobia/low motivation and low neophobia/high motivation (low exploring and high exploring rats, LE- and HE-rats, respectively) with relatively few animals occupying the middle ground. These characteristic traits are consistent over time. We have shown that LE-rats are more anxious in plus-maze test and display more passive coping strategies in the forced swimming test (Mällo et al., 2007a). Additionally, the LE- and HE-rats have distinct cerebral metabolic activity in areas that are involved in defensive behaviours and cognitive processing of sensory stimuli (Matrov et al., 2007), furthermore, they differ in gene expression patterns, e.g. involving several glutamatergic and serotonergic genes in brain regions implicated in mood disorders (Alttoa et al., 2010).

Several neurochemical measures have been shown to be associated with inter-individual differences in response to novel stimuli. Compared to low responders to novelty, high responders have higher baseline and stimulated DA release in the NAcc (Hooks et al., 1991; Rougé-Pont et al., 1998) and additionally, lower density of D_2 receptor mRNA in the NAcc and the striatum (Hooks et al., 1994b). It has been reported that after mild stress of an exposure to a novel environment, HR-rats exhibit a prolonged corticosterone response and higher stress-induced levels of mesolimbic DA neurotransmission (Dellu et al., 1996; Piazza et al., 1989). Thiel et al. (1999) reported that in their LR/HR-rat model, which is based on the vertical activity (rearings) in a novel open field, HR-rats have higher DA levels in ventral striatum and lower 5-HT concentration in the medial prefrontal cortex. Differences in NA system have been also associated with its hippocampal release in stressful conditions (Rosario and Abercrombie, 1999). It has been also demonstrated that inter-individual variations exist in the glutamatergic system if animals with different novelty-related behaviour are compared. Shakil et al. (2005) examined striatal glutamate baseline levels in mice with low vs high locomotor responses to novelty, and found low responders mice to have on average 35% higher baseline extracellular glutamate levels.

The exploration box test was originally developed in order to have a suitable tool for assessing behaviour of animals with LC denervation. While the LC activity closely follows any change in surroundings, the effects of near complete LC denervation had been found surprisingly hard to detect in standard tests, as the inter-individual variability of response increased. Indeed, intact NA-ergic projections ascending from the LC have were found to be very

important for normal exploration in rats using this test, as after destruction of these projections a very significant increase of neophobia was observed, but also reduction in motivation to explore (Harro et al., 1995). Indeed, extensive denervation by neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) treatment rendered virtually all rats LE-like.

The locus coeruleus gives rise to the dorsal NA-ergic bundle, which projects to many brain regions, being the only source of NA in the cortex and hippocampus (Robinson et al., 1977). The neurotoxin DSP-4 has a selective effect on NA-axonal terminal fields originating from the LC (Grzanna et al., 1989; Jonsson et al., 1981) and thus can serve a valuable tool in studies of functional significance of LC-derived NA in the brain. Previously, we have found that both behavioural and neurochemical responses to NA-ergic depletion are dose-dependent and in some cases bidirectional. Usually smaller dose (10 mg/kg) of DSP-4 reduces NA levels by 30%, and enhances immobility time in the forced swimming test, whereas the near complete denervation achieved with doses 30–50 mg/kg reduced immobility (Harro et al., 1995; Harro and Oreland, 1996).

It is well known that DA is participating in the locomotion activating and reinforcing effects of psychostimulants such as cocaine and amphetamine. Different studies have produced evidence that NA has also a substantial role in modulating psychostimulant-induced behaviours such as locomotor activity and reward (Tassin, 2008; Weinshenker and Schroeder, 2007). Up till now, there are only a few studies investigating the possible role of NA in psychostimulant-induced conditioned place preference. Mice lacking the NA transporter develop a more pronounced conditioned place preference (CPP) to cocaine than controls (Xu et al., 2000). Concurrently, site targeted NA depletions produced by administration of 6-hydroxydopamine (6-OHDA) to neonatal rats, which caused a severe and long-lasting depletion of NA without affecting central DA content, were shown to not alter the CPP by cocaine (Spyraki et al., 1982).

Although there is no doubt in NA-ergic involvement in psychostimulant-induced behaviour, it is not clear which factors determine the exact role of NA in regulation of behaviour and in drug effects. Several studies utilizing behavioural, biochemical and electrophysiological approaches have demonstrated NA-ergic stimulatory input from LC to the mesencephalic DA-ergic neurons (Andén and Grabowska, 1976; Grenhoff et al., 1993; Lategan et al., 1992). Additionally, the lesions of the LC can reduce DA release. Thus, alterations in the LC projection systems elicit lasting adaptive changes in DA-ergic neurotransmission that can serve as a substrate for psychiatric disorders. Altogether it appears that the response to novelty concept can be used in research of addiction, because these behaviours share some similar neurobiological substrate (Bardo et al., 1996; Blanchard et al., 2009), but many of the necessary pieces of the puzzle are still missing.

1.3.2. Addictive behaviour and inter-individual differences

Addictive disorders such as alcoholism and drug addiction continue to be a substantial problem in society. According to American Society of Addiction Medicine (2011) the concept of addiction is defined as a primary, chronic disease of brain reward, motivation, and memory circuitries. Dysfunction in these circuits leads to characteristic biological, psychological, social and spiritual manifestations. This is reflected in an individual pathologically pursuing reward and/or relief by substance use and other behaviours. Aviel Goodman (2008) defines addiction as a condition in which a behaviour that can function both to produce pleasure and to reduce painful affects is employed in a pattern that is characterized by two key features: (1) recurrent failure to control the behaviour, and (2) continuation of the behaviour despite significant harmful consequences.

Although research during the past two decades has increased the understanding of the neural processes that underlie addictive behaviour (Weiss and Porrino, 2002), this field still requires more investigations and development of new treatment strategies. Most research has not considered how affective processing may mediate addictive behaviours. Until, researchers have focussed more on the neurobiological substrates underlying addiction than on its affective components, and paid less attention to how the dysregulation of brain reward and stress systems bias addicted individuals towards continued substance use (Cheetham et al., 2010).

Much of the recent progress in understanding the mechanisms of addiction has derived from the study of animal models of addiction on specific drugs, such as stimulants and alcohol (Shippenberg and Koob, 2002). While no animal model of addiction fully emulates the human condition, animal models do permit investigation of specific elements of the process of drug addiction. Such elements can be defined by models of different systems, models of psychological constructs such as positive and negative reinforcement, and models of different stages of the addiction cycle (Koob, 2006).

Long-term repeated consumption of ethanol causes adaptation of the brain, which may lead to a habitual or even a compulsive use of the drug (Belin et al., 2009; Everitt et al., 2008). The influence of early environmental factors, such as parental loss, physical abuse etc, and other alterations that affect the developing brain functions may as a result increase propensity for ethanol addiction (Anand and Scalzo, 2000; De Bellis, 2002; de Kloet et al., 2005; Kendler et al., 2000). The conditions that lead to excessive alcohol consumption in some individuals but not in others are complex interactions between environmental, psychosocial, genetic and neurobiological factors (Dick and Foroud, 2003; Kreek et al., 2004) and are suggested to be the cause of variations in vulnerability to addiction between individuals (DiFranza et al., 2007; Le Strat et al., 2009). In order to examine the early-life environment impact on ethanol consumption, underlying neurochemical mechanisms and behavioural functions later in life, the maternal separation model is commonly used (de Kloet et al., 2005; Holmes et al., 2005; Ladd et al., 2000; Pryce and Feldon, 2003). Early social interactions, such as

closeness to mother, are known to be essential in order to have a normal social behaviour and physiological development (Hofer, 1994; Krinke, 2000). Therefore, different manipulations during the three first postnatal weeks, such as maternal separation, alter these developmental processes. It is possible to simulate variable relevant environmental settings using controlled experimental conditions in order to evaluate the short- or long-term outcomes (Ladd et al., 2000; Pryce et al., 2005).

Ethanol intake has an effect on different organs and, in the brain, on several neurochemical systems, such as those using DA, 5-HT, gamma-aminobutyric acid (GABA), glutamate, CRF etc as their neurotransmitter (reviewed in Dick and Foroud, 2003). There is strong evidence that monoamine systems are involved in addiction process. The DA system appears to be included in ethanol-induced effects (Imperato and Di Chiara, 1986; Koob and Le Moal, 2001). Many studies have reported that the initial rewarding effects of ethanol comprise the activation of the mesocorticolimbic DA pathway (also called the reward pathway), where DA neurons projecting from the ventral tegmental area (VTA) terminate in the forebrain target area, comprising amygdala, NAcc and frontal cortex (Everitt and Robbins, 2005; Koob, 2006; Wise, 2008). Additionally, DA system has been indicated to be sensitive to early-life environment. Brake et al. (2004) presented that prolonged litter-wise maternal separation have lower DA transporter density in the mesolimbic DA pathway in adult male rats. Alterated DA levels are further suggested following prolonged litter-wise maternal separation compared to short litter-wise (Matthews et al., 2001).

Many animal and clinical studies are supporting the involvement of the 5-HT system in ethanol reward (Boehm et al., 2005; Oreland, 2004; Yoshimoto et al., 1992). A dysfunction in central 5-HT neurotransmission has been implicated in the pathogenesis of ethanol addiction, although mechanisms remain still unclear (Boehm et al., 2005; Heilig and Egli, 2006; Yoshimoto et al., 1992). Quite recently, a number of gene and early-life environment interaction studies in humans and animals have been carried out (Advani et al., 2007; Nilsson et al., 2005). These studies have been suggested that 5-HTT gene variants and early-life environment affects the vulnerability to ethanol intake.

In addition, brain stress response system is hypothesized to be activated by acute excessive alcohol consumption, to be sensitized during repeated withdrawal, and to contribute to the compulsivity of alcoholism. Brain stress system is mediated by CRF system and is dysregulated by chronic administration of all major drugs with dependence or abuse. As a result, a common response of increased corticosterone and amygdala CRF during acute withdrawal from chronic drug administration may be observed (Koob, 2008; Rasmussen et al., 2000).

The investigation of inter-individual differences in vulnerability to drug effects became more significant in 1990s as a response to the work of Piazza et al. (1989) who observed that differences in response to novel environment, as measured by locomotor activity, would predict the sensitivity to psychostimulants. Compared to LR-rats, HR-rats were found to have higher locomotor

activity in the novel test environment and, response to amphetamine and cocaine administration, and stronger behavioural sensitization to repeated drug treatment. Psychostimulants such as cocaine and amphetamine produce large conditioned place preference (Mackey and Van der Kooy, 1985; Spyraki et al., 1982) and it is generally accepted that DA-ergic neurotransmission is involved (Tzschentke, 1998). Since then, the LR/HR feature has been proposed to model human trait of vulnerability to drug addiction and sensation seeking (Blanchard et al., 2009; Kabbaj, 2006).

1.3.3. Rat ultrasonic vocalization: inter-individual differences

Animal models of affective disorders usually focus on states resembling fear, anxiety, neophobia, depressiveness and other expressions of negative affect (Harro, 2010; Singewald, 2007) but in recent years, research on depression has increasingly paid attention to positive affect (Geschwind et al., 2011; Forbes and Dahl, 2005; Fredrickson, 1998; Watson and Naragon-Gainey, 2010). Positive affect is defined as a trait that refers to stable individual differences in the experience of positive emotions and active engagement with surroundings. Positive and negative affect are relatively independent of one another, which means that they can occur in a widely varying range of combinations (e.g. an individual can be high or low in both traits) (Brudzynski, 2007; Watson and Naragon-Gainey, 2010). They may be taken as the subjective components of larger biobehavioural systems that have evolved to promote the survival of animals. Positive affect is related to the behavioural facilitation system, an approach system that directs towards rewarding and pleasurable situations for organisms, such as food, shelter, and sex (Naragon and Watson, 2009).

Nevertheless, animal models for positive affect are scarce: intracranial self-stimulation (Carlezon and Chartoff, 2007; Sagara et al., 2010), conditioning with addictive drugs (Koob, 2009), measurements of sexual behaviour (García-Horsman et al., 2008; Paredes and Martínez, 2001) and consumption of preference of palatable food (Agmo and Marroquin, 1997). However, these techniques have confounding factors, such as homeostasis-related motivation and general activity. Studying the emotional systems of the animals via possibly affectively valenced vocalizations has been a sphere of interest for almost thirty years. It is generally accepted that ultrasonic vocalizations (USVs) elicited by animals carry a sociobiological significance. Studying these USVs creates an opportunity to have a better understanding about their behaviour, nervous system functions and affective states, and thereby use this knowledge in modelling human affective states (Panksepp, 2007).

Rats elicit ultrasonic vocalizations in many different social and emotional situations and these USVs have been studied mainly in the context of sexual and aggressive behaviour (Takahashi et al., 1983), anxiety, after maternal separation in rat pups (Brunelli and Hofer, 1996; Sales and Pye, 1974) but also in a few other affective states and conditions in rats (Blanchard et al., 1991; Vivian and Miczek, 1991). These USVs vary in frequency and duration and it is known by

now that we can distinguish at least three different types of ultrasonic vocalizations in rats: 1) the 40-kHz vocalizations in rats pups that are maternally separated (Hofer and Shair, 1978; Panksepp, 1998; Sales and Pye, 1974); 2) USVs with long duration and frequency approximately below the 30-kHz (usually termed „22-kHz USVs“) that seem to be the adult counterpart of 40-kHz calls that are evident in distressing situations (Noirot, 1968; Nyby and Whitney, 1978) and 3) short and high-frequent 50-kHz USVs or “chirps“exhibited by adolescent and adult rats (Miczek et al., 1991; Sales and Pye, 1974). These 22-kHz and 50-kHz vocalizations have been suggested to express opposite affective states like aversion and anticipation in adult rats (Burgdorf et al., 2001; Knutson et al., 1999). Longer-lasting 22-kHz USVs in adult rats are considered to reflect negative affective states and are elicited e.g., during social defeat and in response to danger (Blanchard et al., 1991; Sánchez, 2003; Tonoue et al., 1986; Vivian and Miczek, 1991). In contrast, vocalizations in the around 50-kHz frequency range (the so-called "rat laughter") reflect the positive affective states such as in observed playfulness, joyfulness (Burgdorf and Panksepp, 2001; Knutson et al., 1999; Panksepp and Burgdorf, 2003) and in case of sexual behaviour (Barfield et al., 1979; Sales and Pye, 1974). It was found that these chirps can be easily induced by tickle-like stimulation delivered by an experimenter. This experimental animal tickling procedure mimics the stimulation exerted by natural rough-and-tumble play in rats (Burgdorf and Panksepp, 2001; Burgdorf et al., 2005; Panksepp and Burgdorf, 2003). These 50-kHz vocalizations have a positively reinforcing effect as demonstrated by elevated operant behaviour and changes in CPP in response to tickling (Burgdorf and Panksepp, 2001).

It has been demonstrated that individuals persistently differ in emotional (positive and negative) reactivity and that these individual differences have implications for affect, social relationships and well-being (Gross and John, 2003; Tellegen, 1985). It is clear by now that the 50-kHz ultrasonic vocalizations are individually stable (Mällo et al., 2007b, 2009; Schwarting et al., 2007) and there are differences in the predisposition of animals to respond to tickling with low (LC) or high (HC) levels of 50-kHz USVs that are related to more general tendency for emotional reactivity of animals over different situations (Mällo et al., 2007b, 2009). Different negatively valenced stimuli reduce 50-kHz USVs (Knutson et al., 1998). If HC- and LC-rats were exposed to a chronic stress regime, stress suppressed the 50-kHz vocalizations, but the levels of 22-kHz USVs were increased in LC male rats (Mällo et al., 2009), suggesting that in males low inherent positive affectivity predisposes to anxiety and affective disorders.

Brain pathways involved in production of these different types of ultrasound can be analyzed through their component neurotransmitter mechanisms. Many investigations suggest that anxiogenic stimuli elicit the release of 5-HT, but that distinct 5-HT-ergic pathways can make a different contribution (Millan, 2003; Morilak and Frazer, 2004). The hippocampal 5-HT-ergic system has been acknowledged to mediate the anxiogenic response (File et al., 2000), while

chronic stress profoundly affects hippocampal 5-HT-ergic neurotransmission in this region, especially in the dentate gyrus (Joëls et al., 2004; McKittrick et al., 1995). Additionally, changes in 5-HT and its metabolite levels/turnover or release following chronic stress in other brain regions have been observed (Bekris et al., 2005; Gamaro et al., 2003; Mangiavacchi et al., 2001). It is also suggested that 50-kHz USVs are associated with positive affect and reward-seeking behaviour, which is believed to be mediated by DA-ergic transmission (Burgdorf et al., 2000; Mällo et al., 2007b). Indeed, activation of the ascending DA-ergic system induces the state with 50-kHz calls, while activation of the relevant portion of the ascending cholinergic system invariably induces the negative state and elicits 22-kHz calls (Brudzynski, 2007). Fu and Brudzynski (1994) showed that injecting of glutamate into the anterior hypothalamic-preoptic area of the rat brain induced 50-kHz USVs while injection of carbachol (a muscarinic cholinominetic agent) into the same brain area contrarily induced 22-kHz USVs, suggesting that these USVs have different neurophysiological and neurochemical mechanisms. Recent studies have revealed that in the medial prefrontal cortex, production of the hedonic 50-kHz vocalizations is determined by the glutamate system and depends on NMDA receptors levels that is increased by rough and tumble play (Burgdorf et al., 2011).

2. HYPOTHESES AND AIMS OF THE THESIS

Inter-individual differences in animal depression-related behaviour and expression of naturally occurring behavioural phenotypes create an opportunity to have a better understanding about the causes of behaviour, nervous system function and affective states. This knowledge can be used for modelling human affective states. The studies presented in this thesis are focussed on relating inter-individual differences in responses of animals to novel environment and imitated social stimuli to underlying neurotransmitter system involvement. More specific aims of the present thesis are:

- The noradrenergic projections arising from the locus coeruleus are necessary for the high expression of exploratory behaviour. To test the hypothesis that intact projections of the locus coeruleus are important for full expression of the effects of psychostimulants acting directly on the dopaminergic system, the effect of denervation of the locus coeruleus with neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) on cocaine-induced locomotion and place preference in rats was examined (**Paper I**).
- In order to test the hypothesis that early environmental experiences would cause long-term changes in monoamine systems, and as a consequence, would alter the response to ethanol during later life, serotonin and dopamine tissue levels in maternal separated male rats after ethanol consumption were studied (**Paper II**).
- As serotonergic neural circuits have been associated with anxiety- and depression-related conditions, the hypothesis that there are differences in serotonergic regulation in rats with different exploratory activity was tested (**Paper III**).
- Glutamatergic neurotransmission has become a target in the development of novel antidepressants. To test the hypothesis that there is an involvement of glutamate system in individual variation of traitwise expression of exploratory behaviour, the glutamate levels in rats with different exploratory activity were measured (**Paper V**).
- In order to test the hypothesis that there are differences in serotonergic regulation between animals with inherent differences in positive affectivity after chronic variable stress, the levels of extracellular serotonin, and corticosterone levels, were measured in low and high chirping rats under chronic variable stress conditions (**Paper IV**).

3. MATERIALS AND METHODS

3.1. Animals and housing conditions

Male (**Papers I, III-V**) or female (**Paper II**) Wistar rats from Scanbur BK AB, Sweden (**Papers I–V**) or Harlan Laboratories, the Netherlands (**Papers I and V**), were used. All animals arrived to our animal facilities approximately at the age of 3 weeks (except in **Paper II**). The behavioural experiments started when the animals were at least 2 months old, except when the juvenile animals were the subjects. Rats were housed in groups of four (**Papers I**, **III, IV** and **V)** or single-housed (**Paper II**) in standard transparent polypropylene (**Papers I**, **III–V**) or makrolon cages (**Paper II**) under controlled light cycle (lights on from 08:00 h to 20:00 h, and temperature (19–22 °C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). All behavioural experiments were carried out when lights were on in an isolated experimental room. The experimental protocol was approved by the Ethics Committee of the University of Tartu and Uppsala Ethics Committee and the Swedish Animal Protection Legislation.

3.2. Drug administration

DSP-4 was administered in the dose of 10 or 50 mg/kg intraperitoneally (i.p.) (expressed as for hydrochloride) (**Paper I**). Each dose was weighed separately, dissolved in distilled water (2 ml/kg) and injected immediately. Cocaine hydrochloride was dissolved in distilled water and administered in the dose of 15 mg/kg in the volume of 2 ml/kg i.p. immediately before conditioned place preference trainings (**Paper I**). Citalopram hydrobromide (1μM) diluted in Ringer solution was administered locally via reverse dialysis (**Papers III** and **IV**). DL-p-chloroamphetamine (PCA) was administered i.p. in the dose of 2 mg/kg (**Paper III**). PDC (4 mM) diluted in Ringer solution was perfused via the microdialysis probe (**Paper V**). Control animals received a vehicle injection (**Paper I**).

3.3. Chemicals

Drugs: cocaine hydrochloride was purchased from Synapharm GmbH & Co. KG, Germany; citalopram hydrobromide from H. Lundbeck A/S, Copenhagen, Denmark; *N*-(2-chloroethyl)-*N*-ethyle-2-bromobenzylamine (DSP-4) from AstraZeneca, Södertälje, Sweden; L-*trans*-pyrrolidine-2,4-dicarboxylic acid was purchased from Sigma, St. Louis, MO, USA. Different standard substances for high performance liquid chromatography (HPLC) analysis: 5-hydroxytryptamine from Sigma, Germany; 3-hydroxytyramine Fluka, Germany; homovanillic acid (HVA), normetanephrine (NMN), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), and noradrenaline from Sigma, Switzerland; glutamate from Applichem, Germany.

Solvents: methanol, ethanol and acetonitrile from Rathburn, Finland. Other chemicals for preparing HPLC eluents or Ringer solution: calcium chloride-2-hydrate and potassium chloride from Riedel-de Haën GmbH, Germany; magnesium chloride from Acros, Belgium; sodium chloride from Reanal, Budapest; sodium phosphate dibasic and boric acid from Applichem, Germany; sodium hydroxide from Applichem, Germany; sodium octanesulphonate from Fisher Scientific, UK; ethylenediaminetetraacetic acid from Serva Electrophoresis GmbH, Germany; sodium phosphate monobasic dihydrate, citric acid monohydrate and sodium metabisulfite from Fluka, Germany. The β-mercaptoethanol (β-ME) (Applichem, Germany) and *o*-phthaldialdehyde (OPA) (Sigma, Austria) were used to derivatizate glutamate.

3.4. Behavioural models and tests

3.4.1. Maternal separation

On the day of birth (day 0) (see **Figure 1**), the sex of each pup was identified before they were culled into litters with five males and four females. The litters were randomly assigned to one of three rearing environments: 1) daily 15 min of individual maternal separation (MS15, n=6 litters), 2) daily 360 min of individual maternal separation (MS360, n=6 litters), or 3) standard animal facility rearing conditions (AFR, n=7 litters). The separations occurred once daily between postnatal days (PND) 1 and 21 as previously described in detail (Gustafsson et al., 2008). First, the dam and then the pups were removed from the nest. The rat pups were placed individually in makrolon cages (26×20× 14 cm) containing wood chip bedding material in a warm cabinet (31.5±0.2 °C) to avoid hypothermia. These cages were changed every third day. The rat pups were separated from each other using a metal cross that divided each cage into four compartments. Separation sessions were performed during the light period with the first MS15 litter starting at 09:00 and the first MS360 litter starting at 09:30. During the separations, the dams in the MS15 groups were moved to other cages and the pups were returned before the dams. In the MS360 groups, the dams were returned to the home cages during the separations and moved again before the pups were returned. All litters were weighed on PNDs 0, 7, 16 and at weaning PND 22. These days were the only occasions when the AFR animals were handled before weaning. The litters were weighed with the pups kept together and the mean pup per litter was calculated. On PND 22, the males were weaned and housed in the same experimental groups with 4–5 males per cage (59×38×20 cm) during a period of seven weeks with standard animal facility rearing with changes of cages and water bottles once a week from PND 23 to 67.

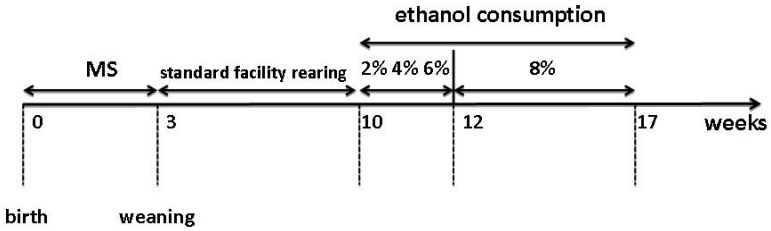

Figure 1. The scheme of maternal separation and ethanol consumption procedure.

3.4.2. Two-bottle free choice between ethanol and water

The consequences of repeated individual maternal separation on long-term voluntary ethanol consumption were examined in adult male rats using the two-bottle free-choice drinking paradigm (see **Figure 1**). The rat pups were subjected to the MS15, MS360 and AFR conditions until weaning. At age of 10 weeks, the male rats were divided into water-consuming and ethanol-consuming groups. The adult male rats were weighed before access to ethanol and then every third day during the ethanol-drinking period. Food intake was measured every third day. During the ethanol-drinking period (7 weeks) the rats were singly housed (59x38x20 cm cages) with a wooden house per cage a wood chip bedding material. The different ethanol concentrations were prepared from 95% ethanol diluted in tap water and were room temperature when placed in the cages. The rats were given access to a two-bottle choice between water and ethanol for seven weeks. The ethanol concentration was gradually increased during the first two weeks with access to 2% (v/v) ethanol for four days and four days of 4% ethanol followed by six days of 6% ethanol. Thereafter, the rats had free access to 8% ethanol and water for five additional weeks. The water-consuming AFR, MS15 and MS360 groups served as control groups and were housed under the same condition but had access to two containing water bottles only. Plastic 250-ml bottles with ball-valve nipples (Scanbur BK AB, Sollentuna, Sweden) were used. Bottle positions were randomised every day to avoid position preferences and were weighed daily during the seven weeks to measure the fluid consumption. All measurements were made in the afternoon.

