The impact of psychostimulant administration during development on adult brain functions controlling motivation, impulsivity and cognition.

Mathieu Di Miceli

Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

July 2016

Thesis conducted under the supervision of
Dr. Benjamin S. Gronier, first supervisor and
Dr. Tyra S. Zetterström, second supervisor.

Department of Pharmacology,
School of Pharmacy,
De Montfort University,
Leicester, LE1 9BH,
United Kingdom

Abstract

ADHD pharmacotherapy uses methylphenidate (MPH), D-amphetamine (D-amph), two psychostimulants targeting dopamine transporters, or atomoxetine (ATX), specifically targeting norepinephrine transporters. We have assessed the pharmacological mechanisms of these three drugs on the *in vitro* efflux of neurotransmitters in rat prefrontal cortex (PFC) and striatal slices as well as on the *in vivo* electrical activities of PFC pyramidal neurons, striatal medium spiny neurons, ventral tegmental area dopamine neurons or dorsal raphe nucleus serotonin neurons, using single cell extracellular electrophysiological recording techniques. We have also tested whether chronic methylphenidate treatment, during either adolescence or adulthood, could have long-lasting consequences on body growth, depression and neuronal functions.

Release experiments showed that all ADHD drugs induce dose-dependent dopamine efflux in both the PFC and striatum, with different efficacies, while only D-amph induced cortical norepinephrine efflux. Atomoxetine induced an unexpected massive dopamine outflow in striatal regions, by mechanisms that depend on physiological parameters.

Our electrophysiological studies indicate that all three drugs equally stimulate the excitability of PFC pyramidal neurons, in basal and NMDA-evoked conditions, when administered acutely (3 mg/kg). While the electrophysiological effects elicited by psychostimulants may be dependent on D_1 receptor activation, those induced by atomoxetine relied on different mechanisms. In the ventral tegmental area (VTA), methylphenidate (2 mg/kg), but not atomoxetine, induced firing and burst activity reductions, through dopamine D_2 autoreceptor activation. Reversal of such effects (eticlopride 0.2 mg/kg) revealed an excitatory effect of methylphenidate on midbrain dopamine neurons that appear to be dependent on glutamate pathways and the combination of D_1 and alpha-1 receptors. Finally, acute intraperitoneal psychostimulant

injections increased vertical locomotor activity as well as $NMDA_{2B}$ protein expression in the striatum.

Some animals chronically treated with intraperitoneal administrations (methylphenidate 4 mg/kg/day or saline 1.2 ml/kg/day) showed decreased body weight gain. Voluntary oral methylphenidate intake induces desensitisation to subsequent intravenous methylphenidate challenges, without altering dopamine D_2 receptor plasticity. Significant decreases in striatal $NMDA_{2B}$ protein expression were observed in animals chronically treated.

After adolescent MPH treatment, midbrain dopaminergic neurons do not display either desensitisation or sensitisation to intravenous methylphenidate re-challenges. However, partial dopamine D_2 receptor desensitisation was observed in midbrain dopamine neurons. Using behavioural experiments, cross-sensitisation between adolescent methylphenidate exposure and later-life D-amphetamine challenge was observed. Significant decreases in striatal $NMDA_{2B}$ protein expression were observed in animals chronically treated, while striatal medium spiny neurons showed decreased sensitivities to locally applied NMDA and dopamine.

While caffeine is devoid of action on baseline spike generation and burst activity of dopamine neurons, nicotine induces either firing rate enhancement, firing rate reduction, or has no consequences. Adolescent methylphenidate treatment leads to decreased neuronal sensitivities to the combination of nicotine, MPH and eticlopride, compared to controls. Finally, nicotine partially prevented D-amphetamine-induced increase of rearing activities.

Our results show that increases in the excitability of PFC neurons in basal conditions and *via* NMDA receptor activation may be involved in the therapeutic response to ADHD drugs. Long-term consequences were observed after psychostimulant exposure. Such novel findings strengthen the mixed hypothesis in ADHD, whereby both dopamine and glutamate neurotransmissions are dysregulated. Therefore, ADHD therapy may now focus on adequate balancing between glutamate and dopamine.

Acknowledgements

First I would like to thank De Montfort University for allowing me to follow this Ph-D program, for paying my tuition fees and giving me financial support in the form of a stipend. For the last 3 years, it has been a real pleasure to come to the University and work with such amazing colleagues, to all of whom I express my heartfelt gratitude.

I would also like to thank all the members of staff involved in the radioactivity work: Liz O'Brien, for all the help she provided regarding isotope regulations, radioactivity handling as well as laboratory health and safety; Nazmin Juma and Dr. Ketan Ruparelia for their patience in collecting the radioactive waste and pointing out some mistakes they found in my log book entries while doing so.

This work could never have been done without technical support from the B.S.U. team, including Anita O'Donoghue, Stephen Bowen (†) and Mike Storer. Their assistance in maintaining high standards within the animal unit has allowed us to save time and effort, but has also had an impact on the quality of work that we were able to perform.

I would like to thank all the technicians in room 2.17 for their immense technical support, in particular David Reeder, Amrat Khorana, Claire West, Jo Tonkin and Leonie Hough. By providing gallons of purified water as well as many consumables, this work could not have been done without their almost daily assistance.

On more than one occasion, I have found myself thinking about all the animals used during the last 3 years. I would like to emphasis the help they provide by increasing our knowledge in biology and Science. *Memento Mori*.

I would also like to express my gratitude to my second supervisor, Dr. Tyra Zetterström for her support and ideas from start to end of this Ph-D.

Last but of great importance, I personally thank my supervisor, Dr. Benjamin Gronier, for his three years of support, his extensive scientific knowledge and for increasing my research skills. Pour tout cela et tout le reste, merci beaucoup !

À ma famille, sans laquelle je n'aurais jamais pu arriver jusqu'au doctorat.

Abbreviations

ADHD	Attention Deficit and Hyperactivity Disorder
AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid
ATX	Atomoxetine
BAPTA-AM	Bis(aminophenoxy)ethane-*N*-tetraacetic acid-acetoxymethyl ester
CNVs	Copy number variation(s)
D-amph	Dextro-amphetamine
^{o}C	Degree Celsius
DAT	Dopamine transporter
EC_{50}	Dose inducing 50% of maximum response
EPSCs	Excitatory post-synaptic currents
fMRI	Functional magnetic resonance imaging
g - kg	Gram - kilogram
GABA	γ-amino-butyric acid
$GlyT_1$	Glycine reuptake transporter 1
IC_{50}	Dose inducing 50% of maximum inhibition
i.e.	*id est*
IPSCs	Inhibitory post-synaptic currents
K_{ATP}	ATP-gated potassium channel
K_i	Inhibitory constant, binding affinity
K_{IR}	Inwardly rectifying potassium channel
Kv_1	Voltage-gated potassium channel, family 1
l	Litre
LTD	Long-term depression
LTP	Long-term potentiation
mEq	Milliequivalent
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
MΩ	Megaohm

MPH	Methylphenidate
ms	Millisecond
MSN	Medium spiny neurons
nA	Nanoampere
NET	Norepinephrine transporter
nM	Nanomolar
NMDA	N-methyl-D-aspartate
%	Percentage
PFC	Prefrontal cortex
rpm	Rotations per minute
s - sec	Second
SERT	Serotonin transporter
SHR	Spontaneously hypertensive rat
SNPs	Single nucleotide polymorphism(s)
µm	Micrometre
µM	Micromolar
$VMAT_2$	Vesicular monoamine transporter 2
VTA	Ventral tegmental area
v/v	Volume to volume ratio
w/v	Weight to volume ratio

Table of content

Table of figures

List of tables

Introduction

ADHD: statistics and symptoms

Among the worldwide population, Attention-Deficit and Hyperactivity Disorder (ADHD) affects 6-7% of children if diagnosed *via* the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, (Willcutt, 2012)). The prevalence of ADHD in the US child population has been estimated at 8.7% (Froehlich *et al.*, 2007), a rate that is very similar to the UK figure of 8% of school-aged children (McClure, 2013). However, the prevalence rate could climb up to 14%-16% depending on the population studied and the methodology used (Perera *et al.*, 2009; Rader *et al.*, 2009). In an American adult cohort (18-44 years old), ADHD occurred at a prevalence of just over 4% (Kessler *et al.*, 2006). According to the DSM-IV, symptoms of ADHD include impulsivity, inattention as well as social and academic difficulties. Depending upon the symptoms, 3 different types of ADHD can be distinguished: predominantly difficulty in concentration, predominantly hyperactivity and impulsiveness and finally a combination of all of the above (Steinau, 2013). This can be diagnosed in children as well as adults (Gentile *et al.*, 2006). ADHD can include a wide range of symptoms such as restlessness, fidgeting, anxiety, attention deficit, distractibility, excessive talking, forgetfulness and frequent interruption of others (American Psychiatric Association, 2013; Wilens *et al.*, 2009; Wilens *et al.*, 2010). After being diagnosed with ADHD, patients will then either follow a psychosocial therapy or receive medication, or both. Although the pharmacological approach is preferred, there is clear meta-analysis evidence that the psychosocial approach is highly effective (Fabiano *et al.*, 2009). This consists in intervention at school, parent management training but also neurofeedback, which consists in self-training the patient to modify the EEG patterns of his own brain, while under fMRI scanning and while also receiving visual feedback on such EEG patterns (Linden, 2014). Neurofeedback is considered as being "efficacious and specific" (Arns *et al.*, 2009). Evidence for beneficial effects of non-pharmacological approaches now exist, such as behavioural training and modification, dietary programs (supplementation or elimination of some compounds), relaxation exercises, massage

and psychotherapy (Catala-Lopez *et al.*, 2015; Hodgson *et al.*, 2014; Moore *et al.*, 2015; Poncin *et al.*, 2007; Richardson *et al.*, 2015; Roman, 2010). All these alternative treatments have led to statistically significant results (Sonuga-Barke *et al.*, 2013). Physical exercise is also efficient in reducing core symptoms of ADHD (Cerrillo-Urbina *et al.*, 2015; Gapin *et al.*, 2011; Hoza *et al.*, 2015; Pontifex *et al.*, 2013; Silva *et al.*, 2015; Ziereis *et al.*, 2015). Acute exercise at moderate intensity also improves fatigue, confusion and depression, as shown by a recent study (Fritz *et al.*, 2016). And yet, stimulant medications are often preferred. Stimulant drugs can consist of either methylphenidate or amphetamine salts. They have been associated with a high response rate in ADHD patients (Wigal, 2009), improving attention-related symptoms such as vigilance, short-term memory, cognition, restlessness and distractibility (Kolar *et al.*, 2008). An alternative to these stimulants is the drug atomoxetine, which, unlike stimulants, fails to improve academic work in children (Prasad *et al.*, 2013) and has a longer onset of action (Bushe *et al.*, 2014). Atomoxetine can be prescribed to patients with a previous history of substance use or patients displaying serious side effects to psychostimulant therapy (Kolar *et al.*, 2008) because of its significantly lower abuse potential than psychostimulants (Niederhofer, 2010; Wee *et al.*, 2004). However, atomoxetine (1.2-1.8 mg/kg/day) also improves ADHD symptoms such as hyperactivity, inattention and impulsivity (Ledbetter, 2006; Michelson *et al.*, 2001b; Purper-Ouakil *et al.*, 2005).

Aetiology of ADHD

The causes of ADHD are yet to be clarified. Some studies have reported possible genetic factors, involving dopamine and serotonin neurotransmission as well as the BDNF gene (Kebir *et al.*, 2009). Other studies have shown that ADHD may be caused by environmental factors such as low birth weight, premature birth, infections or traumas (Millichap, 2008; Thapar *et al.*, 2012). More studies are needed to determine the exact cause of ADHD, if a specific one could be determined. Genetic studies have found an association between several proteins and ADHD. These proteins are often found to be dopamine transporters (DAT_1), dopamine receptors (D_4, D_5) or serotonin (5-HT_{1B}) receptors (Gizer *et al.*, 2009). A few studies have also focused on glutamate neurotransmission. Indeed, genome scans of ADHD patients have identified the $GRIN_{2A}$ gene, encoding for the ionotropic glutamate NMDA receptor 2A (N-methyl-D-

aspartate), as a risk factor for ADHD (Adams *et al.*, 2004; Banaschewski *et al.*, 2010; Dorval *et al.*, 2007). All the genetic studies highlight single nucleotide polymorphism (SNP) in the aforementioned proteins/genes (Adams *et al.*, 2004; Banaschewski *et al.*, 2010; Dorval *et al.*, 2007; Guan *et al.*, 2009; Kollins *et al.*, 2008; Lasky-Su *et al.*, 2008; Oades *et al.*, 2008). Although the NMDA receptor 2A seems to be linked to ADHD, the association between the NMDA receptor 2B and ADHD remains unclear, as it may only be linked to the inattention and hyperactivity dimensions and has yet to be proven to be associated with both verbal working memory and verbal short-term memory (Dorval *et al.*, 2007). These genetic insights into the cause of ADHD emphasise the importance of serotonin, dopamine and glutamate pathways in the physiopathology of ADHD.

Structural evidence of decreased white and grey matter volumes exists in ADHD patients, if compared to healthy control patients. Regional anatomical abnormalities such as decreased grey matter in the right insula and the right orbitofrontal cortex, combined with decreased connectivity between the insula and the right hippocampus have been observed (Li *et al.*, 2015a). Some studies reported structural abnormalities in the dorsolateral prefrontal cortex and the anterior cingulate cortex, two regions involved in cognition control (Kessler *et al.*, 2014). Cerebellar structural changes have also been analysed in patients with ADHD and have been found to be linked to some of the symptoms the patients suffer from (Valera *et al.*, 2007). Some studies have associated ADHD with decreases in regional brain volumes (Castellanos *et al.*, 2002; Lopez-Larson *et al.*, 2012; Mostofsky *et al.*, 2002; Wolosin *et al.*, 2009), although this has been recently disputed by some other studies (Onnink *et al.*, 2014; Semrud-Clikeman *et al.*, 2012; Stevens *et al.*, 2012). In 2008, Perlov found no differences in the hippocampal and amygdaloid volumes of adults with ADHD, compared to healthy volunteers (Perlov *et al.*, 2008), although another study on children and adolescents (6-18 years old) found increased hippocampal volumes (Plessen *et al.*, 2006). In contradiction, a third study found decreased volumes of the left hippocampus in non-medicated ADHD children, which suggests, according to the authors, increased risks for developing depression (Posner *et al.*, 2014).

Neurotransmitters and brain regions implicated in ADHD

The pathophysiology of ADHD suggests two main neurotransmitter pathway alterations: those involving dopamine neurotransmission and those involving norepinephrine neurotransmission (Bokor *et al.*, 2014). Therefore, two brain regions, where both norepinephrine and dopamine are densely stored inside synaptic terminals, are involved: the prefrontal cortex (PFC) and the ventral tegmental area (VTA), respectively. In the prefrontal cortex, therapies aiming at increasing monoamine neurotransmission have been proven efficient in improving cognition, either *via* alpha-2 receptor activation (Arnsten *et al.*, 2005) or *via* dopamine D_1 receptor enhancement (Gronier, 2011; Levy, 2009), which help to reinforce the noradrenergic theory of ADHD. The dopamine theory is at the centre of understanding ADHD. There is no doubt that psychostimulants either block the dopamine reuptake transporter (DAT) or influence dopamine transmission, as evidenced before (Gonon, 2009). Positron emission tomography performed in ADHD patients suggests altered striatal dopamine transporter/receptor availability (Volkow *et al.*, 2007a; Volkow *et al.*, 2007b; Wang *et al.*, 2013), although this was partly contested by Wang's study in 2013 (Wang *et al.*, 2013). Therefore, one could still challenge the dopamine hypothesis in ADHD. The efficiency of methylphenidate in reducing ADHD symptoms seems to be directly associated with striatal dopamine receptor availability (Crunelle *et al.*, 2013; Kasparbauer *et al.*, 2015; Krause *et al.*, 2005; Rosa-Neto *et al.*, 2005). Finally, a polymorphism located within the enzyme dopamine beta-hydroxylase (rs5320) was hypothesised as being a risk factor for ADHD (Wang *et al.*, 2012), suggesting a possible role for norepinephrine in ADHD. The current literature remains contradictory concerning brain dopamine receptor/transporter availability at baseline levels in ADHD patients.

Growing evidence is now suggesting altered glutamate neurotransmission in ADHD. Indeed, a study performed in children with ADHD revealed decreased glutamate/glutamine ratios in the striatum following chronic (14-18 weeks) methylphenidate and atomoxetine, while ratios in the prefrontal cortex were only altered in patients receiving atomoxetine, but not methylphenidate (Carrey *et al.*, 2002). Besides, compared to healthy controls, ADHD patients present greater baseline levels of glutamate and creatinine in the striatum (Carrey *et al.*, 2007) and reduced

glutamate/glutamine ratios in the cingulate cortex (Perlov *et al.*, 2007). ADHD can be characterised by a hypofunctioning glutamatergic neurotransmission (Carlsson, 2000; Carlsson, 2001). Moreover, variations within CNVs (copy number variants) of glutamate metabotropic receptors have been observed in ADHD patients and appear to be related to the clinical characteristics of ADHD (Akutagava-Martins *et al.*, 2014).

Adequate balancing between excitatory and inhibitory neurotransmitters is crucial for the maintenance of normal brain functioning (Purkayastha *et al.*, 2015). A recent imagery study found decreased cortical GABA concentrations in ADHD children (Edden *et al.*, 2012), although a meta-analysis study (performed on 173 studies) revealed no association between ADHD and GABA levels (Schur *et al.*, 2016). Another study observed elevated plasmatic GABA levels in ADHD patients with compulsive disorders, but not in ADHD patients without such a comorbidity (Prosser *et al.*, 1997). In the cingulate cortex of ADHD children, the functioning of $GABA_A$ receptors seems to be in direct correlation to abnormal behavioural disturbances (Nagamitsu *et al.*, 2015).

Attention and impulsivity are core symptoms of ADHD (Adesman, 2001). In rodents, the whole prefrontal cortex can be divided into three parts: the medial, lateral and ventral prefrontal cortex (Dalley *et al.*, 2004). Within the medial prefrontal cortex, a few subregions can be of interest due to the roles they play in attention or impulsivity. Indeed, in rats, lesions within the prelimbic, infralimbic and cingulate cortices induce impaired attention (Dalley *et al.*, 2004; Muir *et al.*, 1996). Lesions within the anterior cingulate do not seem to induce impulsivity (Cardinal *et al.*, 2001), although another study found increased impulsivity following lesions of the anterior cingulate cortex (Muir *et al.*, 1996). On the other hand, the orbitofrontal cortex does not seem to be implicated in impulsivity (Winstanley *et al.*, 2004). Finally, lesions within the infralimbic cortex tend to increase impulsive choices (Chudasama *et al.*, 2003). Although these substructures can be functionally distinguished, anatomical segregation remains heterogeneous and equivocal (Machens *et al.*, 2010).