3.4.3. Place preference conditioning and assessment of locomotion

Place preference conditioning procedure was conducted as described previously (Kivastik et al., 1996). Animals were randomly assigned either to cocaine or vehicle group and handled in the experiment room for 2 min on 3 consecutive days prior to the introduction to the CPP apparatus. On the 9th and 10th day after the DSP-4 treatment, rats were placed into the white part of the CPP apparatus and were given free access to both compartments for 15 min. The latter session was recorded and served as a baseline for place preference

conditioning. Next, during eight daily conditioning sessions (days 11–18 after the DSP-4 treatment), rats were injected once every other day with either cocaine (cocaine group) or distilled water (vehicle group), and then immediately confined into the nonpreferred (white) compartment for 30 min. On alternate days, all animals were injected with vehicle, and confined into the other (gray) compartment. On the 19th day after the DSP-4 treatment, no injections were given; rats were initially placed into the nonpreferred chamber and had then free access to both compartments for 15 min, their activity being recorded. The following measures from the digital recordings were monitored: (1) number of entries into the white compartment, (2) time spent in the white compartment, (3) line crossings and (4) rearings in the white compartment. In order to estimate the effect of denervation of LC on acute locomotion stimulating effect of cocaine and possible revelation of sensitization to cocaine, each animal's first and last training in the white compartment (11–12th and 17–18th day after the DSP-4 administration) were also recorded and for the first 15 min (1) rearings and (2) line crossings were scored (Experiment 1 of **Paper I**). The Experiment 2 of **Paper I** was an exact replication of the acute phase of Experiment 1. Schematic overview of the experimental set-up for Experiment 1 and Experiment 2 is presented in **Figure 2**. To replicate the effect of denervation of LC on acute locomotion stimulating effect of cocaine, on the 11th day after the neurotoxin treatment, rats were either injected with cocaine (cocaine group) or vehicle (control group), restricted into the white compartment and for 15 min, their activity was recorded and subsequently scored. The animals were sacrificed 3 days after the last drug-free CPP test.

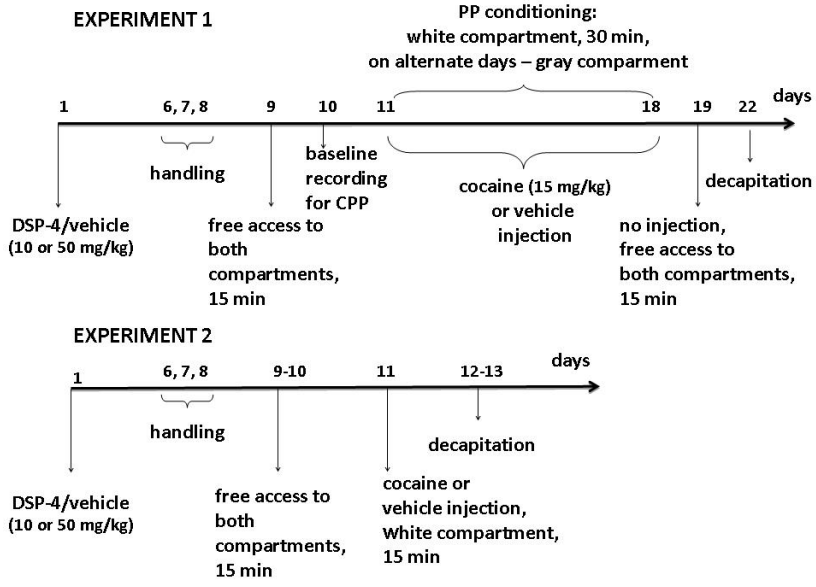

Figure 2. The principal scheme of place preference conditioning and assessment of locomotion in Experiments 1 and 2.

3.4.4. Exploration box test

The exploration box test was conducted as described previously (Harro et al., 1995; Otter et al., 1997). The exploration box was made of metal and consisted of a 0.5×1 m open area (side walls 40 cm) with a small compartment (20×20×20 cm) attached to one of the shorter sides of the open area. The open area was divided into eight squares of equal size. In the open area, four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden handle and a food pellet) were situated in certain places (which remained the same throughout the experiment). The small compartment had its floor covered with wood shavings and was directly linked to the open area through an opening (size 20×20 cm). The apparatus was cleaned with a dampened cloth after each animal. The exploration test was initiated by placing a rat into the small compartment, which was then covered with a lid for the time of the test. A single test session lasted 15 min and the following behavioural parameters were registered: (1) latency (of entering open area with all four paws), (2) number of entries into the open area, (3) time spent exploring on the open area, (4) line crossings, (5) rearings, and (6) number of unfamiliar object investigations. To provide an index of exploration the scores of line crossing, rearing and object investigation were summed for each animal and thus (7) the sum of exploratory events obtained. Animals were classified as high or low explorers (HE and LE, respectively) as previously described (Alttoa et al., 2005). All animals were exposed to the exploration box test on two consecutive days and the categorization as HE and LE was based on the sum of the exploratory events on the second day of testing.

3.4.5. Tickling-induced ultrasonic vocalization and its measurement

The single-housed rats were given daily sessions of tickling-like stimulation that mimics natural rough-and-tumble play in juvenile rats (Burgdorf and Panksepp, 2001) and elicits high levels of 50-kHz chirping (Burgdorf et al., 2005). The tickling procedure was carried out as previously described (Mällo et al., 2007b, 2009) and lasted for 14 days. Animals were individually removed from home cage into an empty and smaller cage and given 15 s for habituation, followed by 15 s of tickling by experimenter. Altogether, four 15 s sessions of stimulation were given over 2 min, after which animals were returned to home cage and test cage cleaned. A microphone was located about 20 cm from the floor of the tickling cage, recording high frequency audio files of the tickling sessions, which were later analyzed with the Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany) software, creating spectrograms from which USVs were manually counted. Fifty kilohertz USVs were counted and animals classified as emitting high or low number of 50-kHz USVs by the median split of the average response on days 12–14 of tickling, providing the high chirping (HC) and low chirping (LC) groups.

3.4.6. Chronic variable stress procedure

At the age of two months the animals were submitted to CVS. The CVS procedure used was developed on the basis of previous experiments (e.g. Harro et al., 2001). CVS regimen lasted for 4 weeks and comprised of seven different stressors each of which was intermittently used once every week: 1) imitation of peritoneal injection using special glove with syringe without needle being pressed to the body of animal for several seconds; 2) stroboscopic light (for 14 h, 10 Hz, 2 lx); 3) tail-pinch with a clothes-pin placed 1 cm distal from the base of tail (5 min); 4) cage tilt at 45° (for 24 h); 5) strong illumination (900 lx) during predicted dark phase (for 12 h); 6) cold (4°C) water and wet bedding (400 ml of water was poured on the rats, and the sawdust bedding was kept wet for the following 22 h); 7) movement restriction in a small cage (11x16x7 cm) for 2 h. The stressors were administered during the light phase of the cycle (except for the stressors that lasted overnight).

3.5. Chemical methods

3.5.1. Monoamine measurement from tissue samples with high-performance liquid chromatography

Monoamines and their metabolites were assayed by high performance liquid chromatography with electrochemical (amperometric) detection. The rat brain tissues were homogenized with an ultrasonic homogenizer (Bandelin Sonopuls, Bandelin Electronic, Berlin, Germany) in ice-cold solution of 0.1 M perchloric acid (30 or 50 µl/mg) containing 5 mM of sodium bisulphite and 0.4 mM EDTA to avoid oxidation. The homogenate was then centrifuged at 14.000 rpm for 10 min at 4°C. Aliquots (10 µl) of the obtained supernatant were chromatographed on a Luna C18(2) column (150 x 2 mm, 5 µm). The separation was done in isocratic elution mode at column temperature of 30 °C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulphonate and 7.5% acetonitrile. The chromatography system consisted of an isocratic pump (Hewlett Packard HP 1100), a temperature-regulated autosampler, a temperature-regulated column compartment and an HP 1049 electrochemical detector (Agilent, Waldbronn, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.7 V versus the Ag/AgCl reference electrode. The limits of detection at signal-to-noise ratio (S/N)=3 were as follows: 0.08 pmol/mg tissue for DA; 0.10 pmol/mg tissue for HVA; 0.05 pmol/mg tissue for DOPAC; 0.08 pmol/mg tissue for 5-HT; 0.04 pmol/mg tissue for 5-HIAA.

3.5.2. Monoamine measurement from microdialysates with high-performance liquid chromatography

After four weeks of stress regimen, *in vivo* online microdialysis procedure was carried out as previously (Mällo et al., 2007a) in awake and freely moving animals. The principle of working of microdialysis probe (Ungerstedt, 1991) is presented in **Figure 3**. In short, the animals were anaesthetized with chloral hydrate (350 mg/kg i.p.) and mounted in a Kopf stereotactic frame. A self-made concentric Y-shaped microdialysis probe with 4 mm (**Papers III and IV**), 5 mm (**Paper III**), 7.0 mm or 7.5 mm (**Paper V**) shaft length; and 1 mm (**Papers III–V**) or 3.0 mm (**Paper V**) active tip or active membrane on the whole length (**Paper III**) were implanted into brain areas with the following coordinates: in PFC: AP, +3.3; ML, +0.8; DV, −5.0 (**Paper III**); in DG: AP -4.3; ML: +2.2; DV: –3.8 (**Papers III and IV**); in CA1: AP: –6.0; ML: –5.6; DV: –6.4 (**Paper V**) and in striatum: AP: +0.7; ML: +3.0; DV: –7.0 (**Paper V**) (according to Paxinos and Watson, 1986). The dialysis membrane used was polyacrylonitrile/sodium methalyl sulphonate copolymer [Filtral 12, inner diameter (ID): 0.22 mm; outer diameter: 0.31 mm; AN 69, Hospal, Bologna, Italy]. Two stainless steel screws and dental cement were used to fix the probe to the scull. After the surgery, rats were placed in individual cages (21x36x 18 cm) in which they remained throughout the rest of the experiment and given about 24 h for recovery. After recovery, the animal was connected to the microdialysis system and the perfusate with Ringer solution (140 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, pH=7.2) at flow rate 1.5 µl/min discarded during the first 60 min to allow stabilization. Then 7 samples of microdialysate were collected, followed by perfusion of the 5-HT reuptake inhibitor citalopram (1 µM) for 2.5 h (**Papers III and IV**) or glutamate uptake inhibitor PDC (4 mM) for 1 h (**Paper V**). Fourteen more samples were collected after the cessation of drug administration. All samples were collected in 15 min periods directly into a 50 µl loop (**Papers III and IV**) or inserts (**Paper V**) and were measured online (**Papers III and IV**) or offline (**Paper V**) by using HPLC with electrochemical detection.

All microdialysates were collected in 15 min periods directly into a 50 µl loop of the electrically actuated injector, injected automatically into the column in order to measure the quantity of monoamine in the samples online. The chromatography system consisted of a Shimadzu LC-10AD series solvent delivery pump, a Luna C18(2) column (150*2 mm, 5 µm) kept at 30ºC and Decade II digital electrochemical amperometric detector (Antec Leyden BV, Netherlands) with electrochemical flow cell VT-03 (2 mm GC WE, ISAAC reference electrode, Antec Leyden BV, Netherlands). The mobile phase consisted of 0.05 M sodium citrate buffered at pH 5.3, 2 mM KCl, 0.02 mM EDTA, 4.9 mM sodium octanesulphonate and 18.5% acetonitrile. The mobile phase was filtered through a 0.22 µm pore size filter (type GV, Millipore, USA) and was pumped through the column at a rate of 0.2 ml/min. Serotonin eluted

from the column (retention time 5 min) were measured with a glassy carbon working electrode maintained at a potential of +0.4 V versus Ag/AgCl reference electrode. Data were acquired using a Shimadzu LC Solution system. Concentrations of 5-HT were estimated by comparing peak heights from the microdialysates with those of external standards of known concentration. Baseline levels of monoamines were defined as average of four samples (3–6).

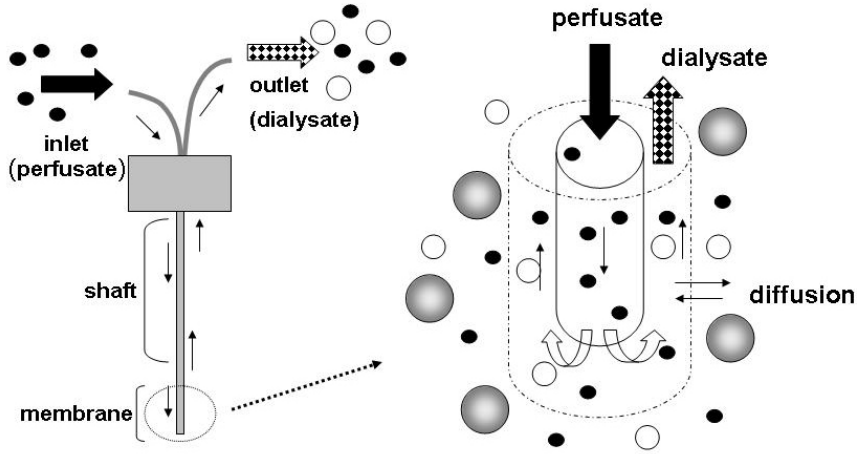

Figure 3. The principle of the microdialysis probe.

3.5.3. Glutamate measurement from microdialysates with high-performance liquid chromatography

The concentration of glutamate in the microdialysates was determined by using high performance liquid chromatography with electrochemical detection as described by Donzanti and Yamamoto (1988), with some modifications. A sample (7.5 µl) of collected microdialysate was derivatized by with 3.8 µl 40 mM *o*-phthaldialdehyde solution (0.04 M OPA; boric buffer pH=9.3; 10 % ethanol; 0.04 mM β-ME) for 1.5 min. The derivatization reaction is presented in **Figure 4**. Thereafter, a 10 µl pre-treated sample was injected into the HPLC system (Hewlett Packard HP 1100). Samples were chromatographed (retention time 6.8 min) (see **Figure 5**) on a Luna C18(2) column (150×2 mm, 5 µm, Phenomenex). The separation was done in isocratic elution mode at column temperature of 30 °C using the mobile phase (0.3 ml/min) containing 0.1 M phosphate buffer (23 % methanol). The chromatography system consisted of a Hewlett Packard HP 1100 Series isocratic pump (G 1310A ISO), a thermostatted autosampler (G 1329A ALS), a thermostatted column compartment and an HP 1049A electrochemical detector (Agilent, Waldbronn, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode. Concentrations of glutamate were estimated from a calibration graph by comparing peak heights from the microdialysates with those of external standards of

known concentration of glutamate. The detection limit of glutamate was 1 µM for a 2:1 signal-to-noise ratio. Calibration graphs were prepared for each series of measurement.

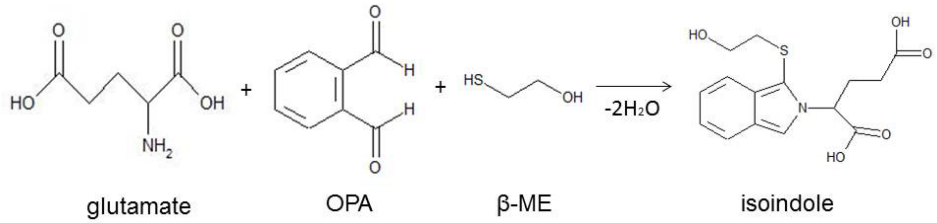

Figure 4. Derivatization of glutamate with *o*-phthaldialdehyde (OPA) and β-mercapto-ethanol (β-ME).

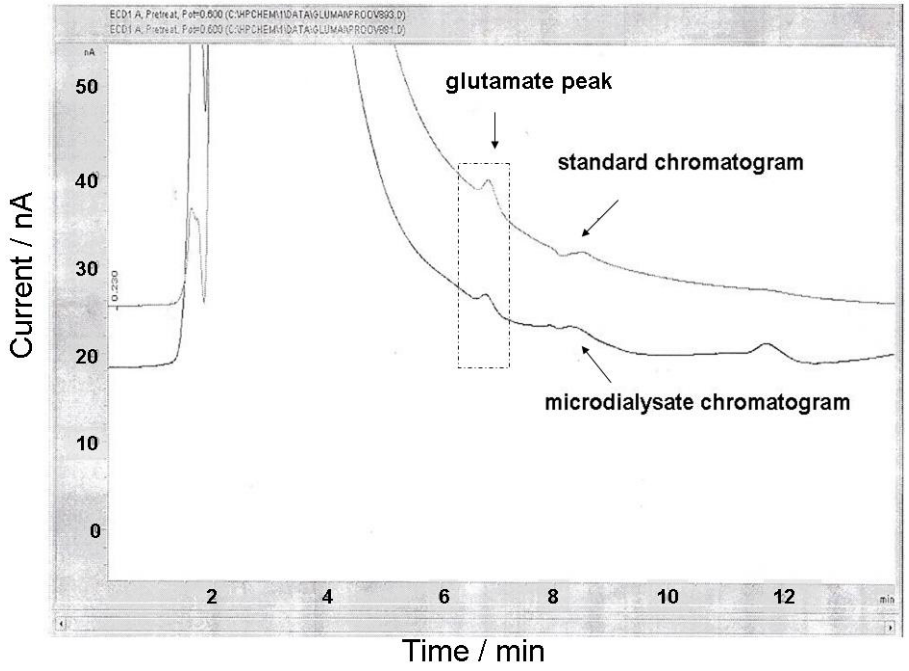

Figure 5. Chromatograms of standard and microdialysate.

3.5.4. Radioligand binding to serotonin transporter

Samples from the PFC and hippocampus of LE- and HE-rats were collected for the determination of 5-HTT levels by specific binding of [*N*-methyl-^{3}H]citalopram as described in Mällo et al. (2007b). The PFC samples were collected from the right hemisphere (contralateral to the microdialysis site) of the frozen brains of the animals that had undergone the microdialysis experi-

ment immediately before the probe localization determination, while the hippocampus samples were collected from the right hemisphere of naive animals that were decapitated and brains immediately dissected on ice. The cortical tissue were homogenized in 5 ml of homogenization buffer (50 mM Tris-HCl, pH=7.4) by Bandelin Sonopuls sonicator (three passes, 10 s each). The membrane fragments were collected by centrifugation at 30 000×g for 20 min at 4 ºC and washed by homogenization and centrifugation for two more times. The final pellet was resuspended in 100 vol (ww/v) of the incubation buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH=7.4). Binding of [*N*-methyl-^{3}H]citalopram (75 Ci/mmol, Amersham Biosciences) was carried out by incubating membranes (1 mg of tissue per tube) in incubation buffer with different concentrations of radioligand (0.3–3.5 nM) for 60 min at 25 °C. Non-specific binding was determined in the presence of 1 μM nonradioactive fluoxetine. The reaction was stopped by rapid filtration through GF/B glass-fiber filter (Whatman Int. Ltd., presoaked with 0.3% polyethyleneimine before filtration) and the filter were washed three times with ice-cold incubation buffer. The filter were kept in 4 ml of scintillation cocktail (OptiPhase HiSafe3, Wallac Perkin Elmer Life Sciences, Cambridge, UK) overnight and counted using a RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD, USA). Raw data was analyzed by means of a non-linear least squares fittings using GraphPad PRISM™ (GraphPad Software, San Diego, CA, USA) software.

3.5.5. RNA isolation, cDNA synthesis and quantitative real-time PCR

Brains of LE- and HE-rats, not used in any experiments but for the selection, were dissected and used for total RNA isolation and cDNA synthesis as described previously (Pruunsild et al., 2007). Levels of total BDNF and NGF mRNA were quantified with qPCR Core kit for SYBR® Green I No ROX (RT-SN10–05NR, Eurogentec, Belgium). All reactions were performed on LightCycler 2.0 thermocycler (Roche) using the following temperature cycling conditions: 10 min at 95°C (initial denaturation step), then 45 cycles of 5 s at 95 °C, 10 s at 55 °C and 10 s at 72 °C. All PCR reactions were performed in triplicate and normalized to β-actin (ACTB) mRNA levels. The following primers were used: BDNF_cod_s GGCCCAACGAAGAAAACCAT, BDNF_ cod_as AGCATCACCCGGGAA GTGT, NGF_s TTGCCAAGGACGCAGCTTTCTA, NGF as CAACATG GACATTACGCTATGCA, ACTB_s ATGCAATCCTGTGGCATCCAT and ACTB_as CCACCAGACAGCACTGTGTTG. The Q-PCR data were expressed as BDNF or NGF mRNA levels relative to the reference β-actin mRNA levels, with the expression level of 1.0 for a randomly selected sample.

3.5.6. Plasma corticosterone measurement

Animals were decapitated immediately after the collection of the last microdialysis sample, and trunk blood was collected into pre-cooled tubes containing K_3 EDTA. The blood samples were kept on ice and centrifuged after every 4 animals (4 000 × g for 10 min at 4°C). Plasma was pipetted into Eppendorf tubes and stored at –80°C until the assay. Plasma samples were thawed on ice and lightly vortexed and diluted 15 times. Plasma corticosterone was measured by ELISA (Correlate-EIA™, Assay Design, Inc., Ann Arbor, MI 48108, USA) according to directions of manufacturer. The sensitivity of this assay is 27 pg⁄mL. Upon completion of the assay, 96-well plates were read at 405 nm on a Labsystems Multiskan MCC/340 microplate reader.

3.6. Data analysis

In **Paper I**, data from the behavioural test and HPLC measurements were analyzed with two-factor analysis of variance (ANOVA), the Toxin (0 vs 10 vs 50 mg/kg of DSP-4) and Treatment (Vehicle vs Cocaine) as independent variables. Where appropriate, a repeated measures factor (Day) was added.

In **Paper II**, overall differences between MS15, MS360 and AFR groups assessed using the Kruskal-Wallis analysis followed by the Mann-Whitney U-test. Overall changes in fluid intake during the five-week access to 8% ethanol were analyzed using the Friedman test followed by the Wilcoxon signed rank test. Between-group differences in changes over time were compared using the Kruskal-Wallis analysis. Two-way ANOVA was used to examine the effect of rearing environment (MS15 vs MS360 vs AFR) and age (PND 0 vs 7 vs 16 vs 22) on the mean pup weight in litters during the postnatal period. The individual body weight in adult rats before and after water/ethanol consumption was compared using a repeated ANOVA. Differences in the levels of monoamine and their metabolites were analyzed using ANOVA. The effect of ethanol intake (ethanol vs water) the effect of rearing environment (MS15 vs MS360 vs AFR) and the interaction between ethanol and rearing environment were examined using two-way ANOVA. Fisher`s Protected Least Significant Difference (PLSD) test was used for *post-hoc* comparisons. Statistical analyses were performed with Statview v5.01 (SAS Institute Inc., Cary, NC).

In **Paper III**, data were analyzed with one-factor (Exploration) ANOVA, with a repeated measure (Sample) added for microdialysis data. Data of the 5-HTT binding were analyzed by means of non-linear least squares regression using a commercial program GraphPad PRISM™ (GraphPad, San Diego, CA, USA) and obtained parameters compared with t-test. Q-PCR data are expressed as BDNF or NGF mRNA levels relative to the reference β-actin mRNA levels and were analyzed with a two-factor ANOVA. When appropriate, *post hoc* comparisons were made with Fisher's PLSD test. All statistics were made using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA).

In **Paper IV**, data were analyzed with a two-factor (Chirping x Stress) ANOVA with repeated measures for the weight gain and microdialysis data and with a two-factor (Chirping x Stress) ANOVA for the corticosterone data. When appropriate, *post hoc* comparisons were made with Fisher's PLSD test. Weight gain for a particular day *n* was calculated by subtracting the day 1 weight from the day *n* weight.

In **Paper V**, data were analyzed with repeated measures ANOVA. Group differences after significant ANOVAs were measured by *post hoc* Fisher's PLSD test. All statistics were made using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA).

Statistical significance was set at $p<0.05$ in all analyses.

4. RESULTS AND DISCUSSION

4.1. Neurobiological and behavioural effects of denervation of the noradrenergic projections from locus coeruleus (Paper I)

4.1.1. Neurotoxin effect on tissue contents of monoamines and their metabolites

Monoamine and their metabolite tissue levels were measured in hippocampus and cortex after administration of two doses (10 mg/kg or 50 mg/kg) of the neurotoxin DSP-4, which has a selective effect on NA-axonal terminal fields originating from the locus coeruleus (LC).

More than 3 weeks (23 days) (Experiment I) after the administration of DSP-4, the higher dose (50 mg/kg) had reduced the tissue content of NA significantly in both hippocampus (74%) and frontal cortex (53%) (**Table 1**), that is, to a comparable extent with previous findings (Harro et al., 2000; Kask et al., 1997). However, after the lower dose (10 mg/kg) of DSP-4 treatment, NA levels did not differ significantly from that of control rats. Similar effect has been found in an experiment (Harro et al., 2003) where after 3 days the treatment with the same dose of 10 mg/kg DSP-4 reduced NA levels in the frontal cortex by 22%, but 1 month later the NA levels were found to be completely recovered. Still, 20–30% reduction of NA content in the same areas after 3–4 weeks with 10 mg/kg of DSP-4 treatment has also been observed (Alttoa et al., 2005, 2007). It is known that gradual recovery of NA levels after neurodegeneration elicited by DSP-4 treatment can occur (Hallman et al., 1984; Srinivasan and Schmidt, 2004; Szot et al., 2010). The tissue levels of NA were recovered in the DSP-4 (10 mg/kg) group by the time of sacrifice, but in behaviour – and in DA turnover – the functional effects were still observable and roughly similar to the effect of the higher DSP-4 dose. Still, NA levels, either at tissue levels or measured in extracellular space do not reflect well alterations in NA pathways, probably because of large compensatory capacities (Hughes and Stanford, 1998; Kask et al., 1997). However, changes in other neurotransmitter systems can reflect even small lesions in the NA system (Häidkind et al., 2002), and in the present work this is indicated by the reduction of DA turnover after DSP-4 treatment irrespective to the dose used (see **Table 1**).