The cerebellum, located beneath the occipital lobe, is important for motor coordination and information processing. It is composed of GABAergic Purkinje neurons and glutamatergic granule neurons (Hibi *et al.*, 2012). Its role in ADHD is

slowly emerging. Indeed, a few studies found loss of volume in the superior cerebellar vermis of ADHD patients (Berquin *et al.*, 1998; Mackie *et al.*, 2007; Mostofsky *et al.*, 1998). Stimulants appear to alleviate such differences in a time-dependent manner, where the longer the treatment, the stronger the effect on cerebellar volumes (Ivanov *et al.*, 2014). The severity of ADHD symptoms is also directly correlated to decreased cerebellar hemisphere volumes (Ivanov *et al.*, 2014). Moreover, a recent study using fMRI demonstrated that ADHD patients display prominent cerebro-cerebellar functional connectivity, an effect that was barely observed in matched controls, highlighting the importance of interactions between the frontal cortex and the cerebellum in a normally functioning brain (Kucyi *et al.*, 2015). Finally, postural abnormalities have been reported in ADHD children (Bucci *et al.*, 2014; Buderath *et al.*, 2009; Kooistra *et al.*, 2009; Pitcher *et al.*, 2003; Shorer *et al.*, 2012) and adults (Hove *et al.*, 2015), an effect partially due to cerebellar gray matter abnormalities (Hove *et al.*, 2015).

ADHD drugs pharmacology

Three main drugs are available: methylphenidate, D-amphetamine, the two being stimulants, and atomoxetine, a non-stimulant medication.

Methylphenidate

Methylphenidate is known to block the Dopamine Transporter (DAT, with an IC_{50} of 0.19 µM) and the Norepinephrine Transporter (NET, with an IC_{50} of 0.034 µM, Table 1) (Bymaster *et al.*, 2002; van der Marel *et al.*, 2014). These two mechanisms induce concentration increases of dopamine and norepinephrine inside the synaptic cleft. Pharmacokinetics studies have shown differences between the two threo-methylphenidate enantiomers (Kimko *et al.*, 1999), although all forms of methylphenidate easily cross the brain-blood barrier. In therapy, only racemic mixtures of d-threo-methylphenidate and l-threo-methylphenidate are administered while erythro enantiomers are not found in any formula. Because of different metabolic stereoselective clearances, d-threo-methylphenidate becomes more potent than its enantiomer counterpart. Methylphenidate is considered a short-acting drug because of its limited half-life (between 2 to 3 hours). The duration of action can extend to 4 hours

maximum, but peak methylphenidate concentration is achieved after 2 hours (Kimko *et al.*, 1999).

Table 1: Binding properties of the 3 main ADHD drugs and other compounds.
Please refer to in-text references. DAT: dopamine reuptake transporter, NET: norepinephrine reuptake transporter, SERT: serotonin reuptake transporter, $VMAT_2$: vesicular monoamine transporter, K_{IR}: inward rectifying potassium channel, A_x receptors: adenosine receptor types, $\alpha_x\beta_y$ receptors: nicotinic acetylcholine receptor types, IC_{50}: half maximum inhibitory concentration, K_i: binding affinity, EC_{50}: half maximum excitatory concentration.

Compound	Target	IC_{50}	K_i	EC_{50}
Methylphenidate	DAT	0.19 µM	0.06 µM	
	NET	0.03 µM	0.1-0.4 µM	
D-amphetamine	$VMAT_2$	3.3 µM	2 µM	
	DAT		35 nM	
	NET		39 nM	
Atomoxetine	DAT	1451 nM	2.3 µM	
	NET	5 nM	5 nM	
	SERT	77 nM		
	K_{IR}	30-50 µM		
	$VMAT_2$		3.4 µM	
Caffeine	A_1, A_2, A_3 receptors		10-800 µM	
Dopamine	DAT		2.5 µM	
	NET		0.67 µM	
Nicotine	α_7			13.2 µM
	$\alpha_4\beta_2$			0.1-2.5 µM
	$\alpha_3\beta_4$			87 µM

Amphetamines

Amphetamines, like methylphenidate, exist in different forms. The well-known methamphetamine (“crystal meth”, “blue meth”, “meth”) is a common addictive substance in western countries like the US and Canada, but also in countries from the European Union (De-Carolis *et al.*, 2015; Lecomte *et al.*, 2014). Levo-amphetamine is used to increase wakefulness while Dextro-amphetamine (D-amph) is prescribed to treat ADHD. The Adderall® formulation contains both types of stereoisomers with a 1:3 L/D ratio (25% L-amphetamine, 75% D-amphetamine). D-

amphetamine exerts its action by firstly penetrating into the synapse through the dopamine transporter. After reaching the cytoplasm, it will block the vesicular dopamine transporter $VMAT_2$, therefore inducing vesicular neurotransmitter depletion as well as increases in intracellular dopamine concentrations (Eiden *et al.*, 2011). This process will result in strong dopamine efflux from the synapse by two distinct mechanisms. Passive dopamine diffusion will occur directly across the membrane as a result of dramatic increases in intracellular dopamine concentrations (Wallace, 2012). Dopamine will also leave the intracellular compartment through reverse dopamine transport *via* the DAT that will switch in a "reverse" mode (Sulzer *et al.*, 1995). Early onset of amphetamine treatment can induce growth deficits in some children (Faraone *et al.*, 2005; Swanson *et al.*, 2007). Amphetamine-like drugs cause neurotoxicity, either by increasing oxidative stress or through consequences of induced epileptic seizures (Berman *et al.*, 2008; Berman *et al.*, 2009b; de la Torre *et al.*, 2000; Steinkellner *et al.*, 2011).

Atomoxetine

Although atomoxetine is not a stimulant, its mechanisms of action are similar to those of MPH by inhibiting the NET (IC_{50} of 5 nM) and to a lower extent the serotonin transporter (SERT, IC_{50} of 77 nM) as well as the DAT (IC_{50} of 1451 nM, Table 1) (Bymaster *et al.*, 2002). ATX also binds to K_{IR} channels (IC_{50} 30-50 µM) and post-synaptic NMDA receptors (IC_{50} of 3.5 µM (Ludolph *et al.*, 2010)). Compared to stimulants, ATX has a longer onset of action, usually 4 to 8 weeks (Bushe *et al.*, 2014). Core symptoms of ADHD patients (hyperactivity, inattention and impulsivity) reduce gradually under ATX treatment. Following 16 weeks of ATX treatment, attention was significantly improved (greater than placebo) (Wietecha *et al.*, 2013a), so was the response inhibition following a 12-week treatment (Shang *et al.*, 2012). Following oral administration, peak plasma levels culminate after 1-2 hours (Sauer *et al.*, 2003). Daily atomoxetine doses are generally within the range of 60-120 mg/day (Simpson *et al.*, 2004). Typical ATX titration is initiated with 0.5 mg/kg on the first day and could be gradually increased up to 1.2 mg/kg on the fourth day (Ledbetter, 2006; Velcea *et al.*, 2004). Patients under 12 generally receive 40 +/- 11 mg/day while patients aged between 12-18 receive 61 +/- 19 mg/day (Bastiaens, 2007). It is important to note

that chronic ATX treatment leads to side effects in more than 10% of all adult ADHD patients (Walker *et al.*, 2015).

The new ADHD drug Metadoxine

Metadoxine was originally designed to treat alcohol intoxication (Addolorato *et al.*, 2003; Diaz Martinez *et al.*, 2002; Shpilenya *et al.*, 2002), but is now targeting other medical conditions such as fatty liver disease (Abenavoli *et al.*, 2014; Caballeria *et al.*, 1998; Stefanini *et al.*, 1999), fragile X syndrome (de Diego-Otero *et al.*, 2014; Schaefer *et al.*, 2015) and ADHD (Manor *et al.*, 2012; Manor *et al.*, 2013; Manor *et al.*, 2014). The molecular formula is composed of a pairing between an ion and vitamin B6 (Guerrini *et al.*, 2006). Because vitamin B6 is necessary for GABA synthesis (Petroff, 2002; Schousboe *et al.*, 2007), Metadoxine may play a crucial role in the regulation of GABA neurotransmission (Bono *et al.*, 1991), the dysregulation of which has been closely linked to ADHD (Bollmann *et al.*, 2015; Ende *et al.*, 2015; Nagamitsu *et al.*, 2015; Purkayastha *et al.*, 2015). Up until 2016, this new molecule is still under clinical trials for its use in ADHD. Core ADHD symptoms were found to be significantly improved following 2 weeks of Metadoxine treatment (1400 mg/day), or after a single acute administration (Manor *et al.*, 2012; Manor *et al.*, 2013; Manor *et al.*, 2014). Side effects of chronic Metadoxine intake are similar to the ones observed following atomoxetine treatment (Manor *et al.*, 2012). Prescriptions of Metadoxine could be indicated for ADHD patients with alcohol abuse (Buoli *et al.*, 2016), because of its efficiency in treating alcohol abuse (Addolorato *et al.*, 2003). In mice, acute Metadoxine exposure (starting at 150 mg/kg) leads to increased dopamine levels in the striatum after 1 hour, but peak levels are observed after 24 hours (Fornai *et al.*, 1993).

Efficiency, safety and side effects of ADHD drugs

Questions arise as to whether chronic psychostimulant treatments could produce deleterious effects on the brain, especially considering that the brain will continue to develop up to the age of 24 (Andersen, 2003). Recent studies conducted on rats have tried to identify possible side effects of such chronic treatments. One study on juvenile rats has shown that methylphenidate administration for 13 weeks leads to weaker and less mineralised bones (Komatsu *et al.*, 2012). It is important to note that, after

termination of the treatment and a recovery period of 5 weeks, all the side effects previously observed were relieved. In Humans, growth is not affected by treatment (Biederman *et al.*, 2010; Faraone *et al.*, 2008; Goldman, 2010; Harstad *et al.*, 2014). Another animal study has focused on the relationship between chronic MPH administration and cortical thickness (van der Marel *et al.*, 2014). This study fails to observe any MPH-induced cortical thinning or functional connectivity loss, but rather highlights significant age-dependent effects of psychostimulant treatment on brain characteristics such as grey and white matter volumes, striatal functional activity and cortical thickness. It will be essential to assess questions regarding the possible addictive effect of MPH on ADHD patients, in order to determine the safety of such medication. Supra-therapeutic doses of MPH, used therefore as a recreational drug, have the potential to induce addiction (Swanson *et al.*, 2003). When used at therapeutic doses, MPH seems to be a safe drug to improve ADHD symptoms in children and adults, although sporadic MPH-induced side effects have been reported such as insomnia, headaches, appetite disturbances, dyskinesia as well as dizziness (Ahmann *et al.*, 1993; Efron *et al.*, 1997; Rappley, 1997; Rodrigues *et al.*, 2008; Senecky *et al.*, 2002). Very surprisingly, a few studies have recently proven that MPH could be prescribed for cocaine-addicted ADHD patients, thus decreasing both addiction-related brain connectivity and cocaine administration, but also improving the inhibitory control of such patients (Collins *et al.*, 2006; Konova *et al.*, 2013; Li *et al.*, 2010). One clinical trial has investigated the efficiency of methylphenidate, or other dopamine uptake blockers, in reducing severe abuse-related effects of amphetamines (Howell *et al.*, 2014). This could be of great interest for preventing craving and withdrawal side effects. While it is easy to understand how MPH can attenuate the effect of D-amph by preventing its penetration into the presynaptic terminal through the dopamine/norepinephrine transporter, the beneficial effect of MPH on cocaine addiction is more difficult to understand, as they both exert DAT blockade.

From 1990 to 1998, the annual number of children diagnosed with ADHD has increased, with discrepancies among genders, ranging from 2 fold for boys to 3 fold for girls (Robison *et al.*, 2002). Unsurprisingly, ADHD drugs consumption has followed the worldwide increase in ADHD diagnoses. This is reflected, for example, in Israel, where D-amphetamine prescriptions have increased by 30% over a 7-year period (Ponizovsky *et al.*, 2014). As with MPH, D-amph has been extensively studied for its use in ADHD

patients. It has been proven that D-amph improves ADHD symptoms in adults, but can also trigger cardiovascular and psychiatric adverse effects, as well as substance abuse (Castells *et al.*, 2011). Although several studies have reported an increased risk of sudden cardiac events in patients under psychostimulant therapy (Munk *et al.*, 2015; Schelleman *et al.*, 2012; Vetter *et al.*, 2008; Winterstein *et al.*, 2009), no consequences were found on the QRS and QT intervals, even after a methylphenidate overdose (Hill *et al.*, 2010). Therefore, the risk of developing abnormal heart rhythms such as "torsades de pointes" is not increased (Hill *et al.*, 2010; Hole *et al.*, 2014; Lamberti *et al.*, 2015; Noda *et al.*, 2004). For children aged 6-12 and adults, daily treatment with amphetamine is safe and efficient in improving symptoms (Hart *et al.*, 2013; Najib, 2009; Spencer *et al.*, 2013). However, 0.25% of all patients under D-amph treatment experience strong adverse effects such as psychosis or delayed motor response (Berman *et al.*, 2009a; Ross, 2006). Nevertheless, D-amph appears today to be a safe and efficient medication for ADHD treatment.

For ADHD patients that do not respond well to the stimulants, atomoxetine may be the medication of choice. As a NET inhibitor, it increases norepinephrine concentration inside the synaptic cleft. However, recent data using the *in vivo* microdialysis technique have shown that it can also increase dopamine efflux in the PFC. Although ATX is also safe and well tolerated, the symptomatology of a subgroup of patients did not improve with medication (Schwartz *et al.*, 2014). Some patients reported adverse effects during ATX treatment with variances amongst onset (Wietecha *et al.*, 2013b), but all could be resolved with an appropriate dose schedule and titration. Some known side effects are insomnia, nausea, sweating and decreased appetite (Kolar *et al.*, 2008). As a sympathetic indirect agonist, this drug may produce undesirable cardiovascular side effects, but may have no action on the motor system and drug abuse liability. However, the onset of action is particularly long compared to that of psychostimulants, usually between 4-8 weeks (Bushe *et al.*, 2014). There is an almost complete lack of scientific evidence of abuse potential for ATX (Upadhyaya *et al.*, 2013). Only one study (Lile *et al.*, 2006) reported a partial effect of ATX on discriminative stimulus in subjects with recent histories of stimulant use. On the other hand, some studies found that ATX remains well tolerated, safe and efficient (Garnock-Jones *et al.*, 2009; Michelson *et al.*, 2001a). One study reported that ATX, ingested in a

suicide attempt at a dose of nearly 3 grams, induced widening of the QRS interval as well as a tonic-clonic seizure, but not death (Kashani *et al.*, 2007).

Overall, the three ADHD drugs MPH, D-amph and ATX are considered as efficient in treating ADHD symptoms and appear safe for use, even during pregnancy (Bro *et al.*, 2015). However, it is wise to remember that some adverse effects have been reported, justifying the need for close monitoring of at-risk patients.

Methylphenidate as a drug of abuse, misuse and addiction

Growing concerns have arisen regarding methylphenidate non-medical diversions (Sembower *et al.*, 2013). As a psychostimulant drug, it can be used for cognitive enhancement by some users and an estimated proportion of 6-8% of all adult students used such cognitive enhancement (Teter *et al.*, 2006). This off-label use is often found in students wanting to improve academic performance (Beyer *et al.*, 2014; Outram, 2010), although methylphenidate is not the only cognitive enhancer in use (Micoulaud-Franchi *et al.*, 2014).

Toxic exposure to methylphenidate is often caused by drug misuse (Jensen *et al.*, 2014). Intentional misuse remains very low whereas intentional overexposure to methylphenidate is often found as a result of suicide attempts (Jensen *et al.*, 2014; Zosel *et al.*, 2013). Due to the nature of the drug and its physiological effects, death after methylphenidate overdose or misuse has never been reported (Zosel *et al.*, 2013).

Addiction to methylphenidate, also called dependence, remains rare (Ozaki *et al.*, 2006). Paradoxically, methylphenidate seems to be efficient in decreasing amphetamine cravings in amphetamine-dependent patients (Solhi *et al.*, 2014; Tiihonen *et al.*, 2007), although this is disputed (Konstenius *et al.*, 2010; Miles *et al.*, 2013; Tiihonen *et al.*, 2007).

Pharmacology of ADHD drugs in animal studies

In animal studies, two brain regions have been particularly scrutinised for their involvement in physiological processes that are fundamental in ADHD symptoms. The first region of interest is the Prefrontal Cortex (PFC), where the brain processes information regarding social behaviour, cognition and executive functions. As a matter of fact, the PFC is responsible for executive functions such as mentation, environment representation and semantic processing (Goldman-Rakic, 1996). Short-term and long-term memory also originate within the PFC (Fletcher *et al.*, 1998; Shimamura *et al.*, 1990). Another major function of the PFC is composed of both the filtering of information and the inhibition of irrelevant activities (Chao *et al.*, 1998; Shimamura, 2000). Finally, the PFC also processes all the sensorimotor information, including language and self awareness (Clemo *et al.*, 2012; Kayser *et al.*, 2012b; Laurienti *et al.*, 2003; Siddiqui *et al.*, 2008; Stephan *et al.*, 2002). Psychostimulants enhance PFC activities, as observed recently (Berridge *et al.*, 2011; Gronier, 2011). The PFC is characterised by dense cellular populations of glutamatergic pyramidal neurons and γ-amino-butyric acid (GABA) interneurons. Pyramidal neurons are modulated by several neurotransmitters, such as dopamine, serotonin, GABA and norepinephrine (Steketee, 2003). The second region of interest is the Ventral Tegmental Area (VTA). This region is involved in reward processes, motivation as well as addiction (Adinoff, 2004) and so is extensively studied in rodents. A functional connectivity exists between the VTA and the PFC. Although these two regions are physically separated, they are able to maintain a physiological connectivity, thus allowing permanent communication between the two structures. This is achieved by axonal projections from the VTA dopaminergic neurons onto PFC pyramidal neurons and interneurons. Thereby, the VTA exerts control over the PFC, through complex mechanisms (Steketee, 2003). In parallel, PFC glutamatergic neurons also project onto VTA dopaminergic neurons *in vivo*, proving a two-way communication between the two structures (Kauer *et al.*, 2007; Lewis *et al.*, 2000). The PFC directly controls burst activity of midbrain dopamine neurons, mainly through descending pathways (Floresco *et al.*, 2003; Gariano *et al.*, 1988; Kalivas, 1993; Murase *et al.*, 1993; Overton *et al.*, 1997; White, 1996). Therefore, glutamate plays a major role in inducing burst-like electrical discharges (Charlety *et al.*, 1991; Chergui *et al.*, 1993; Geisler *et al.*, 2008; Johnson *et al.*, 1992), which will be discussed later on. Within the VTA, dopamine neurons represent 55-60% of all neurons, while GABAergic neurons

represent around 20% of all neurons (Kim *et al.*, 2015). Interestingly, midbrain dopamine neurons can enter a GABA synthesising pathway through activation of an evolutionary alternative using the enzyme aldehyde dehydrogenase 1a1 (Kim *et al.*, 2015). The remaining 20% of all neurons are composed of glutamate neurons (Nair-Roberts *et al.*, 2008; Yamaguchi *et al.*, 2007). Subchronic D-amphetamine exposure (5 mg/kg for 5 days, intraperitoneal) was found to alter midbrain GABAergic neurotransmission in rats (Giorgetti *et al.*, 2002).