Animals need to recover after DSP-4 treatment to replete peripheral stores of NA, and cocaine conditioning takes several days. As levels of NA recover by the time, it appears not possible to measure DSP-4 effect on cocaine conditioning and to use the NA levels to confirm the efficacy of the neurotoxin in the same animals. Therefore another, similar experiment (Experiment 2) was carried out, in which NA levels were measured earlier – 12–13 days after administration with neurotoxin. As we predicted, the NA levels were reduced dose-dependently as compared to the controls. Levels of NA were decreased in frontal cortex and hippocampus in the lower dose group by 32% and 28%,

respectively, and in case of higher dose 74% and 85% respectively (**Table 2**). Therefore, the finding that extensive LC denervation reduces the effect of cocaine was confirmed and we can presume that similar reduction in NA content also occurred in Experiment 1 during the first CPP training (11^{th}–12^{th} day after the neurotoxin administration) as this would also be in line with previous studies (Harro et al., 2003).

Additionally, pre-treatment with DSP-4 has effect not only on the noradrenaline but also on other monoamine systems. In the experiments described in **Paper I**, treatment with DSP-4 time-dependently affected DA and 5-HT levels. In Experiment 1, DA levels in frontal cortex were upregulated. Similar upregulation of DA levels has been reported by others (Hallman et al., 1984; Fortnai et al., 1996) who used near-complete denervation two weeks following DSP-4 treatment. In addition, in Experiment 1, DA turnover was reduced after DSP-4 pre-treatment irrespective of the dose used. Similar outcomes, increased DA levels, was observed in Experiment 2, but the increase was not statistically significant.

Alterations in 5-HT levels also appeared in both Experiments. Higher dose of DSP-4 (50 mg/kg) decreased the 5-HIAA levels in frontal cortex. In Experiment 2, both doses lowered the turnover of 5-HT (as measured by 5-HIAA/5-HT ratio) in frontal cortex. In hippocampus the higher dose of the neurotoxin reduced 5-HT in hippocampus, but enhanced 5-HT turnover. Treatment with DSP-4 has been found to change 5-HT levels and its turnover in several studies (Cassano et al., 2009; Dabrowska et al., 2008; Ferdyn-Drosik et al., 2010). Nevertheless it is still questioned whether this effect is direct or indirect. It seems that this effect is secondary and has no direct established behavioural importance. Nevertheless, a possible part of changes in 5-HT system and behavioural effects after the DSP-4 treatment still has to be considered.

Table 1. Monoamines in the rat brain 23 days after DSP-4 administration[a]

	Frontal cortex			Hippocampus		
	Vehicle	**DSP-4–10 mg/kg**	**DSP-4–50 mg/kg**	**Vehicle**	**DSP-4–10 mg/kg**	**DSP-4–50 mg/kg**
NA	2.11 ± 0.05	1.95 ± 0.06	1.00 ± 0.12$^{****,¤¤¤¤}$	3.09 ± 0.11	2.94 ± 0.10	0.82 ± 0.22$^{****,¤¤¤¤}$
NMN	0.23 ± 0.07	0.21 ± 0.02	0.18 ± 0.03	0.30 ± 0.02	0.29 ± 0.04	0.20 ± 0.03$^{*,¤}$
NMN/NA	0.11 ± 0.03	0.11 ± 0.01	0.19 ± 0.03$^{*,¤}$	0.10 ± 0.01	0.10 ± 0.01	0.60 ± 0.18$^{**,¤¤}$
DA	0.56 ± 0.02	0.68 ± 0.03**	0.63 ± 0.03	0.23 ± 0.06	0.33 ± 0.19	0.12 ± 0.01
DOPAC	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.09 ± 0.01	0.11 ± 0.04	0.08 ± 0.01
DOPAC/DA	0.30 ± 0.02	0.22 ± 0.02**	0.23 ± 0.02*	0.55 ± 0.09	0.44 ± 0.07	0.49 ± 0.08
HVA	0.23 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
5-HT	3.20 ± 0.06	3.30 ± 0.10	2.90 ± 0.13$^{¤}$	2.24 ± 0.18	2.26 ± 0.11	1.86 ± 0.15
5-HIAA	1.60 ± 0.02	1.50 ± 0.05	1.30 ± 0.09**	1.82 ± 0.15	1.82 ± 0.08	1.53 ± 0.08
5-HIAA/5-HT	0.51 ± 0.02	0.46 ± 0.01	0.47 ± 0.04	0.82 ± 0.03	0.82 ± 0.04	0.88 ± 0.06

[a] The values (mean ± S.E.M.) are expressed as pmol/mg wet weight tissue.

* $p<0.05$, vs vehicle group.

** $p<0.01$, vs vehicle group.

**** $p<0.0001$, vs vehicle group.

¤ $p<0.05$ vs 10 mg/kg DSP-4 group.

¤¤ $p<0.01$ vs 10 mg/kg DSP-4 group.

¤¤¤¤ $p<0.0001$ vs 10 mg/kg DSP-4 group.

Table 2. Monoamine levels in the rat brain 12–13 days after DSP-4 administration[a]

	Frontal cortex			Hippocampus		
	Vehicle	**DSP-4–10 mg/kg**	**DSP-4–50 mg/kg**	**Vehicle**	**DSP-4–10 mg/kg**	**DSP-4–50 mg/kg**
NA	2.32 ± 0.12	1.57 ± 0.10****	0.60 ± 0.06$^{****,¤¤¤¤}$	3.26 ± 0.15	2.36 ± 0.16****	0.48 ± 0.22$^{****,¤¤¤¤}$
DA	0.54 ± 0.04	0.62 ± 0.04	0.61 ± 0.03	0.16 ± 0.13	0.20 ± 0.05	0.15 ± 0.02
5-HT	3.94 ± 0.16	4.22 ± 0.19	3.83 ± 0.19	2.27 ± 0.08	2.27 ± 0.07	1.72 ± 0.09$^{****,¤¤¤}$
5-HIAA	1.56 ± 0.06	1.47 ± 0.07	1.31 ± 0.03$^{**,¤}$	1.72 ± 0.04	1.61 ± 0.05	1.69 ± 0.07
5-HIAA/5-HT	0.40 ± 0.02	0.35 ± 0.02*	0.35 ± 0.02*	0.77 ± 0.03	0.75 ± 0.02	0.97 ± 0.05$^{****,¤¤¤¤}$

[a] The values (mean ± S.E.M.) are expressed as pmol/mg wet weight tissue.

* $p<0.05$ vs vehicle group.

** $p<0.01$ vs vehicle group.

**** $p<0.0001$ vs vehicle group.

¤ $p<0.05$ vs 10 mg/kg DSP-4 group

¤¤¤ $p<0.001$ vs 10 mg/kg DSP-4 group

¤¤¤¤ $p<0.0001$ vs 10 mg/kg DSP-4 group.

4.1.2. The effect of locus coeruleus denervation on locomotor activity

The results of this experiment indicate a large decrease in locomotor activity effect of cocaine in animals with LC denervation (**Paper I Figure 1**). Smaller dose of DSP-4 was sufficient to reduce the cocaine-stimulated movement during the first training in the white compartment (nonpreferred part for animals) of the CPP apparatus, the experiment carried out 11–12 days after administration of DSP-4. During the last training in the cocaine-paired compartment both doses of DSP-4 reduced the number of cocaine-induced rearings, but only the higher dose had a decreasing effect [$F(2,42)=4.7$, $p<0.05$)] on line crossing. This supports the notion that the recovered NA levels in the 10 mg/kg group were behaviourally relevant. The fact that cocaine still had a substantial stimulating effect in animals with denervation on line crossings indicates that LC-denervated noradrenergic transmission enhances but is not completely required to obtain locomotor responses to cocaine. Although, in Experiment 2, there was a confirmation that higher dose of DSP-4 produces the reduction of locomotor activating effect of cocaine (**Paper I Figure 3**), the effect of the partial denervation was not significant in this experiment. While this could have been because of either different source/age of the animals used in this study, or lower locomotor response to cocaine, it suggests that a minor reduction in NA function has no robust impact on the motor activating effects of cocaine.

4.1.3. The effect of locus coeruleus denervation on cocaine-induced place preference

Cocaine-conditioned place preference was revealed only in rats with an intact projection from the LC. Cocaine CPP as measured by increase of time spent in the previously cocaine-paired chamber during drug-free conditions before and after cocaine-paired trainings, was completely absent after the large lesion of LC projections by high dose of DSP-4 (50 mg/kg) (**Paper I Figure 2**). The cause of this effect needs further investigation because it is not quite clear whether the attenuation of CPP in LC - compromised animals is attributable to attenuation of rewarding properties of cocaine or lesion in contextual learning or both. Studies using DSP-4 have suggested lesions in acquisition of contextual learning in two-way avoidance paradigm (Archer et al., 1984a; 1984b; Ögren et al., 1980). Impaired learning of a spatial discrimination has also been observed following treatment with DSP-4 (Archer et al., 1983). On the other hand, several studies have been presented that elevation of noradrenergic function improve the performance of rodents in attentional set-shifting tasks (Lapiz and Morilak, 2006; Lapiz et al., 2007). However, as the locomotor response to cocaine was attenuated in the 50 mg/kg DSP-4 group, it can be deduced that a crucial part in this effect is due to compromised NA system. It has also been reported that local prefrontal cortical NA reduction, which impairs cocaine-induced DA release, eliminates CPP in mice (Ventura et al., 2007). If NA levels

do not to reflect completely the behavioural effects of DSP-4, this might be because of mediation by compensatory changes in other neurotransmitter systems, such as the dopaminergic pathways. As recent studies have revealed that activity in VTA dopaminergic cells is sufficient to mediate place preference conditioning (Tsai et al., 2009), one may propose that psychostimulant reward depends on both noradrenergic and dopaminergic neurotransmission in this brain area.

4.2. Ethanol–induced effects on monoamine systems in the rat depending of early-life experiences (Paper II)

4.2.1. Influence of the early environmental effects and voluntary ethanol drinking on body weight

The body weight was measured throughout the experiment to examine whether the maternal separation *per se* resulted in body weight changes and also to examine any effects of ethanol consumption on body weight gain.

In pups, there was a significant effect of rearing environment [$F(2, 64)=14.37$, $p<0.0001$]. Although, all rats showed an increase in body weight as supported by the significant effect of age [$F(3,64)=1133.98$, $p<0.0001$], no significant interaction between rearing environment and age was observed, which indicated similar weight gain in the MS15-, MS360- and AFR-rats. In adult age, the body weight differed between MS15, MS360 and AFR groups [$F(5,85)=2.49$, $p=0.04$]. The MS360-rats had lower body weight both in young age and in adulthood compared to MS15- and AFR-rats before access to the two-bottle free choice between ethanol and water. This indicates that the MS360 regime was stressful. There were no significant differences between the rats assigned for ethanol or water consumption. Additionally, all rats had an increase in weight during the seven-week drinking period [$F(1,85)=3784.73$, $p<0.0001$] and the weight gain was similar. After drinking period, no differences observed between ethanol-drinking AFR-, MS15- and MS360-rats.

4.2.2. Early environmental effects on adult ethanol and water consumption

Ethanol and water intake were measured daily during seven weeks of continuous access to a free choice between ethanol (concentrations differed between days as seen in **Figure 1**) and water. No differences in voluntary ethanol intake were observed between the MS15-, MS360- and AFR-rats during the two weeks of increasing ethanol concentration. During the last five weeks before sacrifice, the rats had free choice between 8% ethanol and water. No differences in ethanol intake between MS15-, MS360- and AFR-rats were seen during these five weeks.

An overall significant increase of ethanol consumption over time was observed in all groups (**Figure 6A**). The ethanol intake continuously increased over time and stabilised towards the end of the drinking period. All groups consumed more ethanol during the last week compared to the first week but still no differences in consumption increase were observed between groups. Therefore, the assessment of the neurobiological consequences of voluntary ethanol consumption could be performed without the confounding effects of differences in ingested ethanol.

There were no differences between the experimental groups in water consumption. However, water intake was lower during the last week compared to the first week in all groups [MS15 ($Z=-3.40$, $p=0.0007$), MS360 ($Z=-2.90$, $p=0.004$), AFR ($Z=-3.18$, $p=0.002$)], with a similar decrease. Additionally, there was no change in total fluid consumption between the first and last week during access to 8% ethanol.

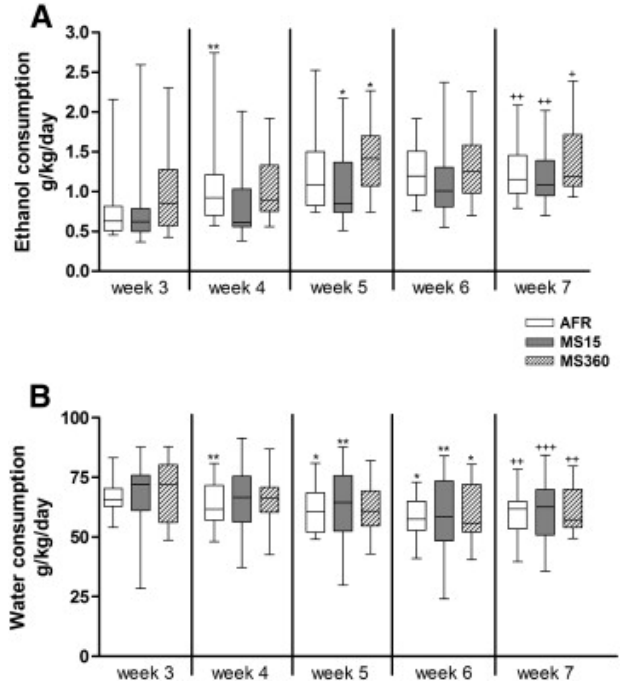

Figure 6. Ethanol and water consumption in adult rats. The rats were exposed to individual maternal separation for either 15 min (MS15) or 360 min (MS360) or AFR during the postnatal period. The rats had free access to ethanol and water using a two-bottle paradigm for seven weeks. The graphs illustrate the weekly 8% ethanol (A) and water (B) consumption during the last five weeks before the neurochemical analysis. Data are shown as medians and 1st and 3rd quartiles (box). The whiskers extend to show the highest and lowest values. *$p<0.05$; $p<0.01$; compared to the ethanol intake the preceding week in each group (Wilcoxon signed rank test). +$p<0.05$; ++$p<0.01$; compared to the first week with 8% ethanol in each group (Wilcoxon signed rank test).

4.2.3. Early environmental and ethanol effects on levels of dopamine and serotonin

The experiment herein revealed that voluntary ethanol consumption for seven weeks induced a few region-and transmitter-specific changes depending on the ingested ethanol dose. DA, 5-HT and their metabolites were measured in the frontal cortex, cingulate cortex, nucleus caudatus, NAcc, VTA, amygdala, hippocampus and dorsal raphe nucleus (DRN).

The amount of metabolite formed is indirectly reflected in the changes of the amount of neurotransmitter being released. Therefore, alterations in the metabolite formation are often used as indicators of neuronal activity (McBride et al., 1989). The results of turnover of tissue levels of 5-HT and DA and statistical tests are presented in **Table 3**. In mesocorticolimbic (largely dopaminergic) areas, including NAcc, VTA, DRN, cortex and amygdala, the results pointed to environmental-induced alterations in 5-HT and DA transmission.

In nucleus accumbens, the control MS360-rats had lower 5-HT levels in the NAcc than both MS15 and AFR groups, indicating environmentally induced adaptation in this system that might lead to changes in adult behaviour. Lower 5-HT levels in rats subjected to the longer separation, which served as a risk environment, is in line with previous studies in rats with different innate propensity for high voluntary ethanol consumption (Arborelius and Eklund, 2007). The facts that ethanol-drinking MS15-rats had more significant lowering of 5-HT levels compared to water-drinking MS15-rats (**Paper II Figure 2A**), and that 5-HIAA levels (**Paper II Figure 2B**), were not affected by ethanol, combined in an increased 5-HIAA/5-HT (**Table 3**) ratio in MS15-rats. An increase in 5-HT turnover could indicate an increase of 5-HT activity after ethanol consumption.

Many studies accept a regulating role of 5-HT on the central DA activity (reviewed in Di Matteo et al., 2008). Also it is suggested that 5-HT inhibits DA-dependent reward networks in the NAcc, and that 5-HT plays an important role in reward-related behaviour (Liu and Ikemoto, 2007; Nakamura et al., 2008). However, in the present work, no differences in DA measures after seven weeks of ethanol consumption were observed in this region.

It is indicated that the serotonergic innervation of the NAcc originates from the DRN (Van Bockstaele et al., 1993). In the present work, there were no changes either in 5-HT or 5-HIAA levels in the DRN area as a result of ethanol consumption. However, the DOPAC+HVA/DA ratio was increased, indicated an increase in dopaminergic activity. Ethanol-induced effects in DRN were observed similarly to NAcc only in MS15-rats. Dopamine-containing nerve terminals in the DRN are demonstrated to regulate 5-HT neurons (Ferré and Artigas, 1993) that are further linked with the NAcc in an interactive neuronal circuit (Brown and Molliver, 2000; Daw et al., 2002). Altogether, shorter time maternal separated rats were more sensitive to ethanol-induced changes in the NAcc and DRN compared to other groups and there was dependence on previous early environmental experiences in the DRN response to ethanol.

Table 3. 5-HT turnover (A) and DA turnover (B) expressed as the ratio between the transmitter and metabolite(s) in adult rats. The rats were subjected to three different rearing conditions during the first three postnatal weeks, 15 min individual maternal separation (MS15), 360 min individual maternal separation (MS360) and animal facility rearing (AFR). The tissue levels were measured in different brain areas after seven weeks of voluntary ethanol intake (E) and water intake (W).

Brain region		AFR	MS15	MS360
A. 5-HIAA/5-HT				
Frontal cortex	W	0.310±0.015	0.288±0.012	0.286±0.010
	E	0.299±0.012	0.295±0.011	0.274±0.008
Cingulate cortex	W	0.687±0.072	0.706±0.097	0.728±0.068
	E	0.689±0.058	0.731±0.061	0.749±0.039
Dorsal raphe nucleus	W	1.358±0.044	1.353±0.094	1.414±0.075
	E	1.389±0.065	1.489±0.075	1.591±0.119
Nucleus accumbens	W	0.809±0.026	0.707±0.038	0.811±0.045
	E	0.848±0.028	0.820±0.030[a]	0.826±0.033
Ventral tegmental area	W	0.890±0.071	0.804±0.053	0.978±0.077
	E	0.968±0.081	0.863±0.046	1.029±0.082
Amygdala	W	0.426±0.037	0.472±0.037	0.551±0.020
	E	0.510±0.033	0.503±0.031	0.437±0.031[a,b]
B. DOPAC+HVA/DA; DOPAC/DA in amygdala				
Frontal cortex	W	0.811±0.051	0.611±0.076	0.539±0.051
	E	0.667±0.067	0.755±0.086	0.684±0.058
Cingulate cortex	W	0.647±0.028	0.732±0.027	0.743±0.043
	E	0.718±0.037	0.695±0.030	0.703±0.039
Dorsal raphe nucleus	W	0.540±0.058	0.380±0.045	0.463±0.045
	E	0.557±0.046	0.551±0.044[a]	0.528±0.036
Nucleus accumbens	W	0.180±0.005	0.178±0.010	0.180±0.006
	E	0.186±0.005	0.189±0.006	0.183±0.006
Ventral tegmental area	W	0.890±0.071	0.804±0.053	0.978±0.077
	E	0.968±0.081	0.863±0.046	1.029±0.082
Amygdala	W	0.183±0.030	0.244±0.055	0.248±0.031
	E	0.263±0.033	0.181±0.028	0.158±0.025[b]

Data are presented as mean ± SEM

[a] $p<0.05$ compared to the corresponding water drinking group

[b] $p<0.05$ compared to the corresponding AFR group

In frontal cortex, adult water-drinking MS360-rats had lower levels of 5-HIAA compared to the water-drinking MS15-rats (**Paper II Figure 3B**). Low frontal cortical 5-HIAA levels have been linked to impulsiveness (Higley et al., 1991), which in turn is closely linked to addictive behaviour both as a cause and a consequence (de Wit, 2009). Thus changed 5-HT transmission in the MS360-rats could have been affected their ethanol consumption in a long-term

perspective. Interestingly, the differences in the levels of 5-HIAA between MS15- and MS360-rats were eliminated in ethanol-drinking rats, which demonstrates that 5-HIAA levels increased as a result of ethanol consumption in MS360-rats. It has been reported that the ethanol-preferring P line rats had increased 5-HIAA levels in the frontal cortex after ethanol injections (Murphy et al., 1988). Thus, results from the present study indicate that ethanol elicited effects in MS360-rats reared in a risk environment resembling rats that had been selectively bred for ethanol preference. Finding of no effect on DA levels in ethanol-drinking rats in this area is consistent with results described by Murphy et al. (1988), that acute ethanol had no influence on DOPAC and HVA levels in ethanol-tolerant rats.

In amygdala, which is implicated in development of addiction, probably because of its key position in regulation of emotion (Koob, 2011; Weiss and Porrino, 2002), the water-drinking MS360-rats had similar 5-HIAA levels, but lower 5-HT levels compared to AFR-rats (**Figure 7**). Thus, as a result the 5-HIAA/5-HT ratio was higher in the MS360-rats (**Table 3**). Therefore, the present work provided evidence of different ethanol-induced effects in the amygdala depending on the early environmental rearing condition. Additionally, these results may indicate altered 5-HT neurotransmission in rats reared in a more stressful environment (longer maternal separation), and this is consistent with the notion that amygdala is sensitive to early stress (Pechtel and Pizzagalli, 2011; Tottenham and Sheridan, 2009). Several studies with animals using maternal separation paradigm have also shown that amygdala is a target for postnatal influence (Davis and Whalen, 2001; Ebner et al., 2005; Gustafsson et al., 2007).

Voluntary ethanol consumption resulted in opposite effects between MS360- and AFR-rats: ethanol caused higher 5-HT levels in the MS360-rats but lower 5-HT levels in the AFR-rats (**Figure 7**). These results present definite evidence of the influences of early environmental factors on adult responses to voluntary ethanol drinking in the 5-HT system. Interestingly, MS15-rats were seemingly unresponsive to ethanol consumption in terms of neurochemistry in the amygdala compared to MS360- and AFR-rats. This outcome is exciting in the light of the proposed role of the amygdala in adaptive processes during chronic drug intake (Everitt et al., 2008; Koob, 2003).

The DOPAC/DA ratio was also affected by ethanol consumption differently dependent upon maternal separation. In MS360-rats the DOPAC/DA ratio was lower (see **Table 3**), due to lower DOPAC and higher DA levels, which may indicate a lower DA activity.

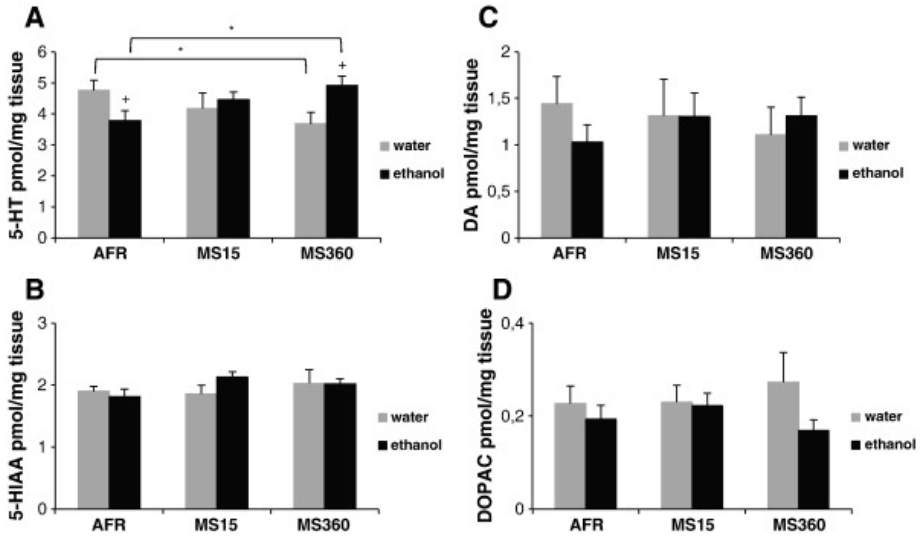

Figure 7. Tissue levels of 5-HT, DA and metabolites in the amygdala. The 5-HT (A), 5-HIAA (B), DA (C) and DOPAC (D) levels (mean±SEM) in the amygdala of adult water- and ethanol-drinking rats are shown. The rats were subjected to different rearing conditions during postnatal days 1–21: MS15, MS360 or ARF. *p<0.05, **p<0.01, +p<0.05 compared to water-drinking rats within each rearing group (Fisher *post hoc* test).

Altogether, these findings on levels of 5-HT, DA and their metabolites suggest that maternal separation induces long-term changes in different brain regions and transmitter systems. It is known that the effects of a drug, for instance alcohol, possibly at the level of the amygdala, modulate individual decision-making whether to continue drug intake (Belin et al., 2009; Robbins et al., 2008). The effects of voluntary ethanol consumption on 5-HT and DA measures in brain areas that are related to reward and addiction processes were dependent on early environmental experience. In this experiment, these effects were most distinct in the amygdala. Environmentally induced alterations in response to ethanol may contribute to the individual differences in vulnerability for addiction.

4.3. Neurobiological differences in rats with high and low exploratory activity (Papers III and V)

4.3.1. Extracellular serotonin and serotonin transporter

The 5-HT-ergic system is well known to play an important role in emotional reactivity and anxiety in animals and the greatest interest has been paid to the 5-HT-ergic function in PFC and hippocampus (Giorgi et al., 2003; Keck et al., 2005).