Pharmacology studies have been performed in the PFC and the VTA, using MPH, ATX, D-amph or other psychostimulants.

In the PFC, methylphenidate dose-dependently enhances signal processing, underlying the therapeutic effects of MPH for social behaviour and cognition (Devilbiss *et al.*, 2008). Indeed, 0.5 mg/kg of acute MPH improved working memory, while 2 mg/kg impaired performance. Such behavioural effects were also positively correlated to prefrontal cortex neuronal activities, where low doses of MPH (0.25-2 mg/kg) enhanced electrical activities while higher doses (15 mg/kg) reduced spontaneous discharges (Devilbiss *et al.*, 2008). Today, it is generally admitted that the mechanism of action of MPH within the PFC is triggered through dopamine D_1 and adrenergic $\alpha 2$ receptor modulations (Gronier, 2011). However, acute administration of such a stimulant (2.5 and 10 mg/kg) to rodents lead to behaviour sensitisation, described here as increased stereotypic movements as well as an increase in horizontal activity (Salek *et al.*, 2012; Yang *et al.*, 2007). Though dopamine in the prefrontal cortex is known to control a broad spectrum of brain activities, dopamine innervations are relatively sparse in the prefrontal cortex, compared to other monoamines innervations (Devoto *et al.*, 2006) such as norepinephrine. Because dopamine has a higher affinity for the NET at norepinephrine terminals than for the dopamine transporter itself, it is believed that a significant part of the dopamine released in prefrontal areas originates from norepinephrine terminals (Devoto *et al.*, 2006; Horn, 1973). Evidence for this hypothesis includes experiments in dopamine transporter knockout mice, where dopamine efflux is stimulated in the PFC by cocaine and norepinephrine uptake blockers (Moron *et al.*, 2002). Prefrontal dopamine, at the adequate concentration range, is thought to play a major positive role in cognition, attention and working memory, mainly *via* stimulation of dopamine D_1 receptors (Floresco, 2013). Activation of

dopamine D_1 receptors in the prefrontal cortex initially suppress inhibitory post-synaptic currents (IPSCs), which is followed by facilitation of such IPSCs (Paul *et al.*, 2013). Such biphasic effects are also observed following amphetamine exposure. As a matter of fact, acute D-amphetamine (0.01-3 mg/kg) exposure facilitates long-term potentiation (LTP) in pyramidal neurons of the PFC *via* D_1, but not D_2, receptor stimulation, while 10 mg/kg impairs LTP (Xu *et al.*, 2010).

In the VTA, methylphenidate modulates dopamine neurotransmission by decreasing dopaminergic neuron activities through dopamine D_2 receptor stimulation *in vivo*. The electrophysiological effects of psychostimulants all seem to trigger decreases in VTA dopaminergic neurons activity, as observed with MPH and cocaine (Bunney *et al.*, 2001), but can also lead to the uncoupling of D_2/D_3 midbrain receptors from $G\alpha_i2$ proteins when amphetamine is applied, while leaving striatal D_2/D_3 receptors unchanged (Calipari *et al.*, 2014). This is of great importance considering that receptor alterations can lead to synaptic reorganisation and altered brain plasticity.

In the dopaminergic synapse, D-amphetamine is known to cause dopamine release by interacting mainly with the membrane dopamine transporter and with the vesicular dopamine uptake system (VMAT), causing vesicular dopamine depletion and increases in cytoplasmic dopamine, as already mentioned. In return, these increases promote reverse transport of dopamine through the DAT (Robertson *et al.*, 2009) but may also increase extracellular dopamine levels by other mechanisms, such as promoting rapid internalisation of dopamine carrier (El-Kabbani *et al.*, 2004) or "passive membrane diffusion" (Wallace *et al.*, 2008), although this passive dopamine diffusion has only been demonstrated in a computational model. Passive diffusion of relatively small and neutrally-charged hydrophobic molecules can occur directly across the phospholipidic bi-layer (Bergstrom *et al.*, 2003; Camenisch *et al.*, 1998; Cooper, 2000). However, no direct *in vivo* evidence of such a process has yet been observed. In the PFC, D-amph has a strong impact on synapse plasticity, inducing increases in the presynaptic membrane area, spine density and postsynaptic membrane area, but also inducing a reduced synaptic vesicle density (Uranova *et al.*, 1989). The drug also induces behavioural sensitisation, defined here as an enhancement of the behavioural activating effects of the drug when repeated injections are performed, as well as a long-lasting hypersensitivity to later environmental or pharmacological challenges (Cador *et*

al., 1999). The study by Cador also shows that when an NMDA receptor antagonist was co-administered with D-amph within the VTA, behavioural sensitization was abolished, underlying the involvement of the glutamatergic neurotransmission in inducing sensitization, as well as PFC-VTA innervations (Cador *et al.*, 1999).

Pharmacologically, systemic administrations of ATX (3 mg/kg) led to increases in prefrontal norepinephrine and dopamine levels, but not serotonin (Ago *et al.*, 2014). This study underlines the monoamine system as a target for ATX. The drug also targets the NMDA (N-methyl-D-aspartate) neurotransmission in the striatum, as revealed by a marked reduction of the NMDA receptor 1 mRNA after 2 months of ATX treatment, as well as an increased $NMDA_{2B}$ receptor expression (Udvardi *et al.*, 2013). These transcriptions/translations of the NMDA receptor and the norepinephrine transporter in the rat brain might contribute to ATX's clinical effects in the treatment of ADHD, in which synaptic processes and especially a dysregulated glutamatergic metabolism seem to be involved. Another *in vitro* study has shown that 3 µM of ATX could block NMDA-induced membrane currents in a voltage-dependent manner, suggesting an open-channel blocking mechanism by ATX (Ludolph *et al.*, 2010). Surprinsingly, the bioavailability of ATX in the rat is only around 4% when absorbed through the gastro-intestinal tract, probably due to their powerful first-pass metabolism capacities (Mattiuz *et al.*, 2003).

ADHD patients have difficulties in controlling impulsive behaviour (Connor *et al.*, 2010; Ende *et al.*, 2016; Raiker *et al.*, 2012; Urcelay *et al.*, 2012). As a matter of fact, clinical diagnosis of ADHD accounts for impulsive choices (Neef *et al.*, 2005), a trait arising from impaired control inhibition in patients (Schachar *et al.*, 1995). In rodents, different experiments can be used to determine impulsivity. Indeed, rodents can be submitted to a delayed reward task, whereby either a large food reward is given following visual clues or an immediate but small food reward is given following a different visual clue (Schippers *et al.*, 2016; van Gaalen *et al.*, 2006). In this model, impulsivity is assessed as enhanced responses for an immediate but small reward (impulsive) compared to the delayed large reward (non-impulsive). Other experiments used the 5-choice serial reaction task. In this protocol, rodents are trained to perform correct nose poke in one (out of five) previously-illuminated holes following a waiting period. Here, impulsivity corresponds to the amount of premature nose pokes observed

during the waiting period (Pillidge *et al.*, 2016; Young *et al.*, 2004; Young *et al.*, 2009). Another method of assessing impulsivity is by using the go/no-go task. Such a protocol can be applied to rodents and humans (Anker *et al.*, 2008; Bezdjian *et al.*, 2009) and consists of only responding to a precise visual clue ("go") whilst refraining responses ("no-go") to other clues (Gomez *et al.*, 2007). Some studies have shown the impact of psychostimulants on impulsivity. Indeed, D-amphetamine induces impulsivity in control rats but not in already impulsive spontaneously hypertensive animals (Adriani *et al.*, 2003b; Furlong *et al.*, 2016; Hand *et al.*, 2009). Moreover, such an effect is dose dependent at regimens between 0.3 and 1 mg/kg (Paterson *et al.*, 2011). In socially isolated rats, both D-amphetamine (2 mg/kg) and methylphenidate (2.5 mg/kg) decreased impulsivity, while only D-amphetamine increased impulsivity in rats housed socially (Perry *et al.*, 2008). However, one study found decreased impulsivity following intraperitoneal D-amphetamine (Winstanley *et al.*, 2003). Another study has shown that acute methylphenidate (5.6-10 mg/kg) can increase impulsivity in control rats (Wistar-Kyoto) but not in SHR rats (Wooters *et al.*, 2011). Moreover, a low dose of methylphenidate (0.1 mg/kg) can decrease both impulsivity and premature responding (Puumala *et al.*, 1996) while a higher dose (1 mg/kg) fails to do so (Puumala *et al.*, 1996). However, the validity of these models to mimic impulsivity remains arguable, as they may not always accurately reflect human symptomatology (Sontag *et al.*, 2010). In rodents and humans, impulsivity involves the hippocampus and the prefrontal cortex (Cummings, 1993; Kayser *et al.*, 2012a; Kheramin *et al.*, 2002). Finally, D-amphetamine increases impulsivity in healthy volunteers, but not in ADHD patients (Seo *et al.*, 2008; Sostek *et al.*, 1980).

Freely available psychostimulants

Caffeine is one of the few freely available stimulants. It belongs to the Xanthine family and possesses psychoactive effects (Nehlig *et al.*, 1992). Caffeine exerts a blockade of every adenosine receptor (A_1, A_2 and A_3 receptors). In humans, caffeine consumption leads to increased locomotor activity as well as enhanced vigilance but also decreases fatigue (Nehlig *et al.*, 1992). Interestingly, one study reported that supplementation of methylphenidate (10 mg) with a low dose of caffeine, produced a stronger attenuation of behavioural symptoms in children, compared to methylphenidate alone (Garfinkel *et al.*, 1981). In young adults, caffeine use can modulate ADHD

symptoms (Broderick *et al.*, 2004), but a recent study observed that caffeine is only associated with ADHD symptomatology if caffeine is taken in combination with nicotine (Dosh *et al.*, 2010). In young adolescents, one study reported a clear association between high caffeine consumption (4 or more beverages per day) and ADHD (Martin *et al.*, 2008).

In young rats, early caffeine consumption leads to an increased locomotor activity after methylphenidate administration in adulthood, proving a cross-sensitisation (Boeck *et al.*, 2009). However, some studies reported the beneficial effects of caffeine on ADHD symptoms. In an animal model of ADHD (juvenile administration of the dopamine and norepinephrine neurotoxin 6-hydroxy-dopamine), a 14-day chronic caffeine treatment improved spatial attention (Caballero *et al.*, 2011), providing potential evidence for the use of caffeine as an adjuvant to psychostimulants. Another study on adolescent spontaneous hypertensive rats (another animal model of ADHD) revealed that chronic caffeine (2 mg/kg, twice a day) for 21 days can normalise ADHD traits (Pandolfo *et al.*, 2013).

The other freely available psychostimulant is nicotine. Nicotine is a potent agonist of nicotine acetylcholine receptors. Extracted from tobacco plants, this chemical also belongs to the alkaloid family. Although the legal age limit for smoking in the UK is 18 years old, many abuses have been recorded. In fact, a recent European study found that 17.3% of adolescents smoke regularly every week in the UK (Pfortner *et al.*, 2015). Current literature on nicotine and ADHD remains contradictory. While some clinical studies report a positive association between nicotine consumption and ADHD medication (Bron *et al.*, 2013; Symmes *et al.*, 2015), others fail to find any correlation in rats (Justo *et al.*, 2010). A Dutch study reported that smoking amongst ADHD patients is twice the national average and that methylphenidate use increases tobacco consumption (Bron *et al.*, 2013). Acute methylphenidate is also responsible for increases in nicotine consumption in ADHD patients (Vansickel *et al.*, 2011) and non-ADHD patients (Rush *et al.*, 2005). However, few studies have suggested that methylphenidate could be an efficient medication for reducing nicotine intake in ADHD patients (Covey *et al.*, 2010; Gehricke *et al.*, 2011; Hammerness *et al.*, 2013), but one study proved its inefficacy (Hurt *et al.*, 2011). These contradictory findings remain ambiguous and need to be further examined. While caffeine cannot be purely

considered as a cognitive enhancer (Lienert *et al.*, 1966; Nehlig, 2010), nicotine has been proven efficient in enhancing cognition, in both rodents and humans (Griesar *et al.*, 2002; Semenova *et al.*, 2007; Warburton, 1992; Wignall *et al.*, 2011; Young *et al.*, 2004), as well as in young adults with ADHD (Potter *et al.*, 2008).

A link between ADHD and substance abuse?

Substance abuse can also be found as a comorbidity in adult ADHD (Mao *et al.*, 2014) and may be explained by the fact that it gives patients a sense of feeling normal, therefore accepted by society (Nehlin *et al.*, 2014), a feeling that ADHD children have lost (Mueller *et al.*, 2012; Whalen *et al.*, 1981). Adult ADHD patients have also a higher risk of nicotine addiction (Ginsberg *et al.*, 2014). Recent studies on adult ADHD patients have revealed the need to understand substance addiction in ADHD in an attempt to improve the difficult diagnosis in these patients as well as avoiding inadequate treatment (Crunelle *et al.*, 2013; Matthys *et al.*, 2014). Moreover, cocaine-dependent patients diagnosed with ADHD are more severely addicted to cocaine than cocaine-dependent users without ADHD (Daigre *et al.*, 2013).

The relative risk of substance abuse among 208 ADHD adolescents treated with stimulants has been assessed in a recent Danish study (Dalsgaard *et al.*, 2014). Here, the authors found that adolescent ADHD patients, compared to the background population, have a relatively higher risk of substance abuse than the general population as well as alcohol abuse. This study has also shown discrepancies in gender for later-life substance use disorder, females being the most susceptible. Four populations are therefore of interest: ADHD adolescents currently under medication, untreated ADHD adolescents, ADHD adults previously treated during their adolescence and, finally, ADHD adults that never received medication. However, current literature lacks data concerning long-term effects of ADHD medication during adolescence or childhood and substance abuse during later life.

Animal models of ADHD

Different animal models are currently available to mimic ADHD in rodents:

1- The spontaneous hypertensive rat (SHR). This model has been validated to behaviourally and biologically mimic ADHD symptoms (Adriani *et al.*, 2003a; Meneses *et al.*, 2011; Sagvolden, 2000; Sagvolden *et al.*, 2009; Vendruscolo *et al.*, 2009).

2- The 6-hydroxydopamine lesioned rat. Postnatal intracerebroventricular administration of 6-hydroxydopamine (6-OHDA) induces massive destruction of dopaminergic neurons in the substantia nigra, but has less destructive effects on dopamine neurons located in the ventral tegmental area and the prefrontal cortex (Kostrzewa *et al.*, 2015). The loss of dopamine neurons functionally mimics ADHD and Parkinson's disease (Caballero *et al.*, 2011; Kostrzewa *et al.*, 1994; Masuo *et al.*, 2012; Moran-Gates *et al.*, 2005; Russell, 2011; Thiele *et al.*, 2012).

3- In mice, genetic manipulations have led to dopamine transporter knockout mice ($DAT^{-/-}$). Indeed, mice that do not express the dopamine reuptake transporter are used as models for ADHD (Leo *et al.*, 2013; Russell, 2011; Takamatsu *et al.*, 2015; Yamashita *et al.*, 2013).

4- Other models include the Naples high-excitability rat (NHE), the hypo-sexual rat, the Wistar-Kyoto hyperactive rat (WKHA), the polychlorinated biphenyl exposed rat (PCB), the acallosal mouse (corpus callosum agenesis), the lead-exposed mouse and rats reared in social isolation (Davids *et al.*, 2003; Eubig *et al.*, 2010; Johansen *et al.*, 2014; Magara *et al.*, 2000; Russell *et al.*, 2005; Sagvolden *et al.*, 2005).

While these models have proven truly beneficial in the understanding of ADHD, one should not forget that they do not always reflect the exact neurobiology observed in ADHD patients (Alsop, 2007; Sontag *et al.*, 2010). Therefore, such models are limited in precisely mimicking ADHD symptoms. Finally, concerning the SHR strain, the hypertensive aspect can make handling difficult and drug administration inaccurate. Besides, spontaneous mortality in this particular strain occurs frequently, due to cerebral haemorrhage (Dupont *et al.*, 1975).

Objectives

Pharmacotherapies for ADHD involve either methylphenidate, D-amphetamine, atomoxetine or a combination of these drugs. While both stimulants (MPH and D-amph) preferentially target dopamine transporters, the non-stimulant ATX potently targets both norepinephrine and dopamine transporters. However, the exact mechanisms underlying the efficiency of such pharmacotherapies are not yet completely understood. Glutamate neurotransmission appears to be altered in patients suffering from ADHD (Carrey *et al.*, 2002; Grados *et al.*, 2015; MacMaster *et al.*, 2003; Moore *et al.*, 2006), but some results have linked ADHD drugs to improved glutamatergic functions.

Here, using radiolabelled neurotransmitters, we will compare the *in vitro* effects of atomoxetine, D-amphetamine and methylphenidate on prefrontal cortex (PFC) and striatal slices in rats. We will also determine the consequences of these drugs on the regulation of the *in vivo* electrical activities of PFC pyramidal neurons, striatal medium spiny neurons, ventral tegmental area dopamine neurons and dorsal raphe nucleus serotonin neurons, using single cell extracellular electrophysiology. We will also test whether chronic methylphenidate treatment, during either adolescence or adulthood, could induce long-lasting effects on body growth, neuronal functions and drug cross-sensitisation. Moreover, depression-like behaviour in animals previously exposed to methylphenidate will be examined as well as sensitivity to D-amphetamine. Finally, we will assess if pre-treatments with either nicotine or caffeine can modulate neuronal responses to methylphenidate.

Chapter I – ADHD drugs induce *in vitro* neurotransmitter release.

I-1- Introduction

The mechanism by which ADHD drugs exert their therapeutic effects, particularly on attention and cognition processes, is not well understood. The paradoxical effect of ADHD drugs resides in both their abilities to calm hyperactivity while also improving cognition (De Sousa *et al.*, 2012). Although apparently safe to use, ADHD drugs require adequate dosing to avoid negative consequences (Spiller *et al.*, 2013; Vitiello, 2008; Wigal, 2009). The usefulness of such medication for ADHD patients is unquestionable, providing enhancement of both cognition and attention (Swanson *et al.*, 2011) as well as improvements of some of the ADHD symptoms. Many drugs are available to treat ADHD, such as methylphenidate, amphetamine salts, atomoxetine, bupropion, clonidine and reboxetine, although not all have received approval from the Food and Drug Administration (De Sousa *et al.*, 2012). However, the latter three are rarely given to patients. Both MPH and D-amph have immediate effects on ADHD symptoms, whereas ATX has a longer onset of action, usually between 4 to 8 weeks (Bushe *et al.*, 2014; Kolar *et al.*, 2008).