It has previously been found that LE-rats have higher levels of metabolic activity in DRN (Matrov et al., 2007) that is a major source of forebrain 5-HT-ergic innervation, but lower levels of 5-HT in frontal cortical tissue samples (Alttoa et al., 2005). Thus, in order to learn more about differences in 5-HT-ergic regulation in LE- and HE-rats, *in vivo* microdialysis was used in the present work to monitor extracellular 5-HT levels in PFC and DG at baseline and also in response to manipulations on the 5-HT-ergic system.

The baseline levels of 5-HT overflow in PFC and DG were similar in HE- and LE-rats, and no differences were detected in parachloroamphetamine-induced depolarization-independent 5-HT release in these regions (**Paper III Figure 1**). Interestingly, in frontal cortex, the density of 5-HT transporter sites was higher in the LE-rats (**Paper III Figure 2**). Indeed, after local infusion with citalopram, the increase in extracellular 5-HT levels in LE animals was higher in PFC (**Figure 8A**). Probably the density of the 5-HT-ergic projections to the PFC is higher in LE-rats, resulting in higher extracellular 5-HT levels after blockage of 5-HT transporter, while at baseline conditions the greater release of 5-HT is balanced by the increased reuptake in the LE-rats that we found to have higher levels of 5-HTT in this region. Petty et al. (1994) reported that rats with higher increase in extracellular levels of 5-HT caused by an inescapable stress were more likely to become helpless than rats with less 5-HT increase in frontal cortex, although the basal 5-HT levels did not predict the behavioural outcome. Giorgi et al. (2003) have reported that higher levels of [^{3}H]-citalopram binding to 5-HT reuptake sites and greater increase in 5-HT availability elicited by local application of a selective 5-HT reuptake inhibitors in the frontal cortex in Roman high avoidance rats. However, some reports have come to the conclusion that Roman high avoidance rats reveal less anxiety-related behaviour under stressful conditions and also higher novelty-directed, exploratory behaviour than Roman low avoidance rats (Pawlak et al., 2008). Thus, it appears that both increased and decreased 5-HT-ergic activity in cortex, which has extensive influences on many components of forebrain circuits regulating anxiety behaviour (Millan, 2003, for review), can be found in animals revealing more anxiety-related behaviour.

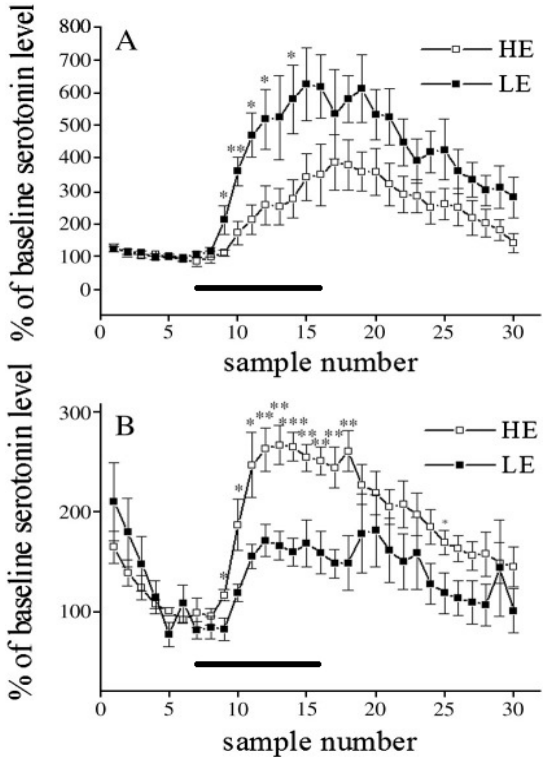

Figure 8. Extracellular serotonin levels in the medial prefrontal cortex (A) and dentate gyrus (B) of HE-and LE-rats, after infusion of citalopram. Samples were collected every 15 min and are presented as percentage of baseline levels (mean of samples 4.–6.). Infusion with 1 μM solution of citalopram was made during the collection of samples 7.–16. *– $p<0.01$; ** – $p<0.01$; *** – $p<0.0001$ difference between HE and LE groups. HE – high exploratory activity rats, LE – low exploratory activity rats. Data are presented as mean±SEM.

Contrarily, in DG (**Figure 8B**), during citalopram perfusion, the LE-rats had lower increase in extracellular 5-HT levels, but this was not accompanied by lower binding capacities of 5-HTT in this area. The hippocampal 5-HT-ergic system has been associated to mediate anxiogenic responses (File et al., 2000) and with dysfunction in hippocampal region, particularly in DG which plays an important role in stress response (Airan et al., 2007; Tanti and Belzung, 2010). The Wistar-Kyoto strain of rats, that is more anxious and passive in the plus-maze test than the spontaneously hypertensive rat strain (Durand et al., 1999; Pollier et al., 2000), has been reported to have lower levels of the 5-HTT binding sites in the hippocampus. Additionally, their extracellular 5-HT levels were lower after citalopram perfusion as compared to less anxious spontaneously hypertensive rats (Pollier et al., 2000). On the other hand, inbred Lewis rats, that are also more anxious in plus-maze, did not differ with regard to

[^{3}H]-citalopram binding in hippocampus (Kulikov et al., 1997). In another animal model, high anxiety-related behaviour rats showed lower 5-HT release in the dorsal hippocampus, which was apparent in response to emotional stressors but not under baseline conditions. Nevertheless, these high anxiety-related behaviour, rats had increased number of 5-HTT binding sites in the hippocampus as compared with low anxiety-related behaviour rats (Keck et al., 2005).

A possible interpretation for different release of 5-HT after infusion of citalopram in DG between HE- and LE-rats may be through differential activity of 5-HT_{1B} autoreceptors, which regulate the release of 5-HT by inhibitory feedback and have been hypothesized to be hypersensitive in depression and anxiety (Moret and Briley, 2000). Additionally, 5-HT_{1B} autoreceptor activity has previously been found to be different in PFC and DG (Adell et al., 2001), and in learned helpless rats, these receptors are found to be up-regulated in the cortex and hippocampus, and down-regulated in the hypothalamus, compared to control animals (Edwards et al., 1991).

Results from other researchers and the present work altogether indicate that there are complex relations between anxiety and 5-HT/5-HTT levels, whereas both increased and decreased levels of 5-HT are associated with anxiety-related behaviours. This is compatible with human studies that have suggested that it is rather the deviation from the mean in the serotonergic system that is driving anxiety-related behaviour (e.g., Harro et al., 2004).

4.3.2. Growth factors

In left prefrontal cortex, the relative levels of BDNF mRNA were higher in LE-rats (**Paper III Figure 4**) as compared to HE-rats, suggesting that BDNF gene expression is increased on the level of transcription or mRNA stability. There was no difference in NGF mRNA levels or BDNF mRNA and NGF mRNA levels in hippocampus between the LE and HE groups. This fits with findings that BDNF promotes the function and growth of 5-HT-containing neurones in the brain (Altar, 1999). Still, it should be acknowledged that our results are not indicative of any direct association between BDNF and 5-HTT expression.

Nevertheless, these results contradict the common BDNF hypothesis of depression, based on findings that stressful conditions decrease BDNF mRNA levels (Altar, 1999; Licinio and Wong, 2002). However, several studies have found increased BDNF levels in depressive-like states. Angelucci et al. (2000) found altered levels of neurotrophins in Flinders Sensitive Line rats, including increased levels of BDNF and NGF in cortex and hypothalamus. Some studies in rats have found that chronic stress resulted in upregulation of BDNF mRNA or protein in hippocampus (Larsen et al., 2010; Schulte-Herbrüggen et al., 2009). Furthermore, bulbectomized mice that have depressed-like phenotype have significantly increased levels of BDNF protein in frontal cortex and hippocampus (Hellweg et al., 2007).

Clearly, these contradictory results suggest that the participation of neurotrophic factors in depression-related disorders is more complex than originally presumed. It has to be considered that the change in BDNF levels in rodent models of anxiety and depression might indicate an attempt towards neurochemical adaptation. Castrén et al. (2007) suggested that neurotrophic factors act in the activity-dependent modulation of neuronal networks, whereas a gradual network recovery through activity-dependent neuronal plasticity induces the antidepressant effect.

4.3.3. Differences in extracellular glutamate levels in the striatum and hippocampus in rats with high and low exploratory activity

The baseline levels of glutamate were comparable with results of others authors, both in striatum (Del Arco et al., 2001; Ho et al., 2000; Shakil et al., 2005) and hippocampus (Chefer et al., 2011; Matsuno and Inoue, 2008; Takeda et al., 2006).

At baseline, there were no differences in striatal extracellular glutamate levels between LE- and HE-rats, either in 3-months-old or 11-months-old animals. In response to glutamate transporter inhibition with PDC (4 mM), glutamate levels raised both in striatum (**Figure 9**) and in hippocampus (**Figure 10**), but the increase in striatum was proportionally higher. Differences in glutamate levels between LE- and HE-rats were revealed in striatum, as levels of glutamate increased (up to 600%) to a proportionately larger extent after PDC infusion in 3-month-old HE-rats [$F(1,411)=2.08$, $p=0.0035$]. Similar proportional increase (up to 500%) was seen in 11-month-old rats, and again the response in HE- and LE-rats was significantly different [$F(1,168)=2.4$, $p=0.0014$]. Although, the response to inhibition with PDC has previously been found to decrease with aging (Del Arco et al., 2001), there was no significant difference in increase of glutamate levels between younger and older HE and LE groups.

The glutamate transporter inhibitor PDC blocks the glial glutamate transporter EAAT2. It is believed that EAAT2 plays a primary role in maintaining extracellular glutamate concentrations below excitotoxic levels (Holmseth et al., 2012; Liu et al., 2012; Tanaka, 2000). In an animal model in which this glutamate transporter was genetically deleted, EAAT2 was shown to be necessary for brain development through regulation of extracellular glutamate concentration (Matsugami et al., 2006). Behrens et al. (2002) observed that transgenic mice expressing an N-terminal fragment of mutant huntingtin (R6/2) had higher striatal glutamate levels compared with controls after inhibition by PDC. Additionally, in the R6/2 mice the decrease of EAAT2 mRNA expression compared to wild type group was also observed, obviously resulting in a reduction of transporter function. The fact that blockade of EAAT2 reveals differences in extracellular levels in HE- and LE-rats suggests that differences may exist in glutamate release and transport between animals

with high vs. low novelty-related activity. It could be speculated that there is a higher presynaptic output of glutamate in HE-rats, which is compensated for by increased glutamate uptake. On the other hand, HE-rats had slightly lower (data not shown) striatal baseline levels compared to LE-rats, that contributed to the difference in proportional levels after perfusion with PDC. However, if any possible natural stimulus affected the balance between release and uptake of glutamate, the HE-rats would be influenced to a greater extent.

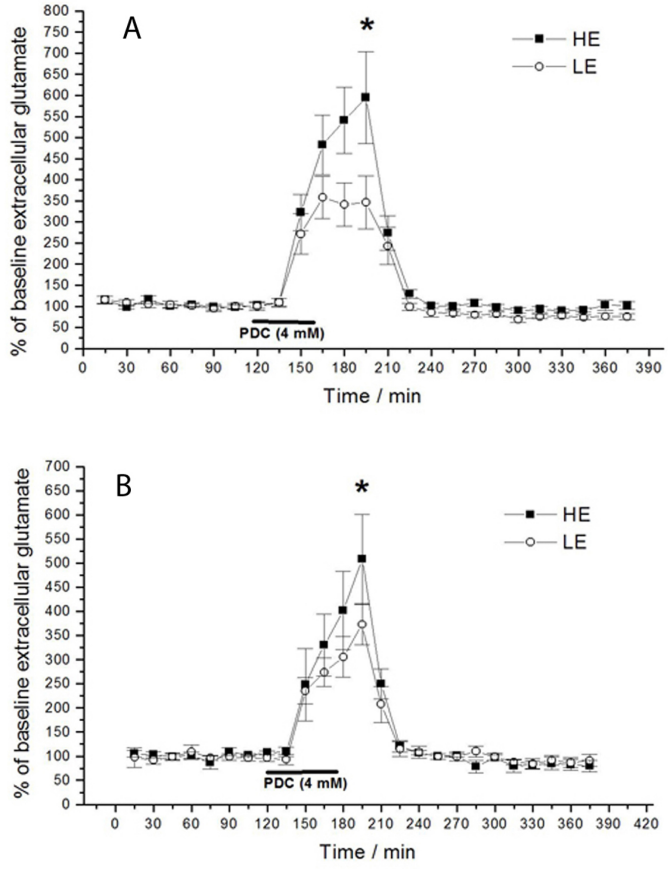

Figure 9. Extracellular glutamate levels in the striatum of 3-month-old (A) and 11-month-old (B) LE- and HE-rats after perfusion of 4 mM PDC. Samples were collected every 15 min and are presented as percentage of baseline (mean of samples 4.-7.) levels. Perfusion with PDC was made during the collection of samples 8.-11. * $p<0.05$ difference between LE and HE group. LE – low exploring rats; HE – high exploring rats.

No difference in baseline glutamate levels between HE and LE group was found in hippocampus. After perfusion with PDC glutamate levels increased a bit more than twofold, but without difference between LE- and HE-rats (**Figure 10**). Previous reports have suggested that increased hippocampal glutamate levels are associated with response to novel environment (Bianchi et al., 2003; Takeda et al., 2006). However, another study (Giovannini et al., 2001) found no change in hippocampal glutamate levels in response to novelty. In our experiment the response to novelty was not measured, but we could not detect any difference in glutamate levels in animals with different strategies in novel environment, suggesting that the differences observed in striatum were region-specific. In another animal model, the learned helplessness model of depression, the more helpless rat had significantly reduced expression of the EAAT2 in hippocampus (Zink et al., 2010). While the LE-rats display a depression-like phenotype and had lower glutamate levels after uptake inhibition in striatum, this was not found in hippocampus. Nevertheless, the learned helplessness model is different from a number of other depression models at least in terms of cerebral regional oxidative metabolism (Harro et al., 2011).

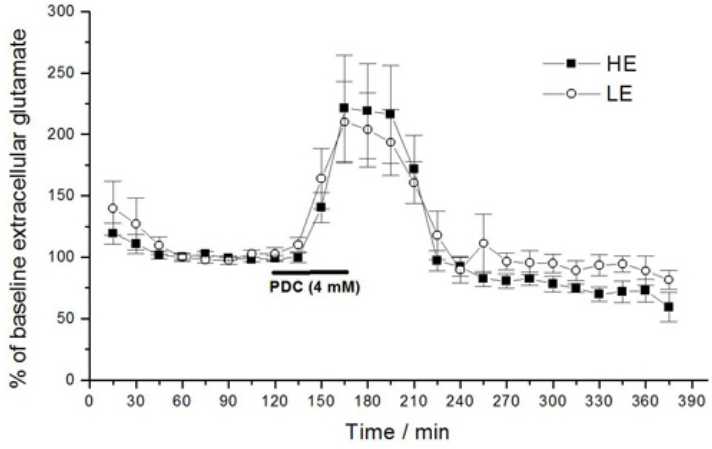

Figure 10. Extracellular glutamate levels in the hippocampus of HE-and LE-rats after infusion with 4 mM PDC. Samples were collected every 15 min and are presented as percentage of baseline (mean of samples 4.-7.) levels. Perfusion with PDC was made during the collection of samples 8.-11. LE – low exploring rats; HE – high exploring rats.

Compared to LE-rats, HE-rats have higher striatal extracellular dopamine levels both in baseline conditions and after administration of amphetamine (Alttoa et al., 2009; Mällo et al., 2007a). It is well known that glutamatergic and dopaminergic neurotransmission interact in the striatum (Smith and Bolam, 1990). There are different mechanisms by which glutamate and dopamine might interact (Hernández et al., 2003; Mora et al., 2003; Seeman et al., 2009). Mora

et al. (2003) showed that increasing levels of glutamate lead to increasing levels of dopamine in striatum, and blockade of ionotropic glutamate receptors attenuates the increase of dopamine levels. The spontaneously hypertensive rats that are more active in the open field had higher glutamate-stimulated dopamine release in substantia nigra, suggesting that altered glutamate regulation of dopamine neurons may contribute to novelty response at different levels (Warton et al., 2009). Nevertheless, there are no single-valued interactions between dopaminergic and glutamatergic transmission (reviewed in David et al., 2005). Thus, further studies should elucidate how the interplay between dopamine- and glutamate-mediated neurotransmission contributes to active vs. passive coping strategies with novelty.

4.3.4. Changes in exploratory behaviour and striatal extracellular glutamate levels

Our previous study indicated that the characteristic levels of exploratory behaviour are highly persistent at least up to the age of 8 months (Mällo et al., 2007a). In the present experiments, exploratory activity of some HE-rats had significantly dropped by 11 months of age (in 3 out of 7). Interestingly, this subgroup differed significantly [$F(2,240)=1.5$, $p=0.028$)] from both HE-and LE-rats at same age, having almost two times higher extracellular striatal glutamate levels at baseline (16.2±3.5 μM) (**Figure 11**). Still, the proportional increase in glutamate levels after PDC in this subgroup [former high exploring (HEF) group] did not significantly differ from HE- and LE-rats.

It is well known that pathologically high levels of glutamate can cause excitotoxicity (Meldrum and Garthwaite, 1990; Rosenberg et al., 1992) and stressful environment might increase extracellular content of glutamate in older animals in different brain areas, including in striatum (Lowy et al., 1995; Moghaddam, 1993; Smith et al., 2002). Moghaddam et al. (1993) have presented a microdialysis study, where 20 min long stressful restraint procedure had an enhancing impact on extracellular striatal glutamate levels in rats. It is thus tempting to speculate that persistently high levels of glutamate could be associated with changes in neuroplasticity that eventually led to the loss of high exploring phenotype in these animals. Ho et al. (2000) examined striatal glutamate levels in olfactory bulbectomized rats. It appeared that under novelty stress condition glutamate levels increased (up to 160%) from the baseline value in bulbectomized rats, while no changes were seen in sham-operated rats. The most prominent behavioural feature of bulbectomized animals is hyperactivity. One could speculate that higher sensitivity to environmental stimuli leads to higher levels of striatal glutamate output and hyperactivity, but excessive over-flow of glutamate eventually causes loss of novelty-related activity. Nevertheless, certainly our finding needs to be re-examined with larger experimental groups, and the causality of the link between extracellular glutamate and changes in novelty-related behaviour experimentally tested.

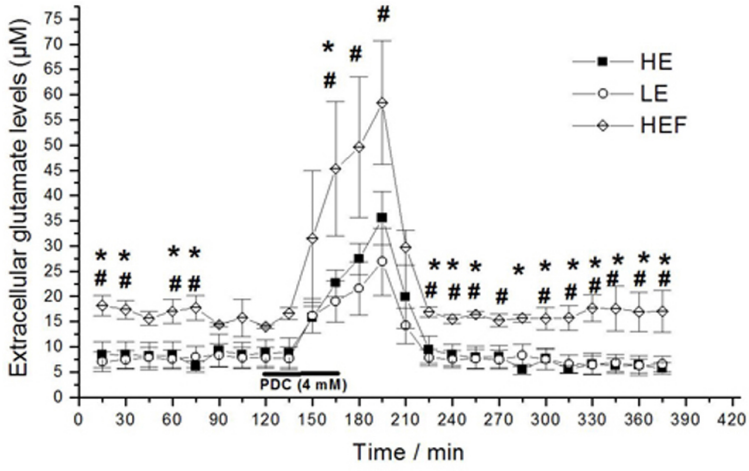

Figure 11. Extracellular glutamate levels in the striatum of 11-month-old LE- and HE-rats after perfusion with 4 mM PDC. Samples were collected every 15 min. Perfusion with PDC was made during the collection of samples 8.–11. * – $p<0.05$ difference between HEF and HE group; # - $p<0.05$ difference between HEF and LE group. LE – low explorating rats; HE – high exploring rats; HEF – former high exploring rats.

4.4. Neurobiological differences in rats with high and low levels of 50-kHz ultrasonic vocalizations (Paper IV)

4.4.1. Effect of chronic stress on hippocampal extracellular serotonin levels in rats with high and low levels of 50-kHz ultrasonic vocalizations

We have earlier found that stable inter-individual differences exist in the expression of 50-kHz USVs, accompanied by behavioural differences in tests used in anxiety and depression studies (Mällo et al., 2007b, 2009). In the present experiment again large persistent inter-individual differences in emission of 50-kHZ USVs were observed. Emission of 22-kHz USVs, as being associated with negative affectivity, was present in only a few animals, even in these individuals at very low levels, and did not differ between the high chirping and low chirping groups.

Chronic variable stress had an effect on body weight gain [$F(26,598)=3.28$, $p<0.001$]. It decreased weight gain in both LC and HC group (**Figure 12**), but the effect was stronger in the LC-rats. This confirms the effectiveness of the applied stress regimen (Harro et al., 2001) and indicates a greater susceptibility to stress in LC-rats. A relative decrease in the body weight of stressed rats after CVS procedures, as compared to controls, has been observed in many studies (Moreau et al., 1992; Nielsen et al., 2000; Willner et al., 1996), including our own (Harro et al., 1999; Mällo et al., 2007b, 2009). Our previous finding that

body weight gain is affected by chronic stress more in LC- than the HC-rats (Mällo et al., 2009) was thus reproduced.

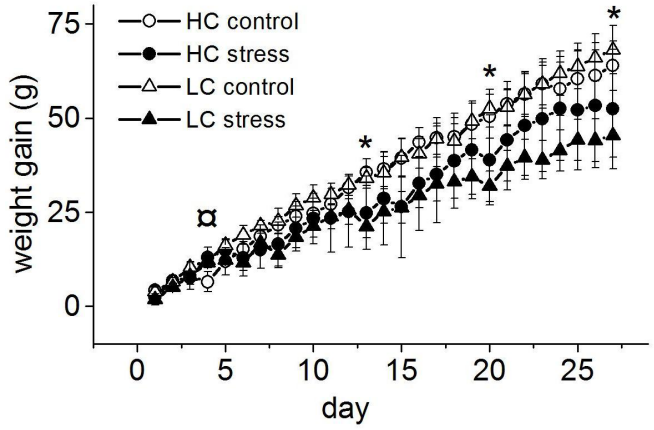

Figure 12. Weight gain in control and stressed LC- and HC-rats (mean ± SEM). **p*<0.05 difference between LC control and LC stress groups; ¤ *p*<0.05 difference between HC control and HC stress groups. LC – low chirping rats; HC – high chirping rats.

In the present study, at baseline extracellular levels of 5-HT, there was no significant difference between LC and HC animals. Nevertheless, there was a main effect of Chirping [$F(1,23)=8.82$, $p<0.01$] on baseline extracellular 5-HT levels, and a Chirping x Time interaction [$F(28,644)=3.25$, $p<0.0001$]; a significant Stress x Time interaction was also found [$F(28,644)=1.79$, $p<0.01$]. This was because after local inhibition of 5-HT uptake by citalopram (1 μM), significant differences between the groups emerged. Thus, the increase in extracellular 5-HT levels after infusion with citalopram, as compared to baseline, was much higher in LC-rats that had been submitted to chronic variable stress (**Figure 13**). The effect of citalopram was also remarkably long-lasting in this group. Stressed LC group might have responded more to microdialysis procedure itself than other groups. Additionally this might explain the slightly higher 5-HT levels in stressed LC group immediately before treatment with citalopram. However, throughout the experiment, treatment with citalopram appeared to be increasing 5-HT overflow especially in the stressed LC-rats, as the baseline differences were minor and, when collapsed, not statistically significant.

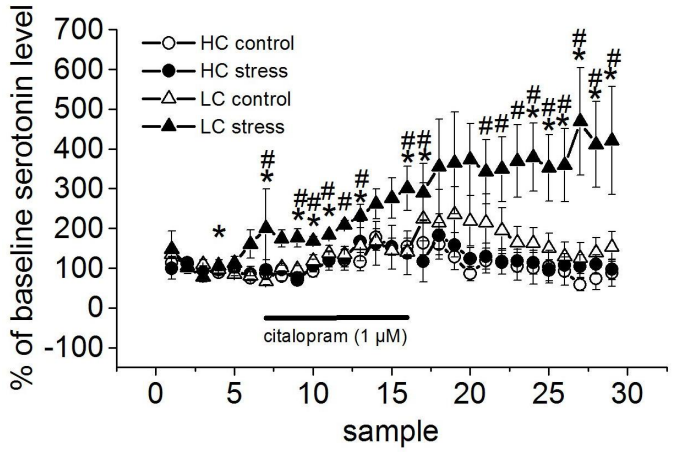

Figure 13. Extracellular serotonin levels in the dentate gyrus of LC- and HC-rats after local infusion of 1 µM citalopram solution (mean ± SEM). Samples were collected every 15 min and are presented as percentage of baseline (mean of samples 4.-6.) levels. Infusion with citalopram was made during the collection of samples 7.-16. * $p<0.05$ difference between LC control and LC stress groups; # $p<0.05$ difference between HC stress and LC stress groups. LC – low chirping rats; HC – high chirping rats.

The reuptake of 5-HT by the serotonin transporter is considered to be the critically important link in 5-HT neurotransmission (Blakely et al., 1994; Lesch and Gutknecht, 2005). Experiments with 5-HTT knockout mice have shown that they have increased anxiety-like behaviours, whereas transgenic over-expression of the 5-HTT reduces extracellular 5-HT levels and decreases these behaviours (Jennings et al., 2006; Wellman et al., 2007). If there were differences in 5-HTT expression, this might explain why LC- and HC-rats had different 5-HT levels after 5-HTT blockade with citalopram. Another possible mechanism for increased 5-HT levels in LC-rats in DG after local infusion of citalopram may be through differential activity of 5-HT_{1B} autoreceptors, which regulate the release of 5-HT by inhibitory feedback and have been hypothesized to be supersensitive in depression and anxiety (Moret and Briley, 2000).