D-amphetamine and methylphenidate are strong inhibitors of the synaptic reuptake of both dopamine and norepinephrine. MPH potently inhibits the dopamine reuptake transporter (K_i = 34 nM) as well as the norepinephrine reuptake transporter (K_i = 339 nM) (Bymaster *et al.*, 2002). Other effects of D-amph include the inhibition of monoamine oxidase and blockade of vesicular transport of catecholamines (K_i = 2 µM, Table 1) (Erickson *et al.*, 1996; Heal *et al.*, 2009). On the other hand, atomoxetine interacts very selectively with the norepinephrine transporter, with a K_i of 5 nM (Table 1) (Bymaster *et al.*, 2002). It is believed that the therapeutic effects of these drugs are associated with their abilities to stimulate dopamine release in the prefrontal cortex (PFC) (dela Pena *et al.*, 2015; Kalivas, 2007). The PFC is considered as one of the main brain regions involved in the behavioural-calming and cognition-enhancing effects of

ADHD drugs (Gamo *et al.*, 2010). It plays a critical role in the control of higher cognitive function such as vigilance, attention, impulsivity and behavioural inhibition (Kieling *et al.*, 2008). As a matter of fact, lesions within the orbitofrontal areas of the prefrontal cortex lead to altered social perception in humans while having anxiolytic effects and increasing impulsiveness in rodents (Deacon *et al.*, 2003; Mah *et al.*, 2004; Schneider *et al.*, 2005). According to microdialysis studies, MPH and ATX, at therapeutic doses (1-3 mg/kg), increase dopamine release preferentially in prefrontal areas, with little or no effect in basal ganglia (Koda *et al.*, 2010). Prefrontal dopamine, at the adequate concentration range, is thought to play a major positive role in cognition, attention and working memory, mainly through stimulation of dopamine D_1 receptors (Floresco, 2013). Nevertheless, dopamine innervations are relatively sparse in the PFC (Devoto *et al.*, 2006). Because dopamine has an affinity for the NET (K_i = 0.67 µM, Table 1) (Giros *et al.*, 1994), it is believed that a significant part of the dopamine released in prefrontal areas is cleared by (or even originates from) norepinephrine terminals (Devoto *et al.*, 2006; Moron *et al.*, 2002). Moreover, dopamine and norepinephrine can be simultaneously co-released in specific noradrenergic terminals (Devoto *et al.*, 2001) while dopamine can even be reuptaken by the norepinephrine reuptake transporter (Moron *et al.*, 2002). In contrast, in the striatum, dopamine innervations are dense (Matsuda *et al.*, 2009). Besides, the striatum is believed to play an important role in ADHD, as increased striatal dopamine transporters and low striatal activity have both been observed in adult ADHD patients (Fusar-Poli *et al.*, 2012; Lou *et al.*, 1989), two effects alleviated by MPH pharmacotherapy (Krause *et al.*, 2000). Moreover, ADHD patients present altered cortico-striatal functional connectivity (Hong *et al.*, 2015), a characteristic that was also observed in rodents following early postnatal dopamine lesions (Braz *et al.*, 2015).

In the present chapter, the *in vitro* neurotransmitter release experiments aim to compare the effects of the main ADHD drugs on dopamine and norepinephrine neurotransmissions, in an attempt to characterise a possible common mechanism by which ADHD drugs alter monoamine neurotransmission. Here, we compare the effects of ADHD drugs on ^{3}H-dopamine and ^{3}H-norepinephrine efflux from rat PFC and striatal slices.

I-2- Material and Method

I-2-A- Subjects

Male Sprague-Dawley rats were purchased from Charles River, UK. Animals were housed in groups of 2-4 animals per cage, maintained at 20-22^{o}C with humidity rates above 40% under a 12:12 L/D cycle with lights ON at 07h00 AM (*Ante Meridiem*). Animals were kept in polypropylene cages measuring 56x38x17 cm. Food and water were provided *ad libitum*. Animals were allowed a 3-day acclimatisation period after delivery. All experiments were performed during the light phase and with permission from the UK Home Office and De Montfort University Ethics Committee under the Project Licence 60/4333 and with the Personal Licence 60/13750.

I-2-B- *In vitro* radio-labelled neurotransmitter efflux

Seventy-one naïve rats weighing between 150 and 250 grams were sacrificed by cervical dislocation. The brain was quickly dissected out and immersed into ice-cold oxygenated Krebs buffer (NaCl 125 mM, $MgSO_4$ 1.2 mM, KCl 2.5 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 10 mM and pargyline 10 µM, an inhibitor of monoamine catabolism, pH 7.4). The brain was then placed on an ice-cold platform for further dissection of either the prefrontal cortex (PFC, Fig. 1A) or the striatum (Fig. 1B). The tissue was then sliced into 350x350 µm prisms using a McIlwain tissue chopper. Constant oxygenation was maintained after this step. Prisms were then left for 20 min to rest at room temperature. Tissue prisms were then loaded for 40 minutes at 37^{o}C with either ^{3}H-dopamine (1.0 µCi/ml, specific activity 28.7 Ci/mmol) or ^{3}H-norepinephrine (1.0 µCi/ml, specific activity 12-15 Ci/mmol; Perkin-Elmer USA) in the presence or absence of desipramine 10 µM to inhibit norepinephrine uptake. Once the loading completed, the prisms were then washed 3 times with fresh Krebs buffer before being divided into 6 even portions and loaded into 6 perfusion chambers. Throughout the experiment, all prisms and superfusion Krebs buffers were maintained at 37^{o}C and under constant oxygenation. An equilibrating period of 40 minutes was initiated by superfusion of the chambers with Krebs buffer at 0.6 ml/min. In order to determine baseline outflow of dopamine, 3-4 samples were collected per chamber at intervals of 4 minutes. Sample were collected into vials and each sample

would hold 2.4 ml of perfusion liquid, to which scintillation liquid was added up to a total volume of 7 ml per vial. At the end of the experiment, all tissues were collected and dissolved with 1 ml of tissue solubiliser. Total tritium (^3H) quantities were measured in a liquid scintillation counter (Hidex, UK), from which disintegrations per minute were extracted. If necessary, the composition of the superfused Krebs buffer was altered. The low Na^+ Krebs buffer consisted of a 20 mEq of NaCl (instead of 125 mEq) substituted by isotonic concentration of choline chloride. The Ca^{2+}-free Krebs buffer was made by omitting the $CaCl_2$ in the buffer ($CaCl_2$ 0 mM). In addition to the calcium-free Krebs buffer, a further intracellular calcium chelation was achieved by adding 50-100 µM of BAPTA-AM, a cell-permeable calcium chelator, to the Ca^{2+}-free Krebs buffer. A depolarisating buffer was also tested by increasing KCl concentration from 2.5 to 20 mM. Superfusion of such potassium-rich buffer is known to induce sudden membrane depolarisation and neurotransmitter release (Ayata *et al.*, 2000; Chen *et al.*, 1996; Khvotchev *et al.*, 2000; Nagai *et al.*, 1998; Okuma *et al.*, 1986; Zhao *et al.*, 2001).

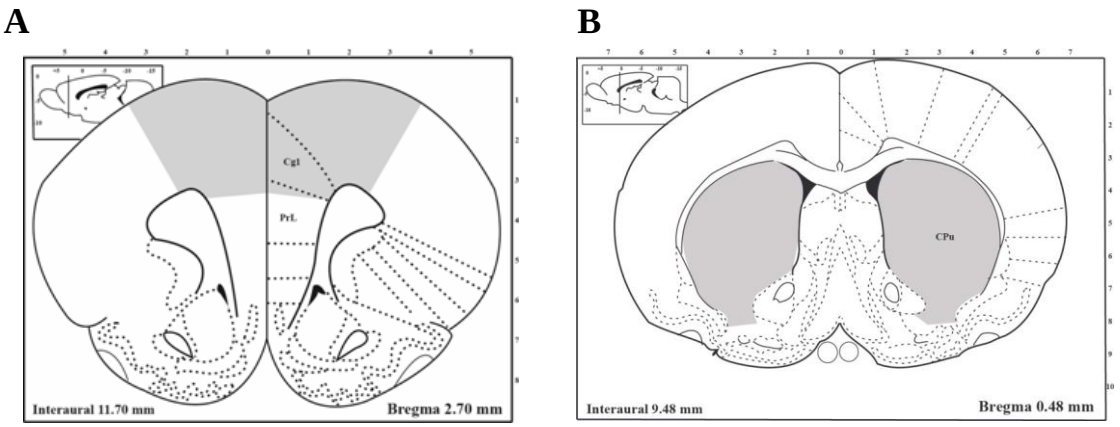


Figure 1: Regions of interest for *in vitro* neurotransmitter efflux experiments.
Prefrontal cortex (**A**) and striatum (**B**) locations. Shaded areas correspond to regions of interest for *in vitro* neurotransmitter releases. Scales represent distances (in mm) from the midline and the surface of the brain. Coronal slices adapted from Paxinos and Watson (1997). Cg1 cingulate cortex area, PrL prelimbic cortex, CPu caudate putamen.

I-2-C- Drugs

All drugs were purchased from Sigma (Sigma-Aldrich, UK) except for BAPTA-AM (Abcam, UK), atomoxetine and reboxetine (Sequoia Research, UK) as well as SCH23390 (Tocris, UK). The drugs were dissolved into normal or modified Krebs buffers, as appropriate, except for BAPTA-AM and desipramine that were firstly dissolved into DMSO (dimethyl-sulfoxide, 20%) as stock solutions before being diluted back into the appropriate buffers. The final concentration of DMSO in the buffer was below 0.02% (v/v).