Whereas the activity of neurons is almost entirely dependent on oxidative metabolism (Wong-Riley et al., 1998), then measurement of differences in oxidative metabolism can be used to reveal the underlying neural substrate of differences in positive affect and resilience to stress. It has been previously found that chronic stress increased oxidative metabolism in male LC-rats compared to HC-rats in several brain regions, including hippocampus (Mällo et al., 2009). Additionally, in many regions in which chronic stress had no significant effect in unselected animals, it increased oxidative metabolism in LC animals only (Kanarik et al., 2008).

Altogether, it may be claimed that CVS appears to modify regulation of hippocampal 5-HT-ergic neurotransmission differently in rats with low positive

affectivity. This finding supports the notion of greater vulnerability to CVS in male rats with low positive affectivity.

4.4.2. Effect of chronic stress on plasma corticosterone levels in rats with high and low levels of 50-kHz ultrasonic vocalizations

Corticosterone is involved in physiological systems such as stress response (Grippo et al., 2005; Strausbaugh et al., 1999), which is mediated by an increased release of glucocorticoids via the activation of the HPA axis that, in turn, is under CRF-ergic control (Arborelius et al., 1999; Koob, 1999). Additionally, stress is strongly associated with multiple changes in 5-HT system (Ressler and Nemeroff, 2000), and drugs that enhance brain 5-HT function alter circulating concentrations of corticosterone are believed to be mediated by the CRF-containing neurons (Grippo et al., 2005; Murphy et al., 1996). It has been reported that acute or chronic stress may have a diverse impact on plasma corticosterone levels in rats ranging from decreasing (Murison and Hansen, 2001) and no change (Azpiroz et al., 1999) to an increase of corticosterone levels in plasma in rats (Abelson et al., 2005; Bielajew et al., 2002; Grippo et al., 2005). The latter is similar to the human cortisol response (Römer et al., 2009).

We found that corticosterone levels measured from full blood after the end of microdialysis were significantly higher in the stressed LC animals as compared to both the unstressed LC group and the respective HC animals (**Figure 14**). While there was no difference between the control LC- and HC-rats, higher corticosterone in the LC-rats submitted to chronic stress, as measured after the microdialysis experiment, indicates that CVS sensitizes the stress axis in rats with low positive affect.

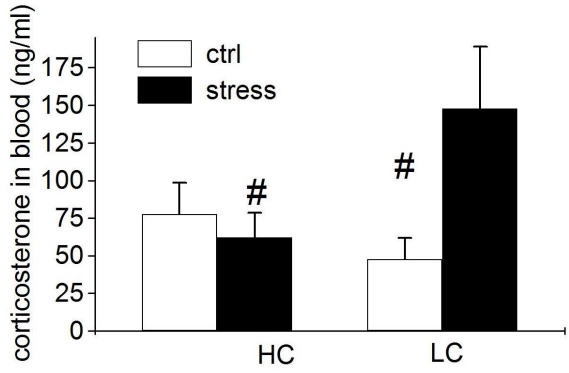

Figure 14. Corticosterone levels in the plasma of LC- and HC-rats (mean ± SEM). # $p<0.05$ as compared to LC stress group. LC – low chirping rats; HC – high chirping rats.

5. GENERAL SUMMARY

The present dissertation focuses on the neurochemical mechanisms associated with behaviour in depression–related states. Inter-individual differences, that are probably caused by variations in the regulation of neurochemical pathways, exist between organisms, and are likely to be significant in the pathogenesis of affective disorders. Thus the main emphasis of this dissertation is on the use of neurochemical techniques in elucidating the biological substrate of inter-individual differences in affect-related behaviour, including experimental manipulations to study possible mediating mechanisms of such differences.

Exploratory behaviour is very much dependent on the function of the noradrenergic projections of the locus coeruleus. All rats with near complete denervation of these projections behave in the exploration box test like the spontaneous LE-phenotype that has been associated with less efficient dopaminergic neurotransmission. The hypothesis was tested that intact projections of the locus coeruleus are important for full expression of the effects of psychostimulants acting directly on the dopaminergic system. It appeared that extensive denervation of the noradrenergic projections from locus coeruleus indeed reduced the effect of cocaine-induced place preference and locomotor activation.

Early life experiences may alter epigenetically the regulatorsof neurochemical mechanisms and thereby influence the behavioural pattern later in life. It is increasingly believed that perinatal events are a major, if not the main, source of inter-individual differences in childhood and adult behaviour that eventually mediate the development of affective disorders. Alcohol use moderates the relationship between behavioural traits and psychiatric disorders. The hypothesis that ethanol-induced effects on monoaminergic system are dependent on early life environment was confirmed. Animals that experienced maternal separation had low 5-HT levels in amygdala and responded with an increase in 5-HT after ethanol intake, which is a key area in addiction processes. Additionally, alterations in dopamine turnover were observed in dorsal raphe nucleus. These results suggest that inter-individual differences in neurochemical responses to ethanol consumption occur, and can be result of early environmental factors. It should be further examined whether such changes can mediate the transition from habitual to compulsive drinking, and contribute to individual vulnerability or resilience to addiction, or the comorbidity of addiction with affective disorders.

Studies in the present dissertation indicate that rats with different exploratory phenotype differ, in addition to catecholaminergic mechanisms, also with regard to regulation of serotonergic and glutamatergic systems. Serotonergic neurotransmission in hippocampus and prefrontal cortex was found to be regionally differentially regulated in HE- and LE-rats. In prefrontal cortex, the LE-rats had higher extracellular 5-HT levels induced by citalopram and higher levels of serotonin transporter binding. In dentate gyrus, contrary, the HE-rats had higher levels of extracellular 5-HT. LE-rats had higher levels of BDNF mRNA in the

prefrontal cortex, supporting the recent claims that the relationship between BDNF expression and depression is not simple linear. These results suggest that the persistently different profiles of anxiety-related behaviour can be related to the differences in the functional qualities of the 5-HT system in these brain regions. Additionally, HE-rats had increased levels of glutamate after blockade of glutamate transporter in striatum. Thus, the hypothesis that inter-individual differences in exploratory behaviour may be related to striatal glutamatergic neurotransmission was confirmed.

Predisposition to express positive affect at low levels had previously been linked to vulnerability to depression. We could confirm the higher sensitivity of the LC-rats that produce less 50-kHz vocalizations to chronic stress: they gained weight more slowly and had higher corticosterone levels measured from full blood after acute stress. The hypothesis that LC-rats have differences in serotonergic mechanisms after chronic stress was supported by the finding that stressed LC-rats had higher extracellular 5-HT levels induced by citalopram in hippocampus, where 5-HT-ergic system has been known to mediate response to anxious stimuli. These findings support the notion that male rats with low 50-kHz USVs response to tickling, having lower positive emotionality, are behaviourally more vulnerable to stress.

Taken together, these results suggest that serotonergic, dopaminergic and glutamatergic neurotransmission contribute to variations in response to novelty and place preference conditioning, and maternal separation and chronic stress in adulthood. Consideration of these neurobiological differences between individuals could lead to novel approaches to more personalized medical treatment of depression-related states.

REFERENCES

Abelson, K.S.P., Adem, B., Royo, F., Carlsson, H.-E., Hau, J., 2005. High plasma corticosterone levels persist during frequent automatic blood sampling in rats. In Vivo 19, 815–819.

Adell, A., Celada, P., Artigas, F., 2001. The role of 5-HT1B receptors in the regulation of serotonin cell firing and release in the rat brain. J. Neurochem. 79, 172–182.

Advani, T., Hensler, J.G., Koek, W., 2007. Effect of early rearing conditions on alcohol drinking and 5-HT1A receptor function in C57BL/6J mice. Int. J. Neuropsychopharmacol. 10, 595–607.

Agmo, A., Marroquin, E., 1997. Role of gustatory and postingestive actions of sweeteners in the generation of positive affect as evaluated by place preference conditioning. Appetite 29, 269–289.

Airan, R.D., Meltzer, L.A., Roy, M., Gong, Y., Chen, H., Deisseroth, K., 2007. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317, 819–823.

Aisa, B., Tordera, R., Lasheras, B., Del Río, J., Ramírez, M.J., 2007. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology 32, 256–266.

Aisa, B., Tordera, R., Lasheras, B., Del Río, J., Ramírez, M.J., 2008. Effects of maternal separation on hypothalamic-pituitary-adrenal responses, cognition and vulnerability to stress in adult female rats. Neuroscience 154, 1218–1226.

Almeida, R.F., Thomazi, A.P., Godinho, G.F., Saute, J.A., Wofchuk, S.T., Souza, D.O., Ganzella, M., 2010. Effects of depressive-like behavior of rats on brain glutamate uptake. Neurochem. Res. 35, 1164–1171.

Altar, C.A., 1999. Neurotrophins and depression. Trends Pharmacol. Sci. 20, 59–61.

Alttoa, A., Eller, M., Herm, L., Rinken, A., Harro, J., 2007. Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D2 receptor function in rats with high or low spontaneous exploratory activity: differences in the role of locus coeruleus. Brain Res. 1131, 138–148.

Alttoa, A., Kõiv, K., Eller, M., Uustare, A., Rinken, A., Harro, J., 2005. Effects of low dose N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine administration on exploratory and amphetamine-induced behavior and dopamine D2 receptor function in rats with high or low exploratory activity. Neuroscience 132, 979–990.

Alttoa, A., Kõiv, K., Hinsley, T.A., Brass, A., Harro, J., 2010. Differential gene expression in a rat model of depression based on persistent differences in exploratory activity. Eur. Neuropsychopharmacol. 20, 288–300.

Alttoa, A., Seeman, P., Kõiv, K., Eller, M., Harro, J., 2009. Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D(2) (High) receptors in the striatum. Synapse 63, 443–446.

Amelang, M., Bartussek, D., Stemmler, G., Hagemann, D., 2006. Differentielle Psychologie und Persönlichkeitsforschung (Differential psychology and personality research), 6th ed. Kohlammer, Stuttgart.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders, (DSM-IV), 4th ed.,Washington, DC: American Psychiatric Press, 1994.

Anand, K.J., Scalzo, F.M., 2000. Can adverse neonatal experiences alter brain development and subsequent behavior? Biol. Neonate 77, 69–82.

Andén, N.E., Grabowska, M., 1976. Pharmacological evidence for a stimulation of dopamine neurons by noradrenaline neurons in the brain. Eur. J. Pharmacol. 39, 275–282.
Angelucci, F., Aloe, L., Vasquez, P.J., Mathé, A.A., 2000. Mapping the differences in the brain concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in an animal model of depression. Neuroreport 11, 1369–1373.
Anisman, H., Zacharko, R.M., 1982. Depression: the predisposing influence of stress. Behav. Brain Sci. 5, 89–99.
Anisman, H., Zacharko, R.M., 1990. Multiple neurochemical and behavioral consequences of stressors: implications for depression. Pharmacol. Ther. 46, 119–136.
APA, 1994. Diagnostic and Statistical Manual of Mental Disorders (4th ed.). American Psychiatric Association, Washington, DC.
Arborelius, L., Eklund, M.B., 2007. Both long and brief maternal separation produces persistent changes in tissue levels of brain monoamines on middle-aged female rats. Neuroscience 145, 738–750.
Arborelius, L., Owens, M.J., Plotsky, P.M., Nemeroff, C.B., 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. J. Endocrinol. 160, 1–12.
Archer, T., Jonsson, G., Ross, S.B., 1984a. A parametric study of the effects of the noradrenaline neurotoxin DSP4 on avoidance acquisition and noradrenaline neurones in the CNS of the rat. Br. J. Pharmacol. 82, 249–257.
Archer, T., Mohammed, A.K., Ross, S.B., Söderberg, U., 1983. T-maze learning, spontaneous activity and food intake recovery following systemic administration of the noradrenaline neurotoxin, DSP4. Pharmacol. Biochem. Behav. 19, 121–130.
Archer, T., Söderberg, U., Ross, S.B., Jonsson, G., 1984b. Role of olfactory bulbectomy and DSP4 treatment in avoidance learning in the rat. Behav. Neurosci. 98, 496–505.
Azpiroz, A., Fano, E., Garmendia, L., Arregi, A., Cacho, R., Beitia, G., Brain, P.F., 1999. Effects of chronic mild stress (CMS) and imipramine administration, on spleen mononuclear cell proliferative response, serum corticosterone level and brain norepinephrine content in male mice. Psychoneuroendocrinology 24, 345–361.
Bardo, M.T., Donohew, R.L., Harrington, N.G., 1996. Psychobiology of novelty seeking and drug seeking behavior. Behav. Brain Res. 77, 23–43.
Barfield, R.J., Auerbach, P., Geyer, L.A., McIntosh, T.K., 1979. Ultrasonic vocalizations in rat sexual behavior. Am. Zool. 19, 469–480.
Behrens, P.F., Franz, P., Woodman, B., Lindenberg, K.S., Landwehrmeyer, G.B., 2002. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain 125, 1908–1922.
Bekris, S., Antoniou, K., Daskas, S., Papadopoulou-Daifoti, Z., 2005. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav. Brain Res. 161, 45–59.
Belin, D., Jonkman, S., Dickinson, A., Robbins, T.W., Everitt, B.J., 2009. Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction. Behav. Brain Res. 199, 89–102.
Belzung, C., 1999. Measuring rodent exploratory behaviour, in: Crusio, W.E., Gerlai, R.T. (Eds.), Handbook of Molecular-Genetic Techniques for Brain and Behaviour Research, Techniques in the Behavioural and Neural Science. Elsevier Science EV, Amsterdam, pp. 738–749.
Bergles, D.E., Diamond, J.S., Jahr, C.E., 1999. Clearance of glutamate inside the synapse and beyond. Curr. Opin. Neurobiol. 9, 293–298.

Berlyne, D.E., 1950. Novelty and curiosity as determinants of exploratory behaviour. Br. J. Psychol. 41, 68–80.
Berton, O., McClung, C.A., DiLeone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanoc, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler, E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–868.
Bianchi, L., Ballini, C., Colivicchi, M.A., Della Corte, L., Giovannini, M.G., Pepeu, G., 2003. Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. Neurochem. Res. 28, 565–573.
Bibel, M., Barde, Y.A., 2000. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. 14, 2919–2937.
Bielajew, C., Konkle, A.T., Merali, Z., 2002. The effects of chronic mild stress on male Sprague-Dawley and Long Evans rats: I. Biochemical and physiological analyses. Behav. Brain Res. 136, 583–592.
Binder, D.K., Scharfman, H.E., 2004. Brain-derived neurotrophic factor. Growth Factors 22, 123–131.
Blakely, R.D., De Felice, L.J., Hartzell, H.C., 1994. Molecular physiology of norepinephrine and serotonin transporters. J. Exp. Biol. 196, 263–281.
Blanchard, R.J., Blanchard, D.C., Agullana, R., Weiss, S.M., 1991. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiol. Behav. 50, 967–972.
Blanchard, M.M., Mendelsohn, D., Stamp, J.A., 2009. The HR/LR model: further evidence as an animal model of sensation seeking. Neurosci. Biobehav. Rev. 33, 1145–1154.
Bleich, S., Römer, K., Wiltfang, J., Kornhuber, J., 2003. Glutamate and the glutamate receptor system: a target for drug action. Int. J. Geriatr. Psychiatry 18, S33–S40.
Boehm, S.L., Valenzuela, F.C., Harris, A.R., 2005. Substances of abuse, in: Lowinson, J.H., Riuz, P., Millman, R.B., Langrod, J.G. (Eds.), Substance abuse: A comprehensive textbook. Lipincott Williams & Wilkins, Philadelphia, pp. 121–468.
Bondi, C.O., Rodriquez, G., Gould, G.G., Frazer, A., Morilak, D.A., 2007. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacology 33, 320–331.
Bonhomme, N., Esposito, E., 1998. Involvement of serotonin and dopamine in the mechanism of action of novel antidepressant drugs: a review. J. Clin. Psychopharmacol. 18, 447–454.
Brake, W.G., Zhang, T.Y., Diorio, J., Meaney, M.J., Gratton, A., 2004. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. Eur. J. Neurosci. 19, 1863–1874.
Bridges, R.J., Stanley, M.S., Anderson, M.W., Cotman, C.W., Chamberlin, A.R., 1991. Conformationally defined neurotransmitter analogues. Selective inhibition of glutamate uptake by one pyrrolidine-2,4-dicarboxylate diastereomer. J. Med. Chem. 34, 717–725.
Briley, M.S., Langer, S.Z., Raisman, R., Sechter, D., Zarifian, E., 1980. Tritiated imipramine binding sites are decreased in platelets of untreated depressed patients. Science 209, 303–305.
Brown, G.W., 1998. Genetic and population perspectives on life events and depression. Soc. Psychiatry Psychiatr. Epidemiol. 33, 363–372.

Brown, P., Molliver, M.E., 2000. Dual serotonin (5-HT) projections to the nucleus accumbens core and shell: relation of the 5-HT transporter to amphetamine-induced neurotoxicity. J. Neurosci. 20, 1952–1963.

Brudzynski, S.M., 2007. Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine-dopamine interaction and acoustic coding. Behav. Brain Res. 182, 261–273.

Brunelli, S.A., Hofer, M.A., 1996. Development of ultrasonic vocalization responses in genetically heterogeneous National Institute of Health (N:NIH) rats. II. Associations among variables and behaviors. Dev. Psychobiol. 29, 517–528.

Burgdorf, J., Knutson, B., Panksepp, J., 2000. Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalizations in rats. Behav. Neurosci. 114, 320–327.

Burgdorf, J., Kroes, R.A., Weiss, C., Oh, M.M., Disterhoft, J.F., Brundzynski, S.M., Panksepp, J., Moskal, J.R., 2011. Positive emotional learning is regulated in the medial prefrontal cortex by GluN2B-containing NMDA receptors. Neuroscience 192, 515–523.

Burgdorf, J., Knutson, B., Panksepp, J., Shippenberg, T.S., 2001. Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. Psychopharmacology 155, 35–42.

Burgdorf, J., Panksepp, J., 2001. Tickling induces reward in adolescent rats. Physiol. Behav. 72, 167–173.

Burgdorf, J., Panksepp, J., Brudzynski, S.M., Kroes, R., Moskal, J.R., 2005. Breeding for 50-kHz positive affective vocalization in rats. Behav. Genet. 35, 67–72.

Carlezon, W.A. Jr., Chartoff, E.H., 2007. Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat. Protoc. 2, 2987–2995.

Cassano, T., Gaetani, S., Morgese, M.G., Macheda, T., Laconca, L., Dipasquale, P., Taltavull, J., Shippenberg, T.S., Cuomo, V., Gobbi, G., 2009. Monoaminergic changes in locus coeruleus and dorsal raphe nucleus following noradrenaline depletion. Neurochem. Res. 34, 1417–1426.

Castrén, E., Võikar, V., Rantamäki, T., 2007. Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7, 18–21.

Chaouloff, F., 2000. Serotonin, stress and corticoids. J. Psychopharmacol. 14, 139–151.

Cheetham, A., Allen, N.B., Yücel, M., Lubman, D.I., 2010. The role of affective dysregulation in drug addiction. Clin. Psychol. Rev. 30, 621–634.

Chefer, V.I., Meis, J., Wang, G., Kuzmin, A., Bakalkin, G., Shippenberg, T., 2011. Repeated exposure to moderate doses of ethanol augments hippocampal glutamate neurotransmission by increasing release. Addict. Biol. 16, 220–237.

Chefer, V.I., Zakharova, I., Shippenberg, T.S., 2003. Enhanced responsiveness to novelty and cocaine is associated with decreased basal dopamine uptake and release in the nucleus accumbens: quantitative microdialysis in rats under transient conditions. J. Neurosci. 23, 3076–3084.

Clements, J.D., Lester, R.A., Tong, G., Jahr, C.E., Westbrook, G.L., 1992. The time course of glutamate in the synaptic cleft. Science 258, 1498–1501.

Coppen, A., 1967. The biochemistry of affective disorders. Br. J. Psychiatry 113, 1237–1264.

Cox, B.M., Alsawah, F., McNeill, P.C., Galloway, M.P., Perrine, S.A., 2011. Neurochemical, hormonal, and behavioral effects of chronic unpredictable stress in the rat. Behav. Brain Res. 220, 106–111.

Crusio, W.E., van Abeelen, J.H., 1986. The genetic architecture of behavioural responses to novelty in mice. Heredity 56, 55–63.

Dabrowska, J., Nowak, P., Brus, P., 2008. Reactivity of 5-HT1A receptor in adult rats after neonatal noradrenergic neurons lesion - implications for antidepressant-like action. Brain Res. 6, 66–76.

Danbolt, N.C., 2001. Glutamate uptake. Prog. Neurobiol. 65, 1–105.

Danbolt, N.C., Storm-Mathisen, J., Kanner, B.I., 1992. An [Na^+ + K^+]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience 51, 295–310.

David, H.N., Ansseau, M., Abraini, J.H., 2005. Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudate-putamen and nucleus accumbens of "intact" animals. Brain Res. Rev. 50, 336–360.

Davis, M., Whalen, P.J., 2001. The amygdala: vigilance and emotion. Mol. Psychiatry 6, 13–34.

Daw, N.D., Kakade, S., Dayan, P., 2002. Opponent interactions between serotonin and dopamine. Neural Netw. 15, 603–616.

De Bellis, M.D., 2002. Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. Psychoneuroendocrinology 27, 155–170.

de Kloet, E.R., Oitzl, M.S., Joëls, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426.

de Kloet, E.R., Sibug, R.M., Helmerhorst, F.M., Schmidt, M.V., 2005. Stress, genes and the mechanism of programming the brain for later life. Neurosci. Biobehav. Rev. 29, 271–281.

Del Arco, A., Segovia, G., Prieto, L., Mora, F., 2001. Endogenous glutamate-taurine interaction in striatum and nucleus accumbens of the freely moving rat: studies during the normal process of aging. Mech. Ageing Dev. 122, 401–414.

Delgado, P.L., Moreno, F.A., 2000. Role of norepinephrine in depression. J. Clin. Psychiatry 61, 5–12.

Dellu, F., Mayo, W., Piazza, P.V., Le Moal, M., Simon, H., 1993. Individual differences in behavioral responses to novelty in rats. Possible relationship with the sensation-seeking trait in man. Person. Individ. Diff. 15, 411–418.

Dellu, F., Piazza, P.V., Mayo, W., Le Moal, M., Simon, H., 1996. Novelty-seeking in rats-biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology 34, 136–145.

de Wit, H., 2009. Impulsivity as a determinant and consequence of drug use: a review of underlying processes. Addict. Biol. 14, 22–31.

Dick, D.M., Foroud, T., 2003. Candidate genes for alcohol dependence: a review of genetic evidence from human studies. Alcohol. Clin. Exp. Res. 27, 868–879.

DiFranza, J.R., Savageau, J.A., Fletcher, K., Pbert, L., O`Loughlin, J., McNeill, A.D., Ockene, J.K., Friedman, K., Hazelton, J., Wood, C., Dussault, G., Wellman, R.J., 2007. Susceptibility to nicotine dependence: the Development and Assessment of Nicotine Dependence in Youth 2 study. Pediatrics 120, e974–e983.

Di Matteo, V., Di Giovanni, G., Pierucci, M., Esposito, E., 2008. Serotonin control of central dopaminergic function: focus on in vivo microdialysis studies. Prog. Brain Res. 172, 7–44.

Donzanti, B.A., Yamamoto, B.K., 1988. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life Sci. 43, 913–922.

Drago, A., Crisafulli, C., Sidoti, A., Serretti, A., 2011. The molecular interaction between the glutamatergic, noradrenergic, dopaminergic and serotoninergic systems informs a detailed genetic perspective on depressive phenotypes. Prog. Neurobiol. 94, 418–460.

Dranovsky, A., Hen, R., 2006. Hippocampal neurogenesis: regulation by stress and antidepressants. Biol. Psychiatry 59, 1136–1143.
Durand, M., Berton, O., Aguerre, S., Edno, L., Combourieu, I., Mormède, P., Chaouloff, F., 1999. Effects of repeated fluoxetine on anxiety-related behaviours, central serotonergic systems, and the corticotropic axis in SHR and WKY rats. Neuropharmacology 38, 893–907.
Dybala, M., Siwek, A., Poleszak, E., Pilc, A., Nowak, G., 2008. Lack of NMDA–AMPA interaction in antidepressant-like effect of CGP 37849, an antagonist of NMDA receptor, in the forced swim test. J. Neural Transm. 115, 1519–1520.
Ebner, K., Bosch, O.J., Krömer, S.A., Singewald, N., Neumann, I.D., 2005. Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. Neuropsychopharmacology 30, 223–230.
Edwards, E., Harkins, K., Wright, G., Henn, F.A., 1991. 5-HT1b receptor in an animal model of depression. Neuropharmacology 30, 101–105.
Everitt, B.J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J.W., Robbins, T.W., 2008. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos. Trans. R. Soc. Lond .B. Biol. Sci. 363, 3125–3135.
Everitt, B.J., Robbins, T.W., 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 8, 1481–1489.
Ferdyn-Drosik, M., Nowak, P., Bojanek, K., Bałasz, M., Kasperski, J., Skaba, D., Muchacki, R., Kostrzewa, R.M., 2010. Neonatal DSP-4 treatment impairs 5-HT1B receptor reactivity in adult rats. Behavioral and biochemical studies. Pharmacol. Rep. 62, 608–620.
Ferré, S., Artigas, F., 1993. Dopamine D2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. J. Neurochem. 61, 772–775.
File, S.E., Hyde, J.R., 1978. Can social interaction be used to measure anxiety? Br. J. Pharmacol. 62, 19–24.
File, S.E., Kenny, P.J., Cheeta, S., 2000. The role of dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. Pharmacol. Biochem. Behav. 66, 65–72.
Forbes, E.E., Dahl, R.E., 2005. Neural systems of positive affect: relevance to understanding child and adolescent depression? Dev. Psychopathol. 17, 827–850.
Fortnai, F., Bassi, L., Torracca, M.T., Alessandri, M.G., Scalori, V., Corsini, G.U., 1996. Region- and neurotransmitter-dependent species and strain differences in DSP-4-induced monoamine depletion in rodents. Neurodegeneration 5, 241–249.
Fredrickson, B.L., 1998. What good are positive emotions? Rev. Gen. Psychol. 2, 300–319.
Fu, X.W., Brudzynski, S.M., 1994. High-frequency ultrasonic vocalization induced by intracerebral glutamate in rats. Pharmacol. Biochem. Behav. 49, 835–841.
Gamaro, G.D., Manoli, L.P., Torres, I.L.S., Silveira, R., Dalmaz, C., 2003. Effects of chronic variable stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem. Int. 42, 107–114.
Gao, S.-F., Bao, A.-M., 2011. Corticotropin-releasing hormone, glutamate, and γ-aminobutyric acid in depression. Neuroscientist 17, 124–144.
García-Horsman, S.P., Agmo, A., Paredes, R.G., 2008. Infusions of naloxone into the medial preoptic area, ventromedial nucleus of the hypothalamus, and amygdala block conditioned place preference induced by paced mating behavior. Horm. Behav. 54, 709–716.

Geschwind, N., Nicolson, N.A., Peeters, F., van Os, J., Barge-Schaapveld, D., Wichers, M., 2011. Early improvement in positive rather than negative emotion predicts remission from depression after pharmacotherapy. Eur. Neuropsychopharmacol. 21, 241–247.

Gillick, B.T., Zirpel, L., 2012. Neuroplasticity: an appreciation from synapse to system. Arch. Phys. Med. Rehabil. 93, 1846–1855.

Giorgi, O., Piras, G., Lecca, D., Hansson, S., Driscoll, P., Gorda, M.G., 2003. Differential neurochemical properties of central serotonergic transmission in Roman high-and low-avoidance rats. J. Neurochem. 86, 422–431.

Giovannini, M.G., Rakovska, A., Benton, R.S., Pazzagli, M., Bianchi, L., Pepeu, G., 2001. Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience 106, 43–53.

Goodman, A., 2008. Neurobiology of addiction: an integrative review. Biochem. Pharmacol. 75, 266–322.

Grenhoff, J., Nisell, M., Ferré, S., Aston-Jones, G., Svensson, T.H., 1993. Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. J. Neural. Transm. Gen. Sect. 93, 11–25.

Grippo, A.J., Sullivan, N.R., Damjanoska, K.J., Crane, J.W., Carrasco, G.A., Shi, J., Chen, Z., Garcia, F., Muma, N.A., Van de Kar, L.D., 2005. Chronic mild stess induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. Psychopharmacology 179, 769–780.

Grønli, J., Murison, R., Fiske, E., Bjorvatn, B., Sørensen, E., Portas, C.M., Ursin, R., 2005. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. Physiol. Behav. 84, 571–577.

Gross, J.J., John, O.P., 2003. Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being. J. Pers. Soc. Psychol. 85, 348–362.

Grzanna, R., Berger, U., Fritschy, J.M., Geffard, M., 1989. Acute action of DSP-4 on central norepinephrine axons: biochemical and immunohistochemical evidence for differential effects. J. Histochem. Cytochem. 37, 1435–1442.

Gustafsson, L., Oreland, S., Hoffmann, P., Nylander, I., 2008. The impact of postnatal environment on opioid peptides in young and adult male Wistar rats. Neuropeptides 42, 177–191.

Gustafsson, L., Zhou, Q., Nylander, I., 2007. Ethanol-induced effects on opioid peptides in adult male Wistar rats are dependent on early environmental factors. Neuroscience 146, 1137–1149.

Häidkind, R., Kivastik, T., Eller, M., Kolts, I., Oreland, L., Harro, J., 2002. Denervation of the locus coeruleus projections by treatment with the selective neurotoxin DSP-4 [N(2-chloroethyl)-N-ethyl-2-bromobenzylamine] reduces dopamine release potential in the nucleus accumbens shell in conscious rats. Neurosci. Lett. 332, 79–82.

Hallman, H., Sundström, E., Jonsson, G., 1984. Effects of the noradrenaline neurotoxin DSP-4 on monoamine neurons and their transmitter turnover in rat CNS. J. Neural Transm. 60, 89–102.

Hammen, C., 2005. Stress and depression. Annu. Rev. Clin. Psychol. 1, 293–319.

Harro, J., 1993. Measurement of exploratory behaviour in rodents, in: Conn, R.A. (Ed.), Methods in Neurosciences. Academic Press, Inc., San Diego, pp. 359–377.

Harro, J., 2010. Inter-individual differences in neurobiology as vulnerability factors for affective disorders: implications for psychopharmacology. Pharmacol. Ther. 125, 402–422.

Harro, J., Fischer, K., Vansteelandt, S., Harro, M., 2004. Both low and high activities of platelet monoamine oxidase increase the probability of becoming a smoker. Eur. Neuropsychopharmacol. 14, 65–69.

Harro, J., Häidkind, R., Harro, M., Modiri, A.R., Gillberg, P.G., Pähkla, R., Matto, V., Oreland, L., 1999. Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. Eur. Neuropsychopharmacol. 10, 5–16.

Harro, J., Kanarik, M., Matrov, D., Panksepp, J., 2011. Mapping patterns of depression-related brain regions with cytochrome oxidase histochemistry: relevance of animal affective systems to human disorders, with a focus on resilience to adverse events. Neurosci. Biobehav. Rev. 35, 1876–1889.

Harro, J., Kiivet, R.-A., Lang, A., Vasar, E., 1990. Rats with anxious or non-anxious type of exploratory behaviour differ in their brain CCK-8 and benzodiazepine receptor characteristics. Behav. Brain Res. 39, 63–71.

Harro, J., Meriküla, A., Lepiku, M., Modiri, A.R., Rinken, A., Oreland, L., 2000. Lesioning of locus coeruleus projections by DSP-4 neurotoxin treatment: effect on amphetamine-induced hyperlocomotion and dopamine D2 receptor binding in rats. Pharmacol. Toxicol. 86, 197–202.

Harro, J., Oreland, L., 1996. Depression as a spreading neuronal adjustment disorder. Eur. Neuropsychopharmacol. 6, 207–223.

Harro, J., Oreland, L., 2001. Depression as a spreading adjustment disorder of monoaminergic neurons: a case for primary implication of the locus coeruleus. Brain Res. Brain Res. Rev. 38, 79–128.

Harro, J., Oreland, L., Vasar, E., Bradwejn, J., 1995. Impaired exploratory behaviour after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation. Eur. Neuropsychopharmacol. 5, 447–455.

Harro, J., Terasmaa, A., Eller, M., Rinken, A., 2003. Effect of denervation of the locus coeruleus projections by DSP-4 treatment on [3H]-raclopride binding to dopamine D(2) receptors and D(2) receptor-G protein interaction in the rat striatum. Brain Res. 976, 209–216.

Harro, J., Tõnissaar, M., Eller, M., Kask, A., Oreland, L., 2001. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. Brain Res. 899, 227–239.

Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry Clin. Neurosci. 64, 341–357.

Hashimoto, K., 2011. The role of glutamate on the action of antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1558–1568.

Heilig, M., Egli, M., 2006. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol. Ther. 111, 855–876.

Heisler, L.K., Chu, H.M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., Tecott, L.H., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc. Natl. Acad. Sci. USA. 95, 15049–15054.

Hellweg, R., Zueger, M., Fink, K., Hörtnagl, H., Gass, P., 2007. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. Neurobiol. Dis. 25, 1–7.

Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adenocortical axis. Trends Neurosci. 20, 78–84.

Hernández, L.F., Segovia, G., Mora, F., 2003. Effects of activation of NMDA and AMPA glutamate receptors on the extracellular concentrations of dopamine,

acetylcholine, and GABA in striatum of the awake rat: a microdialysis study. Neurochem. Res. 28, 1819–1827.
Higley, J.D., Suomi, S.J., Linnoila, M., 1991. CSF monoamine metabolite concentrations vary according to age, rearing, and sex, and are influenced by the stressor of social separation in rhesus monkeys. Psychopharmacology 103, 551–556.
Ho, Y.-J., Chang, Y.-C., Liu, T.-M., Wong, C.-S., Tsai, Y.-F., 2000. Striatal glutamate release during novelty exposure-induced hyperactivity in olfactory bulbectomized rats. Neurosci. Lett. 287, 117–120.
Hofer, M.A., 1994. Early relationships as regulators of infant physiology and behavior. Acta Paediatr. Suppl. 397, 9–18.
Hofer, M.A., Shair, H., 1978. Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. Dev. Psychobiol. 11, 495–504.
Holmes, A., le Guisquet, A.M., Vogel, E., Millstein, R.A., Leman, S., Belzung, C., 2005. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. Neurosci. Biobehav. Rev. 29, 1335–1346.
Holmseth, S., Dehnes, Y., Huang, Y.H., Follin-Arbelet, V.V., Grutle, N.J., Mylonakou, M.N., Plachez, C., Zhou, Y., Furness, D.N., Bergles, D.E., Lehre, K.P., Danbold, N.C., 2012. The density of EAAC1(EAAT3) glutamate transporters expressed by neurons in the mammalian CNS. J. Neurosci. 32, 6000–6013.
Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuro-psyhopharmacology 23, 477–501.
Hooks, M.S., Jones, D.N., Holtzman, S.G., Juncos, J.L., Kalivas, P.W., Justice, J.B. Jr., 1994a. Individual differences in behavior following amphetamine, GBR-12909, or apomorphine but not SKF-38393 or quinpirole. Psychopharmacology 116, 217–225.
Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B., Justice, J.B. Jr., 1991. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9, 121–128.
Hooks, M.S., Juncos, J.L., Justice, J.B. Jr., Meiergerd, S.M., Povlock, S.L., Schenk, J.O., Kalivas, P.W., 1994b. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. J. Neurosci. 14, 6144–6152.
Hughes, Z.A., Stanford, S.C., 1998. A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: a microdialysis study in vivo. Psychopharmacology 136, 299–303.
Huot, R.L., Thrivikraman, K.V., Meaney, M.J., Plotsky, P.M., 2001. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology 158, 366–373.
Imperato, A., Di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.
Jaworski, J.N., Francis, D.D., Brommer, C.L., Morgon, E.T., Kuhar, M.J., 2005. Effects of early maternal separation on ethanol intake, GABA receptors and metabolizing enzymes in adult rats. Psychopharmacology 181, 8–15.
Jennings, K.A., Loder, M.K., Sheward, W.J., Pei, Q., Deacon, R.M., Benson, M.A., Olverman, H.J., Hastie, N.D., Harmar, A.J., Shen, S., Sharp, T., 2006. Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. J. Neurosci. 26, 8955–8964.

Joëls, M., Karst, H., Alfarez, D., Heine, V.M., Qin, Y., van Riel, E., Verkuyl, M., Lucassen, P.J., Krugers, H.J., 2004. Effects of chronic stress on structure and cell function in rat hippocamus and hypothalamus. Stress 7, 221–231.

Jonsson, G., Hallman, H., Ponzio, F., Ross, S., 1981. DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)-a useful denervation tool for central and peripheral noradrenaline neurons. Eur. J. Pharmacol. 72, 173–188.

Kabbaj, M., 2006. Individual differences in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol. Disord. Drug Targets 5, 513–520.

Kabbaj, M., Devine, D.P., Savage, V.R., Akil, H., 2000. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J. Neurosci. 20, 6983–6988.

Kalinichev, M., Easterling, K.W., Plotsky, P.M., Holtzman, S.G., 2002. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacol. Biochem. Behav. 73, 131–140.

Kanarik, M., Matrov, D., Kõiv, K., Eller, M., Tõnissaar, M., Harro, J., 2008. Changes in regional long-term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. Neurochem. 52, 432–437.

Kask, A., Harro, J., Tuomainen, P., Rägo, L., Männistö, P.T., 1997. Overflow of noradrenaline and dopamine in frontal cortex after [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (DSP-4) treatment: in vivo microdialysis study in anesthetized rats. Naunyn. Schemiedebergs Arch. Pharmacol. 355, 267–272.

Katz, R.J., 1982. Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacol. Biochem. Behav. 16, 965–968.

Katz, R.J., Roth, K.A., Carroll, B.J., 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci. Biobehav. Rev. 5, 247–251.

Keck, M.E., Sartori, S.B., Welt, T., Müller, M.B., Ohl, F., Holsboer, F., Landgraf, R., Singewald, N., 2005. Differences in serotonergic neurotransmission between rats displaying high or low anxiety/depression-like behaviour: effects of chronic paroxetine treatment. J. Neurochem. 92, 1170–1179.

Kendler, K.S., Bulik, C.M., Silberg, J., Hettema, J.M., Myers, J., Prescott, C.A., 2000. Childhood sexual abuse and adult psychiatric and substance use disorders in women: an epidemiological and co-twin control analysis. Arch. Gen. Psychiatry 57, 953–959.

Kendler, K.S., Karkowski, L.M., Prescott, C.A., 1999. Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry 156, 837–841.

Kivastik, T., Vuorikallas, K., Piepponen, T.P., Zharkovsky, A., Ahtee, L., 1996. Morphine- and cocaine-induced conditioned place preference: effects of quinpirole and preclamol. Pharmacol. Biochem. Behav. 54, 371–375.

Knutson, B., Burgdorf, J., Panksepp, J., 1998. Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. J. Comp. Psychol. 112, 65–73.

Knutson, B., Burgdorf, J., Panksepp, J., 1999. High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. Physiol. Behav. 66, 639–643.

Koob, G.F., 1999. Corticotropin-releasing factor, norepinephrine, and stress. Biol. Psychiatry 46, 1167–1180.

Koob, G.F., 2003. Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur. Neuropsychopharmacol. 13, 442–452.

Koob, G.F., 2006. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. Addiction 101, 23–30.
Koob, G.F., 2008. A role for brain stress systems in addiction. Neuron 59, 11–34.
Koob, G.F., 2009. Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. Pharmacopsychiatry 42, S32–S41.
Koob, G.F., 2011. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. Curr. Top. Behav. Neurosci. 13, 3–30.
Koob, G.F., Heinrichs, S.C., Pich, E.M., Menzaghi, F., Baldwin, H., Miczek, K., Britton, K.T., 1993. The role of corticotropin-releasing factor in behavioural responses to stress, in: De Souza, E.B., Nemeroff, C.B. (Eds.), Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC Press, Boca Raton, pp. 277–295.
Koob, G.F., Le Moal, 2001. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24, 97–129.
Kreek, M.J., Nielsen, D.A., LaForge, K.S., 2004. Genes associated with addiction: alcoholism, opiate, and cocaine addiction. Neuromolecular Med. 5, 85–108.
Krinke, G.J., 2000. The Laboratory Rat. Handbook of Experimental Animals, Academic Press, San Diego, CA.
Krystal, J.H., Sanacora, G., Blumberg, H., Anand, A., Charney, D.S., Marek, G., Epperson, C.N., Goddard, A., Mason, G.F., 2002. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol. Psychiatry 7, S71–S80.
Kudoh, A., Takahira, Y., Katagai, H., Takazawa, T., 2002. Small-dose ketamine improves the postoperative state of depressed patients. Anesth. Analg. 95, 114–118.
Kugaya, A., Sanacora, G., 2005. Beyond monoamines: glutamatergic function in mood disorders. CNS Spectr. 10, 808–819.
Kulikov, A., Aquerre, S., Berton, O., Ramos, A., Mormede, P., Chaouloff, F., 1997. Central serotonergic systems in the spontaneously hypertensive and Lewis rat strains that differ in the elevated plus-maze test of anxiety. J. Pharmacol. Exp. Ther. 281, 775–784.
Ladd, C.O., Huot, R.L., Thrivikraman, K.V., Nemeroff, C.B., Meaney, M.J., Plotsky, P.M., 2000. Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog. Brain Res. 122, 81–103.
Landgraf. R., Kessler, M.S., Bunck, M., Murgatroyd, C., Spengler, D., Zimbelmann, M., Nussbaumer, M., Czibere, L., Turck, C.W., Singewald, N., Rujescu, D., Frank, E., 2007. Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci. Biobehav. Rev. 31, 89–102.
Lapiz, M.D., Bondi, C.O., Morilak, D.A., 2007. Chronic treatment with desipramine improves cognitive performance of the rats in an attentional set-shifting test. Neuropsychopharmacology 32, 1000–1010.
Lapiz, M.D., Morilak, D.A., 2006. Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. Neuroscience 137, 1039–1049.
Larsen, M.H., Mikkelsen, J.D., Hay-Schmidt, A., Sandi, C., 2010. Regulation of brain-derived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. J. Psychiatr. Res. 44, 808–816.
Lategan, A.J., Marien, M.R., Colpaert, F.C., 1992. Suppression of nigrostriatal and mesolimbic dopamine release in vivo following noradrenaline depletion by DSP-4: a microdialysis study. Life Sci. 50, 995–999.

Lesch, K.P., Gutknecht, L., 2005. Pharmacogenetics of the serotonin transporter. Prog. Neuropsychopharmacol. Biol. Psychiatry 1062–1073.
Lee, S., Jeong, J., Kwak, Y., Park, S.K., 2010. Depression research: where are we now? Mol. Brain 3, 1–10.
Lehmann, J., Feldon, J., 2000. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev. Neurosci. 11, 383–408.
Lejeune, F., Cobert, A., Rivet, J.M., Millan, M.J., 1994. Blockade of transmission at NMDA receptors facilitates the electrical and synthetic activity of ascending serotoninergic neurones. Brain Res. 656, 427–431.
Le Strat, Y., Ramoz, N., Horwood, J., Falissard, B., Hassler, C., Romo, L., Choquet, M., Fergusson, D., Gorwood, P., 2009. First positive reactions to cannabis constitute a priority risk factor for cannabis dependence. Addiction 104, 1710–1717.
Levine, S., 1957. Infantile experience and resistance to physiological stress. Science 126, 405.
Licinio, J., Wong, M.-L., 2002. Brain-derived neurotrophic factor (BDNF) in stress and affective disorders. Mol. Psychiatry 7, 519.
Liu, D., Caldji, C., Sharma, S., Plotsky, P.M., Meaney, M.J., 2000. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. J. Neuroendocrinol. 12, 5–12.
Liu, Z.-H., Ikemoto, S., 2007. The midbrain raphe nuclei mediate primary reinforcement via GABA(A) receptors. Eur. J. Neurosci. 25, 735–743.
Liu, Y.X., Zhang, M., Liu, L.Z., Cui, X., Hu, Y.Y., Li, W.B., 2012. The role of glutamate transporter-1a in the induction of brain ischemic tolerance in rats. Glia 60, 112–124.
Lowy, M.T., Wittenberg, L., Yamamoto, B.K., 1995. Effect of acute stress on hippocampal glutamate levels and spectrin proteolysis in young and aged rats. J. Neurochem. 65, 268–274.
Lukas, M., Bredewold, R., Landgraf, R., Neumann, I.D., Veenema, A.H., 2011. Early life stress impairs social recognition due to a blunted response of vasopressin release within the septum of adult male rats. Psychoneuroendocrinology 36, 843–853.
Mackey, W.B., Van der Kooy, D., 1985. Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. Pharmacol. Biochem. Behav. 22, 101–105.
Maeng, S., Zarate, C.A. Jr., Du, J., Schloesser, R.J., McCammon, J., Chen, G., Manji, H.K., 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol. Psychiatry 63, 349–352.
Maes, M., Meltzer, H.Y., 1995. The serotonin hypothesis of major depression, in: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology. The Fourth Generation of Progress. Raven Press, New York, pp. 933–944.
Malison, R.T., Price, L.H., van Dyck, C.H., Pelton, G.H., Carpenter, L., Sanacora, G., Owens, M.J., Nemeroff, C.B., Rajeevan, N., Baldwin, R.M., Seibyl, J.P., Innis, R.B., Charney, D.S., 1998. Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3beta-(4-iodophenyl)tropane and single photon emission computed tomography. Biol. Psychiatry 44, 1090–1098.
Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, M., Eller, M., Harro, J., 2007a. Rats with persistently low or high exploratory activity: behaviour in tests of anxiety and depression, and extracellular levels of dopamine. Behav. Brain Res. 177, 269–281.

Mällo, T., Matrov, D., Herm, L., 2007b. Tickling-induced 50-kHz ultrasonic vocalization is individually stable and predicts behaviour in tests of anxiety and depression in rats. Behav. Brain Res. 184, 57–71.
Mällo, T., Matrov, D., Kõiv, K., Harro, J., 2009. Effect of chronic stress on behavior and cerebral oxidative metabolism in rats with high or low positive affect. Neuroscience 164, 963–974.
Mangiavacchi, S., Masi, F., Scheggi, S., Leggio, B., De Montis, M.G., Gambarana, C., 2001. Long-term behavioural and neurochemical effects of chronic stress exposure in rats. J. Neurochem. 79, 1113–1121.
Marin, M.T., Cruz, F.C., Planeta, C.S., 2007. Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. Physiol. Behav. 90, 29–35.
Marsden, C.A., 2006. Dopamine: the rewarding years. Br. J. Pharmacol. 147, S136–S144.
Martinowich, K., Lu, B., 2008. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacology 33, 73–83.
Matrov, D., Kolts, I., Harro, J., 2007. Cerebral oxidative metabolism in rats with high and low exploratory activity. Neurosci. Lett. 413, 154–158.
Matsugami, T.R., Tanemura, K., Mieda, M., Nakatomi, R., Yamada, K., Kondo, T., Oqawa, M., Obata, K., Watanabe, M., Hashikawa, T., Tanaka, K., 2006. Indispensability of the glutamate transporters GLAST and GLT1 to brain development. Proc. Natl. Acad. Sci. USA 103, 12161–12166.
Matsuno, A., Inoue, H., 2008. Hippocampal glutamate release on learning and memory in teeth-loss rats. Prosthodont. Res. Pract. 7, 71–77.
Matthews, K., Dalley, J.W., Matthews, C., Tsai, T.H., Robbins, T.W., 2001. Periodic maternal separation of neonatal rats produces region- and gender-specific effects on biogenic amine content in postmortem adult brain. Synapse 40, 1–10.
McBride, W.J., Murphy, J.M., Lumeng, L., Li, T.K., 1989. Serotonin and ethanol preference. Recent Dev. Alcohol. 7, 187–209.
McEntee, W.J., Crook, T.H., 1993. Glutamate: its role in learning, memory, and the aging brain. Psychopharmacology 111, 391–401.
McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 87, 873–904.
McEwen, B.S., Conrad, C.D., Kuroda, Y., Frankfurt, M., Magarinos, A.M., McKittrick, C., 1997. Prevention of stress-induced morphological and cognitive consequences. Eur. Neuropsychopharmacol. 7, S323–S328.
McKittrick, C.R., Blanchard, D.C., Blanchard, R.J., McEwen, B.S., Sakai, R.R., 1995. Serotonin receptor binding in a colony model of chronic social stress. Biol. Psychiatry 37, 383–393.
McLennan, H., Huffman, R.D., Marshall, K.C., 1968. Patterns of excitation of thalamic neurones by amino-acids and by acetylcholine. Nature 219, 387–388.
Meldrum, B., Garthwaite, J., 1990. Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol. Sci. 11, 379–387.
Miczek, K.A., Tornatzky, W., Vivian, J., 1991. Ethology and neuropharmacology: rodent ultrasounds, in: Olivier, B., Mos, J., Slangen, J.L. (Eds.), Animal Models in Psychopharmacology. Birkhauser Verlag, Boston, pp. 409–427.
Millan, M.J., 2003. The neurobiology and control of anxious states. Prog. Neurobiol. 70, 83–244.
Millan, M.J., Lejeune, F., Gobert, A., 2000. Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal

cortex: relevance to the actions of antidepressant agents. J. Psychopharmacol. 14, 114–138.
Misslin, R., Herzog, F., Koch, B., Ropartz, P., 1982. Effects of isolation, handling, and novelty on the pituitary-adrenal response in the mouse. Psyschoneuroendocrinology 7, 217–221.
Miyashita, T., Williams, C.L., 2004. Peripheral arousal-related hormones modulate norepinephrine release in the hippocampus via influences brainsteam nuclei. Behav. Brain Res. 153, 87–95.
Moffett, M.C., Vicentic, A., Kozel, M., Plotsky, P., Francis, D.D., Kuhar, M.J., 2007. Maternal separation alters drug intake patterns in adulthood in rats. Biochem. Pharmacol. 73, 321–330.
Moghaddam, B., 1993. Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. J. Neurochem. 60, 1650–1657.
Monroe, S.M., Slavich, G.M., Torres, L.D., Gotlib, I.H., 2007. Major life events and major chronic difficulties are differentially associated with history of major depressive episodes. J. Abnorm. Psychol. 116, 116–124.
Montague, P.R., Hyman, S.E., Cohen, J.D., 2004. Computational roles for dopamine in behavioural control. Nature 431, 760–767.
Mora, F., Del Arco, A., Segovia, G., 2003. Glutamate-dopamine interactions in striatum and nucleus accumbens of the conscious rat during aging, in: Graybiel, A.M. (Ed.), Basal Ganglia IV. Plenum Press, New York, pp. 615–622.
Moreau, J.L., Jenck, F., Martin, J.R., Mortas, P., Haefely, W.E., 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.
Moret, C., Briley, M., 2000. The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy. Eur. J. Pharmacol. 404, 1–12.
Morilak, D.A., Frazer, A., 2004. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. Int. J. Neuropsychopharmacol. 7, 193–218.
Morón, J.A., Brockington, A., Wise, R.A., Rocha, B.A., Hope, B.T., 2002. Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J. Neurosci. 22, 389–395.
Munck, A., Guyre, P.M., Holbrook, N.J., 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25–44.
Murison, R., Hansen, A.L., 2001. Reliability of the chronic mild stress paradigm: implications for research and animal welfare. Integr. Physiol. Behav. Sci. 36, 266–274.
Murphy, D.L., Aulakh, C., Mazzola-Pomietto, P., Briggs, N.C., 1996. Neuroendocrine responses to serotonergic agonists as indices of the functional status of central serotonin neurotransmission in humans: a preliminary comparative analysis of neuroendocrine endpoints versus other endpoint measures. Behav. Brain Res. 73, 209–214.
Murphy, J.M., McBride, W.J., Gatto, G.J., Lumeng, L., Li, T.K., 1988. Effects of acute ethanol administration on monoamine and metabolite content in forebrain regions of ethanol-tolerant and –nontolerant alcohol-preferring (P) rats. Pharmacol. Biochem. Behav. 29, 169–174.
Musazzi, L., Milanese, M., Farisello, P., Zappettini, S., Tardito, D., Barbiero, V.S., Bonifacino, T., Mallei, A., Baldelli, P., Racagni, G., Raiteri, M., Benfenati, F.,

Bonanno, G., Popoli, M., 2010. Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. PLOS One 5, e8566.
Muscat, R., Willner, P., 1992. Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. Neurosci. Biobehav. Rev. 16, 507–517.
Nakamura, K., Matsumoto, M., Hikosaka, O., 2008. Reward-dependent modulation of neuronal activity in the primate dorsal raphe nucleus. J. Neurosci. 28, 5331–5343.
Naragon, K., Watson, D., 2009. Positive affectivity, in: Lopez, S. (Ed.), The Encyclopedia of Positive Psychology. Wiley-Blackwell, Hobonen, pp. 707–711.
Nemeroff, C.B., Knight, D.L., Franks, J., Craighead, W.E., Krishnan, K.R., 1994. Further studies on platelet serotonin transporter binding in depression. Am. J. Psychiatry 151, 1623–1625.
Neuhauss, S.C.F., Rico, E.P., Gesemann, M., 2010. Nomenclature of glutamate transporters in zebrafish and other vertebrates. Brain Res. Bull. 83, 297.
Nielsen, C.K., Arnt, J., Sánchez, C., 2000. Intracranial self-stimulation and sucrose intake differ as hedonic measures following chronic mild stress: interstrain and interindividual differences. Behav. Brain Res. 107, 21–33.
Nilsson, K.W., Sjöberg, R.L., Damberg, M., Alm, P.O., Ohrvik, J., Leppert, J., Lindström, L., Oreland, L., 2005. Role of the serotonin transporter gene and family function in adolescent alcohol consumption. Alcohol. Clin. Exp. Res. 29, 564–570.
Noirot, E., 1968. Ultrasounds in young rodents. II. Changes with age in albino rats. Anim. Behav. 16, 129–134.
Nyby, J., Whitney, G., 1978. Ultrasonic communication of adult myomorph rodents. Neurosci. Biobehav. Rev. 2, 1–14.
Ögren, S.O., Archer, T., Ross, S.B., 1980. Evidence for a role of the locus coeruleus noradrenaline system in learning. Neurosci. Lett. 20, 351–356.
Oreland, L., 2004. Platelet monoamine oxidase, personality and alcoholism: the rise, fall and resurrection. Neurotoxicology 25, 79–89.
Otter, M.H., Matto, V., Sõukand, R., Skrebuhhova, T., Allikmets, L., Harro, J., 1997. Characterization of rat exploratory behavior using the exploration box test. Methods Find. Exp. Clin. Pharmacol. 19, 683–691.
Owens, M.J., Nemeroff, C.B., 1991. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 43, 425–473.
Pallotta, M., Segieth, J., Whitton, P.S., 1998. N-methyl-D-aspartate receptors regulate 5-HT release in the raphe nuclei and frontal cortex of freely moving rats: differential role of 5-HT1A autoreceptors. Brain Res. 783, 173–178.
Panksepp, J., 1998. Affective Neuroscience, The Foundations of Human and Animal Emotion, Oxford University Press, Inc, New York, pp. 466.
Panksepp, J., 2007. Neuroevolutionary sources of laughter and social joy: modeling primal human laughter in laboratory rats. Behav. Brain Res. 182, 231–244.
Panksepp, J., Burgdorf, J., 2003. "Laughing" rats and the evolutionary antecedents of human joy? Physiol. Behav. 79, 533–547.
Paredes, R.G., Martínez, I., 2001. Naloxone blocks place preference conditioning after paced mating in female rats. Behav. Neurosci. 115, 1363–1367.
Pawlak, C.R., Ho, Y.-J., Scwarting, R.K.W., 2008. Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci. Biobeh. Rev. 32, 1544–1568.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego.

Pechtel, P., Pizzagalli, D.A., 2011. Effects of early life stress on cognitive and affective function: an integrated review of human literature. Psychopharmacology 214, 55–70.

Petty, F., Kramer, G., Wilson, L., Jordan, S., 1994. In vivo serotonin release and learned helplessness. Psychiatry Res. 52, 285–293.

Piazza, P.V., Deminière, J.M., Le Moal, M., Simon, H., 1989. Factors that predict individual vulnerability to amphetamine self-administration. Science 245, 1511–1513.

Piazza, P.V., Le Moal, M., 1997. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res. Rev. 25, 359–372.

Piazza, P.V., Rougé-Pont, F., Deminière, J.M., Kharoubi, M., Le Moal, M., Simon, H., 1991. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res. 567, 169–174.

Pickering, C., Gustafsson, L., Cebere, A., Nylander, I., Liljequist, S., 2006. Repeated maternal separation of male Wistar rats alters glutamate receptor expression in the hippocampus but not the prefrontal cortex. Brain Res. 1099, 101–108.

Ploj, K., Roman, E., Nylander, I., 2003a. Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121, 787–799.

Ploj, K., Roman, E., Nylander, I., 2003b. Long-term effects of short and long periods of maternal separation on brain opioid peptide levels in male Wistar rats. Neuropeptides 37, 149–156.

Pollier, F., Sarre, S., Aquerre, S., Ebinger, G., Mormède, P., Michotte, Y., Chaouloff, F., 2000. Serotonin reuptake inhibition by citalopram in rat strains differing for their emotionality. Neuropsychopharmacology 22, 64–76.

Popik, P., Kos, T., Sowa-Kućma, M., Nowak, G., 2008. Lack of persistent effects of ketamine in rodent models of depression. Psychopharmacology 198, 421–430.

Pozzi, L., Invernizzi, R., Cervo, L., Vallebuona, F., Samanin, R., 1994. Evidence the extracellular concentrations of dopamine are regulated by noradrenergic neurons in the frontal cortex of rats. J. Neurochem. 63, 195–200.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.

Pruunsild, P., Kazantseva, A., Aid, T., Palm, K., Timmusk, T., 2007. Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. Genomics 90, 397–406.

Pryce, C.R., Bettschen, D., Bahr, N.I., Feldon, J., 2001a. Comparison of the effects of infant handling, isolation, and nonhandling on acoustic startle, prepulse inhibition, locomotion, and HPA activity in the adult rat. Behav. Neurosci. 115, 71–83.

Pryce, C.R., Bettschen, D., Feldon, J., 2001b. Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev. Psychobiol. 38, 239–251.

Pryce, C.R., Feldon, J., 2003. Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. Neurosci. Biobehav. Rev. 27, 57–71.

Pryce, C.R., Rüedi-Bettschen, D., Dettling, A.C., Weston, A., Russig, H., Ferger, B., Feldon, J., 2005. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neurosci. Biobehav. Rev. 29, 649–674.

Rasmussen, D.D., Boldt, B.M., Bryant, C.A., Mitton, D.R., Larsen, S.A., Wilkinson, C.W., 2000. Chronic daily ethanol and withdrawal: 1. Long-term changes in the hypothalamo-pituitary-adrenal axis. Alcohol Clin. Exp. Res. 24, 1836–1849.

Ressler, K.J., Nemeroff, C.B., 2000. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress. Anxiety 12, 2–19.

Robbins, T.W., Ersche, K.D., Everitt, B.J., 2008. Drug addiction and the memory systems of the brain. Ann. N.Y. Acad. Sci. 1141, 1–21.

Robinson, T.E., Vanderwolf, C.H., Pappas, B.A., 1977. Are the dorsal noradrenergic bundle projections from the locus coeruleus important for neocortical or hippocampal activation? Brain Res. 138, 75–98.

Roman, E., Gustafsson, L., Hyytiä, P., Nylander, I., 2005. Short and prolonged periods of maternal separation and voluntary ethanol intake in male and female ethanol-preferring AA and ethanol-avoiding ANA rats. Alcohol Clin. Exp. Res. 29, 591–601.

Roman, E., Hyytiä, P., Nylander, I., 2003. Maternal separation alters acquisition of ethanol intake in male ethanol-preferring AA rats. Alcohol Clin. Exp. Res. 27, 31–37.

Roman, E., Nylander, I., 2005. The impact of emotional stress early in life on adult voluntary ethanol intake-results of maternal separation in rats. Stress 8, 157–174.

Römer, B., Lewicka, S., Kopf, D., Lederbogen, F., Hamann, B., Gilles, M., Schilling, C., Onken, V., Frankhauser, P., Deuschle, M., 2009. Cortisol metabolism in depressed patients and healthy controls. Neuroendocrinology 90, 301–306.

Rosario, L.A., Abercrombie, E.D., 1999. Individual differences in behavioral reactivity: correlation with stress-induced norepinephrine efflux in the hippocampus of Sprague-Dawley rats. Brain Res. Bull. 48, 595–602.

Rosenberg, P.A., Amin, S., Leitner, M., 1992. Glutamate uptake disguises neurotoxic potency of glutamate agonists in cerebral cortex in dissociated cell culture. J. Neurosci. 12, 56–61.

Rougé-Pont, F., Deroche, V., Le Moal, M., Piazza, P.V., 1998. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. Eur. J. Neurosci. 10, 3903–3907.

Ruhé, H.G., Mason, N.S., Schene, A.H., 2007. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol. Psychiatry 12, 331–359.

Sagara, H., Sendo, T., Gomita, Y., 2010. Evaluation of motivational effects induced by intracranial self-stimulation behavior. Acta Med. Okayama 64, 267–275.

Sales, G., Pye, D., 1974.Ultrasonic Communication by Animals, Chapman and Hall, London.

Sanacora, G., Treccani, C., Popoli, M., 2012. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology 62, 63–77.

Sanacora, G., Zarate, C.A. Jr., Krystal, J.H., Manji, H.K., 2008. Targeting the glutamatergic systems to develop novel, improved therapeutics for mood disorders. Nat. Rev. Drug Discov. 7, 426–437.

Sánchez, C., 2003. Stress-induced vocalisation in adult animals. A valid model of anxiety? Eur. J. Pharmacol. 463, 133–143.

Sanders, B.J., Anticevic, A., 2007. Maternal separation enhances neuronal activation and cardiovascular responses to acute stress in borderline hypertensive rats. Behav. Brain Res. 183, 25–30.

Sauvage, M., Steckler, T., 2001. Detection of corticotropin-releasing hormone receptor 1 immunoreactivity in cholinergic, dopaminergic and noradrenergic neurons of the murine basal forebrain and brainstem nuclei – potential implication for arousal and attention. Neuroscience 104, 643–652.

Schildkraut, J.J., 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry 122, 509–522.

Schulte-Herbrüggen, O., Fuchs, E., Abumaria, N., Ziegler, A., Danker-Hopfe, H., Hiemke, C., Hellweg, R., 2009. Effects of escitalopram on the regulation of brain-derived neurotrophic factor and nerve growth factor protein levels in a rat model of chronic stress. J. Neurosci. Res. 87, 2551–2560.

Schwarting, R.K.W., Jegan, N., Wöhr, M., 2007. Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. Behav. Brain Res. 182, 208–222.

Schwarting, R.K.W., Pawlak, C.R., 2004. Behavioural neuroscience in the rat: taking the individual into account. Methods Find. Exp. Clin. Pharmacol. 26, 17–22.

Seeman, P., Battaglia, G., Corti, C., Corsi, M., Bruno, V., 2009. Glutamate receptor mGlu2 and mGlu3 knockout striata are dopamine supersensitive, with elevated D2(High) receptors and marked supersensitivity to the dopamine agonist (+)PHNO. Synapse 63, 247–251.

Shachnai, L., Shimamoto, K., Kanner, B.I., 2005. Sulfhydryl modification of cysteine mutants of a neuronal glutamate transporter reveals an inverse relationship between sodium dependent conformational changes and the glutamate-gated anion conductance. Neuropharmacology 49, 862–871.

Shakil, S.S., Holmer, H.K., Moore, C., Abernathy, A.T., Jakowec, M.W., Petzinger, G.M., Meshul, C.K., 2005. High and low responders to novelty show differential effects in striatal glutamate. Synapse 58, 200–207.

Shaw, C.A., Lanius, R.A., van de Doel, K., 1994. The origin of synaptic neuroplasticity: crucial molecules or a dynamical cascade? Brain Res. Rev. 19, 241–263.

Shippenberg, T.S., Koob, G.F., 2002. Recent advances in animal models of drug addiction and alcolism, in: Davis, K.L., Charney, D., Coyle, J.T., Nemeroff, C. (Eds.), Neuropsychopharmacology: the Fifth Generations of Progress. Lippincott, Williams and Williams, Philadelphia, pp. 1381–1397.

Singewald, N., 2007. Altered brain activity processing in high-anxiety rodents revealed by challenge paradigms and functional mapping. Neurosci. Biobehav. Rev. 31, 18–40.

Skolnick, P., Popik, P., Trullas, R., 2009. Glutamate-based antidepressants: 20 years on. Trends Pharmacol. Sci. 30, 563–569.

Smith, A.D., Bolam, J.P., 1990. The neural network of the basal ganglia as revealed by study of synaptic connections of identified neurones. Trends Neurosci. 13, 259–265.

Smith, A.D., Castro, S.L., Zigmond, M.J., 2002. Stress-induced Parkinson`s disease: a working hypothesis. Physiol. Behav. 77, 527–531.

Spyraki, C., Fidiger, H.C., Phillips, A.G., 1982. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253, 185–193.

Srinivasan, J., Schmidt, W.J., 2004. Functional recovery of locus coeruleus noradrenergic neurons after DSP-4 lesion: effects on dopamine levels and neuroleptic induced-parkinsonian symptoms in rats. J. Neural Transm. 111, 13–26.

Stead, J.D., Clinton, S., Neal, C., Schneider, J., Jama, A., Miller, S., Vazquez, D.M., Watson, S.J., Akil, H., 2006. Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. Behav. Genet. 36, 697–712.

Strausbaugh, H.J., Dallman, M.F., Levine, J.D., 1999. Repeated, but not acute, stress suppresses inflammatory plasma extravasation. Proc. Natl. Acad. Sci. USA 96, 14629–14634.

Sümegi, A., 2008. Domino principle – monoamines in bottom-view. Neuropsychopharmacol. Hung. 10, 131–140.

Szot, P., Miguelez, C., White, S.S., Franklin, A., Sikkema, C., Wilkinson, C.W., Uqedo, L., Raskind, M.A., 2010. A comprehensive analysis of the effect of DSP4 on the locus coeruleus noradrenergic system in the rat. Neuroscience 166, 279–291.

Takahashi, L.K., Thomas, D.A., Barfield, R.J.,1983. Analysis of ultrasonic vocalizations emitted by residents during aggressive encounters among rats (Rattus norvegicus). J. Comp. Psychol. 97, 207–212.

Takeda, A., Sakurada, N., Kanno, S., Minami, A., Oku, N., 2006. Response of extracellular zinc in the ventral hippocampus against novelty stress. J. Neurochem. 99, 670–676.

Tanaka, K., 2000. Functions of glutamate transporters in the brain. Neurosci. Res. 37, 15–19.

Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K., Iwama, H., Nishikawa, T., Ichihara, N., Kikuchi, T., Okuyama, S., Hori, S., Takimoto, M., Wada, K., 1997. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276, 1699–1702.

Tanti, A., Belzung, C., 2010. Open questions in current models of antidepressant action. Br. J. Pharmacol. 159, 1187–1200.

Tassin, J.P., 2008. Uncoupling between noradrenergic and serotonergic neurons as a molecular basis of stable changes in behaviour induced by repeated drugs of abuse. Biochem. Pharmacol. 75, 85–97.

Tellegen, A., 1985. Structures of mood and personality and their relevance to assessing anxiety, with an emphasis on self-report.: in: Tuma, A.H., Maser, J.D. (Eds.), Anxiety and the Anxiety Disorders. Erlbaum, Hillsdale, NJ, London, pp. 681–706.

Thiel, C.M., Müller, C.P., Huston, J.P., Schwarting, R.K., 1999. High versus low reactivity to a novel environment: behavioural, pharmacological and neurochemical assessments. Neuroscience 93, 243–251.

Tokarski, K., Bobula, B., Wabno, J., Hess, G., 2008. Repeated administration of imipramine attenuates glutamatergic transmission in rat frontal cortex. Neuroscience 153, 789–795.

Tokita, K., Yamaji, T., Hashimoto, K., 2012. Roles of glutamate signaling in preclinical and/or mechanistic models of depression. Pharmacol. Biochem. Behav. 100, 688–704.

Tõnissaar, M., Philips, M.-A., Eller, M. Harro, J., 2004. Sociability trait and serotonin metabolism in the rat social interaction test. Neurosci. Lett. 367, 309–312.

Tonoue, T., Ashida, Y., Makino, H., Hata, H., 1986. Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: a psychotropic effect. Psychoneuroendocrinology 1, 177–184.

Tottenham, N., Sheridan, M.A., 2009. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. Front. Hum. Neurosci. 3, 1–18.

Tsai, H.-C., Zhang, F., Adamantidis, A., Stuber, G.D., Bonci, A., de Lecea, L., Deisseroth, K., 2009. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324, 1080–1084.

Tzschentke, T.M., 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.
Ungerstedt, U., 1991. Microdialysis – principles and applications for studies in animals and man. J. Intern. Med. 230, 365–373.
Van Bockstaele, E.J., Biswas, A., Pickel, V.M., 1993. Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. Brain Res. 624, 188–198.
Ventura, R., Morrone, C., Puglisi-Allegra, S., 2007. Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward-and aversion-related stimuli. Proc. Natl. Acad. Sci. USA 104, 5181–5186.
Verheij, M.M.M., Veenvliet, J.V., Kormelink, T.G., Steenhof, M., Cools, A.R., 2009. Individual differences in the sensitivity to serotonergic drugs: a pharmacobehavioural approach using rats selected on the basis of their response to novelty. Psychopharmacology 205, 441–455.
Vicentic, A., Francis, D., Moffett, M., Lakatos, A., Rogge, G., Hubert, G.W., Harley, J., Kuhar, M.J., 2006. Maternal separation alters serotonergic transporter densities and serotonergic 1A receptors in rat brain. Neuroscience 140, 355–365.
Vivian, J.A., Miczek, K.A., 1991. Ultrasounds during morphine withdrawal in rats. Psychopharmacology 104, 187–193.
Warton, F.L., Howells, F.M., Russell, V.A., 2009. Increased glutamate-stimulated release of dopamine in substantia nigra of a rat model for attention-deficit/ hyperactivity disorder- lack of effect of methylphenidate. Metab. Brain Dis. 24, 599–613.
Watson, D., Naragon-Gainey, K., 2010. On the specificity of positive emotional dysfunction in psychopathology: evidence from the mood and anxiety disorders and schizophrenia/schizotypy. Clin. Psychol. Rev. 30, 839–848.
Weininger, O., 1954. Physiological damage under emotional stress as a function of early experience. Science 119, 285–286.
Weinshenker, D., Schroeder, J.P., 2007. There are back again: a tale of norepinephrine and drug addiction. Neuropsychopharmacology 32, 1433–1451.
Weiss, F., Porrino, L.J., 2002. Behavioral neurobiology of alcohol addiction: recent advances and challenges. J. Neurosci. 22, 3332–3337.
Wellman, C.L., Izquierdo, A., Garrett, J.E., Martin, K.P., Carroll, J., Millstein, R., Lesch, K.P., Murphy, D.L., Holmes, A., 2007. Impaired stress-coping and fear extinction and abnormal corticolimbic morphology in serotonin transporter knock-out mice. J. Neurosci. 27, 684–691.
WHO, 1992. The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines. World Health Organisation, Geneva.
Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90–110.
Willner, P., Moreau, J.L., Nielsen, C.K., Papp, M., Sluzewska, A., 1996. Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. Physiol. Behav. 60, 129–134.
Wise, R.A., 2008. Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox. Res. 14, 169–183.
Wong-Riley, M.T., Nie, F., Hevner, R.F., Liu, S., 1998. Brain cytochrome oxidase: functional significance and bigenomic regulation in the CNS, in: Gonzalez-Lima,

F.E. (Ed.), Cytochrome Oxidase in Neuronal Metabolism and Alzheimer`s Disease. Plenum Press, New York, pp. 1–53.
Xu, F., Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Bohn, L.M., Miller, G.W., Wang Y.M., Caron, M.G., 2000. Mice lacking the norepinephrine transporter and supersensitive to psychostimulants. Nat. Neurosci. 3, 465–471.
Yoshimoto, K., McBride, W.J., Lumeng, L., Li, T.K., 1992. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 19, 17–22.
Zarate, C.A. Jr., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864.
Zimmerberg, B., Sageser, K.A., 2011. Comparison of two rodent models of maternal separation on juvenile social behavior. Front. Psychiatry 2, 39.
Zink, M., Vollmayr, B., Gebicke-Haerter, P.J., Henn, F.A., 2010. Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. Neuropharmacology 58, 465–473.
Zurita, A., Cuadra, G., Molina, V.A., 1999. The involvement of an opiate mechanism in the sensitized behavioral deficit induced by early chronic variable stress: influence of desipramine. Behav. Brain Res. 110, 153–159.

SUMMARY IN ESTONIAN

Indiviididevahelised erinevused rottide afektiivses käitumises: neurokeemilised uuringud

Käesolev doktoriväitekiri keskendub depressiooniga seotud seisundite ja neurokeemiliste süsteemide seoste leidmisele, kasutades erinevaid analüütilisi meetodeid ning loomkatsemudeleid. Neurokeemiliste, näiteks monoamiinsete süsteemide normaalse funktsioneerimise tasakaalu muutumine võib viia mitmete meeleolu- ja sõltuvushäirete tekkimiseni. Depressioon, ärevus- ja sõltuvusseisundid on ühiskonnas üha levinumad psüühikahäired ning tänu nende mitmepalgelisele olemusele on nende uurimine ning ravi küllaltki keeruline. Peamised sihtmärgid depressiooniga seotud häirete teraapias on siiamaani seotud olnud monoamiinergiliste – serotoniini, dopamiini ja noradrenaliini – süsteemide talitlusega. Kuna psühhofarmakonide mõju avaldumine on pikaajaline ning indiviidid ei pruugi ravile üheselt alluda, siis on kasvanud vajadus täpsemate depressiooni ravimise lähenemisviiside järele.

Depressiooni ja sellega kaasnevaid häireid saab uurida modelleerides nende sümptomeid loomkatsemudelitel. Depressiooni ja ärevusega seotud häirete uurimisel kasutatakse sageli uudistaval käitumisel põhinevaid teste, kuna uudistav käitumine hõlmab uudsest keskkonnast esilekutsutud stiimulit, mis on mõjutatud nii uudishimust kui ka potentsiaalsest tekkivast ohust selles keskkonnas. Lisaks on leitud, et nii uudistava kui ka sõltuvusega seotud käitumise regulatsioonis omavad rolli just monoamiinsed neurotransmitterid, mille süsteemide mõjustamine toob kaasa ka erinevused käitumuslikes aspektides.

Eelnevalt on näidatud, et katseloomad erinevad püsivalt oma käitumises uudses keskkonnas ning indiviididevahelisi erinevusi on ka neurotransmitterite regulatsioonis. Käesoleva töö eksperimentides kasutati uudistava käitumise uurimisel uudiskasti, kus loomal on vaba valik suunduda uudsele avarale väljale või jääda kodupuuri sarnasele kaitstumale alale. Uudistamisaktiivuse põhjal eristatakse loomad kas väheuudistavateks (LE) või paljuuudistavateks (HE) isenditeks. Madalama uudistamisaktiivusega loomi peetakse depressiivsemateks, kuna nad on reeglina ärevamad ning passiivsemad ka teistes käitumistestides. Käesoleva töö raames tehtud mikrodialüüsi katsed näitasid, et hipokampuses (*hippocampus*) ja frontaalkoores (*frontal cortex*) on serotonergiline süsteem erinevalt reguleeritud. LE-rottidel oli frontaalkoores kõrgemad rakuvälised serotoniini tasemed peale serotoniini transpordi inhibeerimist tsitalopraamiga ning lisaks ka kõrgem serotoniini transporteri seondumisvõime. Vastupidised tulemused ilmnesid hipokampuses, kus kõrgemad serotoniini tasemed olid just HE-rottidel. Paljuuudistavatel rottidel olid kõrgemad glutamaadi tasemed peale glutamaadi transporteri inhibeerimist PDC (L-*trans*-pürrolidiin-2,4-dikarboksüülhape)-ga juttkehas (*striatum*), kuid mitte hipokampuses. Seega järeldub, et indiviididevahelised erinevused uudistavas käitumises võivad olla seotud lisaks serotonergilisele ka glutamatergilise närviülekandega.

Uudistav käitumine on suurel määrav sõltuv sinava tuuma (*locus coeruleus*) noradrenaliini projektsioonidest. Rotid, kellel on antud süsteemi kahjustus, käituvad uudiskasti testis nagu LE-fenotüüp, mida on seostatud madalama dopaminergilise neurotransmissiooni efektiivsusega. Käesolevas töös uuriti, kas sinava tuuma projektsioonid on olulised dopaminergilisele süsteemile toimivatele psühhostimulantide efektidele. Leiti, et ulatuslik sinava tuuma projektsioonide kahjustus närvitoksiiniga DSP-4 [*N*-(2-kloroetüül-*N*-etüül-2-bromobensüülamiin] vähendas psühhostimulandi kokaiini poolt stimuleeritud kohaeelistust ja liikumisaktiivsust.

Varasemalt on leitud, et elusündmused varajases elustaadiumis võivad omada mõju neurokeemiliste süsteemidele ning seeläbi mõjustada käitumismustreid hilisemas elus. Seetõttu uuriti antud doktoriväitekirjas, kas alkoholist tingitud efektid monoamiini süsteemidele on sõltuvad varajasest elukeskkonnast. Selleks kasutati varajases eas emast eraldamist, mis mõjub noortele poegadele kui stressirikas keskkond. Lisaks mõõdeti rottidel alkoholitarbimise mõju monoamiinide sisaldusele erinevates ajupiirkondades. Selgus, et nendel alkoholi tarbinud loomadel, kes olid noores eas emast kaua eraldatud, olid serotoniini tasemed madalamad just mandelkehas (*amygdala*), mida seostakse enim just sõltuvushäirete tekkimisega. Siit järeldub, et varajases eas toimuvatel sündmustel on oluline mõju alkoholi tarbimisele ning serotonergilise süsteemi toimimisele.

Viimastel aastatel on negatiivsete afektiivsete seisundite kõrval hakatud spekuleerima positiivse emotsionaalsuse võimaliku rolli üle depressiooni kujunemisel. Vähenenud võime väljendada positiivset emotsionaalsust on eelnevalt seostatud kõrgema tundlikkusega depressioonile. Positiivset emotsionaalsust on võimalik uurida mõõtes rottide ultraheli-häälitsusi. Nüüdseks on leitud, et rottidel on püsivad indiviididevahelised erinevused nende 50-kHz ultraheli häälitsustes (piuksudes) ning rotid eristuvad vähe- (LC) ja palju- (HC) piuksuvateks loomadeks. Käesolevas töös mõõdeti rottidel eksperimentaatori tekitatud kõdistamisest tingitud 50-kHz ultraheli häälitsusi ning uuriti kroonilise stressi mõju serotonergilisele süsteemile hipokampuses ja frontaalkoores. Leidis kinnitust, et LC-rotid on kroonilisele stressile vastuvõtlikumad: nad võtsid kaalus aeglasemalt juurde ning peale stressi olid neil kõrgemad verest määratud stressihormooni, kortikosterooni, tasemed. Hüpotees, et LC-rottidel on erinevused serotonergilises mehhanismis peale kroonilist stressi, sai kinnitust: LC-rottidel olid hipokampuses kõrgemad tsitalopraamist tingitud serotoniini tasemed. Nendest tulemustest saab järeldada, et isased vähepiuksuvad rotid on madalama positiivse emotsionaalsusega ning on stressile vastuvõtlikumad.

Käesoleva töö tulemustest võib kokkuvõttes järeldada, et rottide käitumuslikud erinevused uudses keskkonnas ja erinev tundlikkus stressile, mis on kas eristatud spontaansete püsivate eelistuste mõõtmise kaudu või eksperimentaalselt esile kutsutud, sõltuvad serotonergilise, dopaminergilise ja glutamatergilise närviülekande toimimise iseärasustest. Neid iseärasusi arvesse võttes võib jõuda depressiooniga seotud seisundite uudsete ning personaalsete farmakoloogiliste ravimeetoditeni.

ACKNOWLEDGEMENTS

The studies in this dissertation were supported by the grants from the European Commission Framework 6 Integrated Project NEWMOOD (LSHM-CT-2004–503474), the Estonian Ministry of Education and Science Project 01080027, Hope for Depression Research Foundation and the Institute for the Study of Affective Neuroscience and several grants to collaborating groups. The authors are grateful to Prof. Svante Ross (AstraZeneca) for generous donation of DSP-4. We thank Sadia Oreland (from University of Uppsala) for great collaboration and for performing several experiments for Paper II.

First I wish to express my deepest gratitude to my supervisors Professor Jaanus Harro and Professor Ivo Leito for their guidance, inspiring discussions, patience and support during all these years of our collaboration. Thank You for helping me to overcome different complications over the years and always seeing new opportunities in my research.

My sincere thanks go to all my present and previous colleagues – Tanel, Kadri, Aet, Margus, Denis, Marika, and Laura from bioorganic chemistry laboratory. Thank You for introducing me and let me explore this interesting world of behaviour of animals. I would like to thank You, my dear friends! You have enriched my life during all student years and made this time more enjoyable.

With all my heart I am thankful to my parents, brother and grandparents for support, understanding and for backing me up whenever needed.

PUBLICATIONS

CURRICULUM VITAE

Name: Karita Raudkivi
Date of birth: 6.12.1982
Citizenship: Estonian
E-mail: karita.raudkivi@ut.ee

Education:

2007–…	University of Tartu, *Ph.D.* student *(*analytical chemistry)
2005–2007	University of Tartu, *M.Sc.* in colloid and environmental chemistry
2001–2005	University of Tartu, *B.Sc.* in chemistry
1989–2001	Haapsalu Gymnasium

Professional employment:

2011–…	University of Tartu, Department of Psychology, researcher
2007–2011	University of Tartu, Department of Psychology, extraordinary researcher
2006–2007	University of Tartu, Department of Psychology, laboratorian
2006–2007	University of Tartu, Institute of Chemistry, chemist

Scientific publications:

1. Raudkivi, K., Mällo, T., Harro, J., 2012. Effect of chronic variable stress on corticosterone levels and hippocampal extracellular 5-HT in rats with persistent differences in positive affectivity. Acta Neuropsychiatrica, 24, 208–214.
2. Kõiv, K., Zobel, R., Raudkivi, K., Kivastik, T., Harro, J., 2011. The effect of denervation of the locus coeruleus projections with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on cocaine-induced locomotion and place preference in rats. Behavioural Brain Research, 216, 172–179.
3. Oreland, S., Raudkivi, K., Oreland, L., Harro, J., Arborelius, L., Nylander, I., 2011. Ethanol-induced effects on the dopamine and serotonin systems in adult Wistar rats are dependent on early-life. Brain Research, 1405, 57–68.
4. Mällo, T., Kõiv, K., Koppel, I., Raudkivi, K., Uustare, A., Rinken, A., Timmusk, T., Harro, J., 2008. Regulation of extracellular serotonin levels and brain-derived neurotrophic factor in rats with high and low exploratory activity. Brain Research, 1194, 110–117.

ELULOOKIRJELDUS

Nimi: Karita Raudkivi
Sünniaeg: 6.12.1982
Kodakondsus: Eesti
E-mail: karita.raudkivi@ut.ee

Haridus:

2007–…	Tartu Ülikooli, doktorant (analüütilise keemia erialal)
2005–2007	Tartu Ülikooli, *M.Sc.* (kolloid- ja keskkonnakeemia erialal)
2001–2005	Tartu Ülikooli, *B.Sc.* (keemia)
1989–2001	Haapsalu Gümnaasium

Teenistuskäik:

2011–…	Tartu Ülikool, Psühholoogia Instituut, Psühhofüsioloogia õppetool, teadur
2007–2011	Tartu Ülikool, Psühholoogia Instituut, Psühhofüsioloogia õppetool, erakorraline teadur
2006–2007	Tartu Ülikool, Psühholoogia Instituut, Psühhofüsioloogia õppetool, laborant
2006–2007	Tartu Ülikool, Keemia Instituut, keemik

Teaduspublikatsioonid:

1. Raudkivi, K., Mällo, T., Harro, J., 2012. Effect of chronic variable stress on corticosterone levels and hippocampal extracellular 5-HT in rats with persistent differences in positive affectivity. Acta Neuropsychiatrica, 24, 208–214.
2. Kõiv, K., Zobel, R., Raudkivi, K., Kivastik, T., Harro, J., 2011. The effect of denervation of the locus coeruleus projections with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on cocaine-induced locomotion and place preference in rats. Behavioural Brain Research, 216, 172–179.
3. Oreland, S., Raudkivi, K., Oreland, L., Harro, J., Arborelius, L., Nylander, I., 2011. Ethanol-induced effects on the dopamine and serotonin systems in adult Wistar rats are dependent on early-life. Brain Research, 1405, 57–68.
4. Mällo, T., Kõiv, K., Koppel, I., Raudkivi, K., Uustare, A., Rinken, A., Timmusk, T., Harro, J., 2008. Regulation of extracellular serotonin levels and brain-derived neurotrophic factor in rats with high and low exploratory activity. Brain Research, 1194, 110–117.

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

1. **Toomas Tamm.** Quantum-chemical simulation of solvent effects. Tartu, 1993, 110 p.
2. **Peeter Burk.** Theoretical study of gas-phase acid-base equilibria. Tartu, 1994, 96 p.
3. **Victor Lobanov.** Quantitative structure-property relationships in large descriptor spaces. Tartu, 1995, 135 p.
4. **Vahur Mäemets.** The ^{17}O and ^{1}H nuclear magnetic resonance study of H_2O in individual solvents and its charged clusters in aqueous solutions of electrolytes. Tartu, 1997, 140 p.
5. **Andrus Metsala.** Microcanonical rate constant in nonequilibrium distribution of vibrational energy and in restricted intramolecular vibrational energy redistribution on the basis of slater's theory of unimolecular reactions. Tartu, 1997, 150 p.
6. **Uko Maran.** Quantum-mechanical study of potential energy surfaces in different environments. Tartu, 1997, 137 p.
7. **Alar Jänes.** Adsorption of organic compounds on antimony, bismuth and cadmium electrodes. Tartu, 1998, 219 p.
8. **Kaido Tammeveski.** Oxygen electroreduction on thin platinum films and the electrochemical detection of superoxide anion. Tartu, 1998, 139 p.
9. **Ivo Leito.** Studies of Brønsted acid-base equilibria in water and non-aqueous media. Tartu, 1998, 101 p.
10. **Jaan Leis.** Conformational dynamics and equilibria in amides. Tartu, 1998, 131 p.
11. **Toonika Rinken.** The modelling of amperometric biosensors based on oxidoreductases. Tartu, 2000, 108 p.
12. **Dmitri Panov.** Partially solvated Grignard reagents. Tartu, 2000, 64 p.
13. **Kaja Orupõld.** Treatment and analysis of phenolic wastewater with microorganisms. Tartu, 2000, 123 p.
14. **Jüri Ivask.** Ion Chromatographic determination of major anions and cations in polar ice core. Tartu, 2000, 85 p.
15. **Lauri Vares.** Stereoselective Synthesis of Tetrahydrofuran and Tetrahydropyran Derivatives by Use of Asymmetric Horner-Wadsworth-Emmons and Ring Closure Reactions. Tartu, 2000, 184 p.
16. **Martin Lepiku.** Kinetic aspects of dopamine D_2 receptor interactions with specific ligands. Tartu, 2000, 81 p.
17. **Katrin Sak.** Some aspects of ligand specificity of P2Y receptors. Tartu, 2000, 106 p.
18. **Vello Pällin.** The role of solvation in the formation of iotsitch complexes. Tartu, 2001, 95 p.

19. **Katrin Kollist.** Interactions between polycyclic aromatic compounds and humic substances. Tartu, 2001, 93 p.
20. **Ivar Koppel.** Quantum chemical study of acidity of strong and superstrong Brønsted acids. Tartu, 2001, 104 p.
21. **Viljar Pihl.** The study of the substituent and solvent effects on the acidity of OH and CH acids. Tartu, 2001, 132 p.
22. **Natalia Palm.** Specification of the minimum, sufficient and significant set of descriptors for general description of solvent effects. Tartu, 2001, 134 p.
23. **Sulev Sild.** QSPR/QSAR approaches for complex molecular systems. Tartu, 2001, 134 p.
24. **Ruslan Petrukhin.** Industrial applications of the quantitative structure-property relationships. Tartu, 2001, 162 p.
25. **Boris V. Rogovoy.** Synthesis of (benzotriazolyl)carboximidamides and their application in relations with *N*- and *S*-nucleophyles. Tartu, 2002, 84 p.
26. **Koit Herodes.** Solvent effects on UV-vis absorption spectra of some solvatochromic substances in binary solvent mixtures: the preferential solvation model. Tartu, 2002, 102 p.
27. **Anti Perkson.** Synthesis and characterisation of nanostructured carbon. Tartu, 2002, 152 p.
28. **Ivari Kaljurand.** Self-consistent acidity scales of neutral and cationic Brønsted acids in acetonitrile and tetrahydrofuran. Tartu, 2003, 108 p.
29. **Karmen Lust.** Adsorption of anions on bismuth single crystal electrodes. Tartu, 2003, 128 p.
30. **Mare Piirsalu.** Substituent, temperature and solvent effects on the alkaline hydrolysis of substituted phenyl and alkyl esters of benzoic acid. Tartu, 2003, 156 p.
31. **Meeri Sassian.** Reactions of partially solvated Grignard reagents. Tartu, 2003, 78 p.
32. **Tarmo Tamm.** Quantum chemical modelling of polypyrrole. Tartu, 2003. 100 p.
33. **Erik Teinemaa.** The environmental fate of the particulate matter and organic pollutants from an oil shale power plant. Tartu, 2003. 102 p.
34. **Jaana Tammiku-Taul.** Quantum chemical study of the properties of Grignard reagents. Tartu, 2003. 120 p.
35. **Andre Lomaka.** Biomedical applications of predictive computational chemistry. Tartu, 2003. 132 p.
36. **Kostyantyn Kirichenko.** Benzotriazole – Mediated Carbon–Carbon Bond Formation. Tartu, 2003. 132 p.
37. **Gunnar Nurk.** Adsorption kinetics of some organic compounds on bismuth single crystal electrodes. Tartu, 2003, 170 p.
38. **Mati Arulepp.** Electrochemical characteristics of porous carbon materials and electrical double layer capacitors. Tartu, 2003, 196 p.

39. **Dan Cornel Fara.** QSPR modeling of complexation and distribution of organic compounds. Tartu, 2004, 126 p.
40. **Riina Mahlapuu.** Signalling of galanin and amyloid precursor protein through adenylate cyclase. Tartu, 2004, 124 p.
41. **Mihkel Kerikmäe.** Some luminescent materials for dosimetric applications and physical research. Tartu, 2004, 143 p.
42. **Jaanus Kruusma.** Determination of some important trace metal ions in human blood. Tartu, 2004, 115 p.
43. **Urmas Johanson.** Investigations of the electrochemical properties of polypyrrole modified electrodes. Tartu, 2004, 91 p.
44. **Kaido Sillar.** Computational study of the acid sites in zeolite ZSM-5. Tartu, 2004, 80 p.
45. **Aldo Oras.** Kinetic aspects of dATPαS interaction with $P2Y_1$ receptor. Tartu, 2004, 75 p.
46. **Erik Mölder.** Measurement of the oxygen mass transfer through the air-water interface. Tartu, 2005, 73 p.
47. **Thomas Thomberg.** The kinetics of electroreduction of peroxodisulfate anion on cadmium (0001) single crystal electrode. Tartu, 2005, 95 p.
48. **Olavi Loog.** Aspects of condensations of carbonyl compounds and their imine analogues. Tartu, 2005, 83 p.
49. **Siim Salmar.** Effect of ultrasound on ester hydrolysis in aqueous ethanol. Tartu, 2006, 73 p.
50. **Ain Uustare.** Modulation of signal transduction of heptahelical receptors by other receptors and G proteins. Tartu, 2006, 121 p.
51. **Sergei Yurchenko.** Determination of some carcinogenic contaminants in food. Tartu, 2006, 143 p.
52. **Kaido Tämm.** QSPR modeling of some properties of organic compounds. Tartu, 2006, 67 p.
53. **Olga Tšubrik.** New methods in the synthesis of multisubstituted hydrazines. Tartu. 2006, 183 p.
54. **Lilli Sooväli.** Spectrophotometric measurements and their uncertainty in chemical analysis and dissociation constant measurements. Tartu, 2006, 125 p.
55. **Eve Koort.** Uncertainty estimation of potentiometrically measured ph and pK_a values. Tartu, 2006, 139 p.
56. **Sergei Kopanchuk.** Regulation of ligand binding to melanocortin receptor subtypes. Tartu, 2006, 119 p.
57. **Silvar Kallip.** Surface structure of some bismuth and antimony single crystal electrodes. Tartu, 2006, 107 p.
58. **Kristjan Saal.** Surface silanization and its application in biomolecule coupling. Tartu, 2006, 77 p.
59. **Tanel Tätte.** High viscosity $Sn(OBu)_4$ oligomeric concentrates and their applications in technology. Tartu, 2006, 91 p.

60. **Dimitar Atanasov Dobchev**. Robust QSAR methods for the prediction of properties from molecular structure. Tartu, 2006, 118 p.
61. **Hannes Hagu**. Impact of ultrasound on hydrophobic interactions in solutions. Tartu, 2007, 81 p.
62. **Rutha Jäger.** Electroreduction of peroxodisulfate anion on bismuth electrodes. Tartu, 2007, 142 p.
63. **Kaido Viht.** Immobilizable bisubstrate-analogue inhibitors of basophilic protein kinases: development and application in biosensors. Tartu, 2007, 88 p.
64. **Eva-Ingrid Rõõm.** Acid-base equilibria in nonpolar media. Tartu, 2007, 156 p.
65. **Sven Tamp.** DFT study of the cesium cation containing complexes relevant to the cesium cation binding by the humic acids. Tartu, 2007, 102 p.
66. **Jaak Nerut.** Electroreduction of hexacyanoferrate(III) anion on Cadmium (0001) single crystal electrode. Tartu, 2007, 180 p.
67. **Lauri Jalukse.** Measurement uncertainty estimation in amperometric dissolved oxygen concentration measurement. Tartu, 2007, 112 p.
68. **Aime Lust.** Charge state of dopants and ordered clusters formation in CaF_2:Mn and CaF_2:Eu luminophors. Tartu, 2007, 100 p.
69. **Iiris Kahn**. Quantitative Structure-Activity Relationships of environmentally relevant properties. Tartu, 2007, 98 p.
70. **Mari Reinik.** Nitrates, nitrites, N-nitrosamines and polycyclic aromatic hydrocarbons in food: analytical methods, occurrence and dietary intake. Tartu, 2007, 172 p.
71. **Heili Kasuk.** Thermodynamic parameters and adsorption kinetics of organic compounds forming the compact adsorption layer at Bi single crystal electrodes. Tartu, 2007, 212 p.
72. **Erki Enkvist.** Synthesis of adenosine-peptide conjugates for biological applications. Tartu, 2007, 114 p.
73. **Svetoslav Hristov Slavov**. Biomedical applications of the QSAR approach. Tartu, 2007, 146 p.
74. **Eneli Härk.** Electroreduction of complex cations on electrochemically polished Bi(*hkl*) single crystal electrodes. Tartu, 2008, 158 p.
75. **Priit Möller.** Electrochemical characteristics of some cathodes for medium temperature solid oxide fuel cells, synthesized by solid state reaction technique. Tartu, 2008, 90 p.
76. **Signe Viggor.** Impact of biochemical parameters of genetically different pseudomonads at the degradation of phenolic compounds. Tartu, 2008, 122 p.
77. **Ave Sarapuu.** Electrochemical reduction of oxygen on quinone-modified carbon electrodes and on thin films of platinum and gold. Tartu, 2008, 134 p.
78. **Agnes Kütt.** Studies of acid-base equilibria in non-aqueous media. Tartu, 2008, 198 p.

79. **Rouvim Kadis.** Evaluation of measurement uncertainty in analytical chemistry: related concepts and some points of misinterpretation. Tartu, 2008, 118 p.
80. **Valter Reedo.** Elaboration of IVB group metal oxide structures and their possible applications. Tartu, 2008, 98 p.
81. **Aleksei Kuznetsov.** Allosteric effects in reactions catalyzed by the cAMP-dependent protein kinase catalytic subunit. Tartu, 2009, 133 p.
82. **Aleksei Bredihhin.** Use of mono- and polyanions in the synthesis of multisubstituted hydrazine derivatives. Tartu, 2009, 105 p.
83. **Anu Ploom.** Quantitative structure-reactivity analysis in organosilicon chemistry. Tartu, 2009, 99 p.
84. **Argo Vonk.** Determination of adenosine A_{2A}- and dopamine D_1 receptor-specific modulation of adenylate cyclase activity in rat striatum. Tartu, 2009, 129 p.
85. **Indrek Kivi.** Synthesis and electrochemical characterization of porous cathode materials for intermediate temperature solid oxide fuel cells. Tartu, 2009, 177 p.
86. **Jaanus Eskusson.** Synthesis and characterisation of diamond-like carbon thin films prepared by pulsed laser deposition method. Tartu, 2009, 117 p.
87. **Marko Lätt.** Carbide derived microporous carbon and electrical double layer capacitors. Tartu, 2009, 107 p.
88. **Vladimir Stepanov.** Slow conformational changes in dopamine transporter interaction with its ligands. Tartu, 2009, 103 p.
89. **Aleksander Trummal.** Computational Study of Structural and Solvent Effects on Acidities of Some Brønsted Acids. Tartu, 2009, 103 p.
90. **Eerold Vellemäe.** Applications of mischmetal in organic synthesis. Tartu, 2009, 93 p.
91. **Sven Parkel.** Ligand binding to 5-HT_{1A} receptors and its regulation by Mg^{2+} and Mn^{2+}. Tartu, 2010, 99 p.
92. **Signe Vahur.** Expanding the possibilities of ATR-FT-IR spectroscopy in determination of inorganic pigments. Tartu, 2010, 184 p.
93. **Tavo Romann**. Preparation and surface modification of bismuth thin film, porous, and microelectrodes. Tartu, 2010, 155 p.
94. **Nadežda Aleksejeva.** Electrocatalytic reduction of oxygen on carbon nanotube-based nanocomposite materials. Tartu, 2010, 147 p.
95. **Marko Kullapere.** Electrochemical properties of glassy carbon, nickel and gold electrodes modified with aryl groups. Tartu, 2010, 233 p.
96. **Liis Siinor.** Adsorption kinetics of ions at Bi single crystal planes from aqueous electrolyte solutions and room-temperature ionic liquids. Tartu, 2010, 101 p.
97. **Angela Vaasa.** Development of fluorescence-based kinetic and binding assays for characterization of protein kinases and their inhibitors. Tartu 2010, 101 p.

98. **Indrek Tulp.** Multivariate analysis of chemical and biological properties. Tartu 2010, 105 p.
99. **Aare Selberg.** Evaluation of environmental quality in Northern Estonia by the analysis of leachate. Tartu 2010, 117 p.
100. **Darja Lavõgina.** Development of protein kinase inhibitors based on adenosine analogue-oligoarginine conjugates. Tartu 2010, 248 p.
101. **Laura Herm.** Biochemistry of dopamine D_2 receptors and its association with motivated behaviour. Tartu 2010, 156 p.
102. **Terje Raudsepp.** Influence of dopant anions on the electrochemical properties of polypyrrole films. Tartu 2010, 112 p.
103. **Margus Marandi.** Electroformation of Polypyrrole Films: *In-situ* AFM and STM Study. Tartu 2011, 116 p.
104. **Kairi Kivirand.** Diamine oxidase-based biosensors: construction and working principles. Tartu, 2011, 140 p.
105. **Anneli Kruve.** Matrix effects in liquid-chromatography electrospray mass-spectrometry. Tartu, 2011, 156 p.
106. **Gary Urb.** Assessment of environmental impact of oil shale fly ash from PF and CFB combustion. Tartu, 2011, 108 p.
107. **Nikita Oskolkov.** A novel strategy for peptide-mediated cellular delivery and induction of endosomal escape. Tartu, 2011, 106 p.
108. **Dana Martin.** The QSPR/QSAR approach for the prediction of properties of fullerene derivatives. Tartu, 2011, 98 p.
109. **Säde Viirlaid.** Novel glutathione analogues and their antioxidant activity. Tartu, 2011, 106 p.
110. **Ülis Sõukand.** Simultaneous adsorption of Cd^{2+}, Ni^{2+}, and Pb^{2+} on peat. Tartu, 2011, 124 p.
111. **Lauri Lipping.** The acidity of strong and superstrong Brønsted acids, an outreach for the "limits of growth": a quantum chemical study. Tartu, 2011, 124 p.
112. **Heisi Kurig.** Electrical double-layer capacitors based on ionic liquids as electrolytes. Tartu, 2011, 146 p.
113. **Marje Kasari.** Bisubstrate luminescent probes, optical sensors and affinity adsorbents for measurement of active protein kinases in biological samples. Tartu, 2012, 126 p.
114. **Kalev Takkis.** Virtual screening of chemical databases for bioactive molecules. Tartu, 2012, 122 p.
115. **Ksenija Kisseljova**. Synthesis of aza-β^3-amino acid containing peptides and kinetic study of their phosphorylation by protein kinase A. Tartu, 2012, 104 p.
116. **Riin Rebane.** Advanced method development strategy for derivatization LC/ESI/MS. Tartu, 2012, 184 p.

117. **Vladislav Ivaništšev.** Double layer structure and adsorption kinetics of ions at metal electrodes in room temperature ionic liquids. Tartu, 2012, 128 p.
118. **Irja Helm.** High accuracy gravimetric Winkler method for determination of dissolved oxygen. Tartu, 2012, 139 p.
119. **Karin Kipper.** Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications. Tartu, 2012, 164 p.
120. **Arno Ratas.** Energy storage and transfer in dosimetric luminescent materials. Tartu, 2012, 163 p.
121. **Reet Reinart-Okugbeni**. Assay systems for characterisation of subtype-selective binding and functional activity of ligands on dopamine receptors. Tartu, 2012, 159 p.
122. **Lauri Sikk.** Computational study of the Sonogashira cross-coupling reaction. Tartu, 2012, 81 p.