I-2-D- Data analysis

All data are expressed as the mean±standard error of the mean (S.E.M.). Statistical analyses were performed using paired or unpaired Student's t-tests or one/two-way analysis of variance (ANOVA), followed by appropriate post-hoc Neuman–Keuls (one-way ANOVA) or Bonferroni tests (two-way ANOVA). The mixed model two-way ANOVA was used when sampling size varied across groups. Probabilities smaller than 0.05 were considered to be significant; n values refer to the number of samples used. Fractional efflux for each superfusate sample was calculated by dividing the amount of tritium in each sample by the total tritium left thereafter. The effect of a tested condition was assessed on at least 3 subsequent sample collections and averaged. Normalised efflux values are calculated for each chamber as the ratio between the mean tested values (generally from at least 3 collections) and average baseline values (usually 3-4 collections). All of the experiments presented were repeated on at least 3 animals. Fig. 2 shows an example of a typical experiment testing the effect of ATX on ^{3}H-dopamine efflux from striatal prisms. In this chapter, a distinction between dopamine release and dopamine efflux/outflow was made. Indeed, dopamine release, arising from exocytosis, only occurs under *in vivo* or artificially-stimulated conditions (perfusions of KCl or D-amphetamine), while dopamine efflux/outflow occurs when prisms are not under stimulated conditions (Beani *et al.*, 1984; Kahlig *et al.*, 2005).

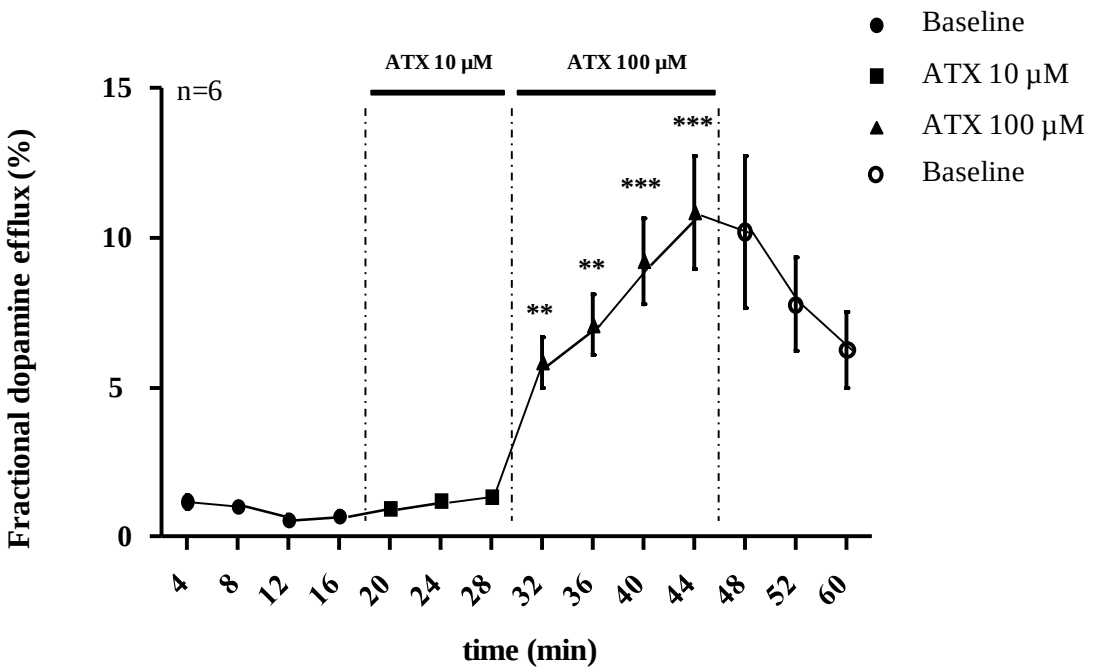


Figure 2: Time course example of a typical ^{3}H-dopamine efflux experiment in the striatum.

Atomoxetine was superfused at 10 and 100 µM. Data shows the average fractional efflux from 6 perfusion chambers over time. In this and the following figures, n values indicate the number of tissue samples which have been tested (from which an average fractional efflux is calculated from 3-4 subsequent collections). **P<0.01, ***P<0.001 *vs.* baseline, Neuman-Keuls after significant repeated measures ANOVA.

I-3- Results

I-3-A- ADHD drugs induce dopamine efflux

I-3-A-1- In the prefrontal cortex

In the prefrontal cortex (PFC), the application of methylphenidate (100 µM), atomoxetine (100 µM) and D-amphetamine (10 µM) significantly increased dopamine efflux (respectively 52%, 300% and 84% over baseline, Fig. 3A). Interestingly, ATX elicited dopamine efflux in the frontal cortex in a dose-dependent manner. Indeed, rising concentrations of ATX, from 10 to 100 µM, all induced significant ($p<0.001$) dopamine efflux (Fig. 3B). Perfusion of ATX at 100 µM produced a considerable dopamine efflux, but may here trigger other mechanisms to explain such a high outflow. At this concentration of 100 µM, the NET blocker ATX elicits a massive release of efflux, increasing baseline levels by 400%. All drugs, except the very selective DAT inhibitor GBR-12909, promoted ^{3}H-dopamine efflux when perfused at 100 µM, including the NET inhibitors desipramine and reboxetine (Fig. 3C). At 100 µM atomoxetine induced dopamine efflux at a significantly greater level (post-hoc test $F_{(2,182)}=11.66$, $p<0.001$) than 100 µM of MPH (Fig. 3A), an effect not observed at 10 µM. Methylphenidate significantly induced dose-dependent dopamine efflux (Fig. 3D).

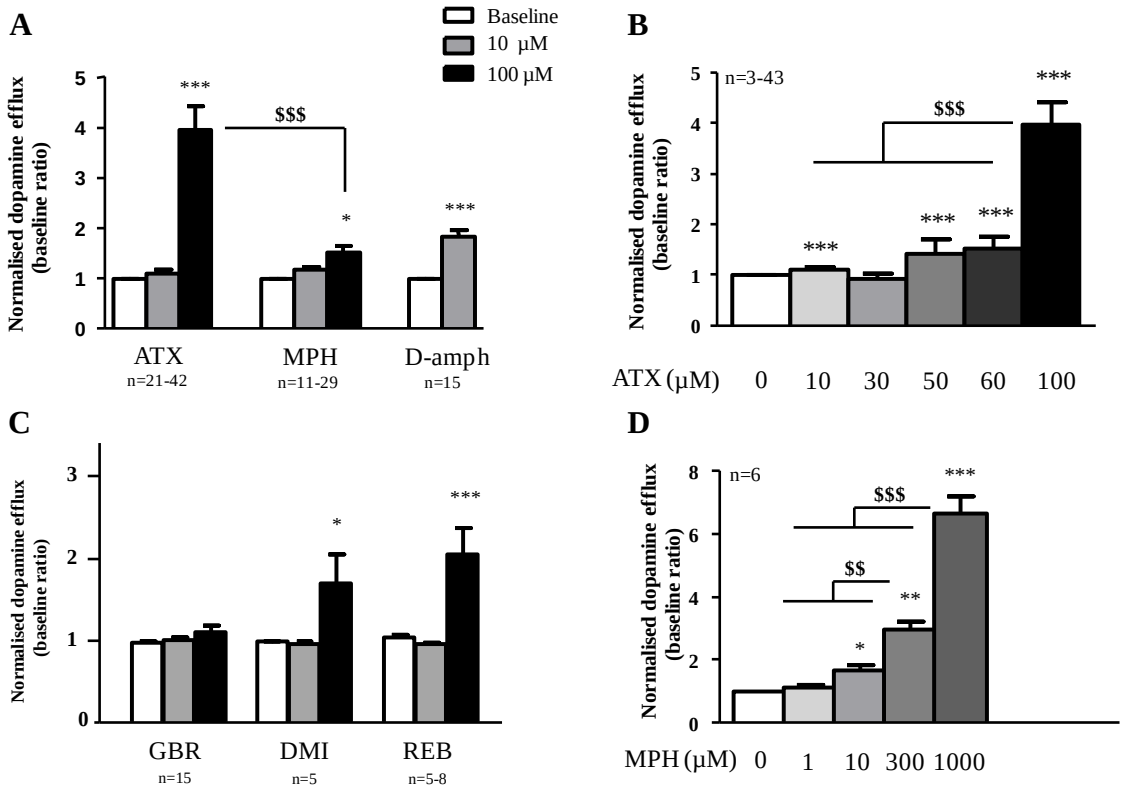


Figure 3: Dose-response of ADHD drugs and other selective uptake inhibitors on prefrontal cortex ^{3}H-dopamine efflux.
(**A**): Superfusion of ATX, MPH or D-amph (100 µM) all significantly induced dopamine efflux with different level of efficacy. (**B**): ATX induces dopamine efflux in a dose-dependent manner. (**C**): Effects of other selective reuptake inhibitors on prefrontal dopamine release. Interestingly, desipramine (DMI) and reboxetine (REB) both significantly induced dopamine efflux when applied at 100 µM, while the selective DAT inhibitor GBR-12909 (10 and 100 µM) did not induce dopamine efflux. (**D**): MPH promotes dopamine efflux in a dose-dependent manner from 10 µM to 1 mM. MPH: methylphenidate, ATX: Atomoxetine, D-amph: D-amphetamine, DMI: desipramine, REB: reboxetine, GBR: GBR-12909. *P<0.05, **P<0.1, ***P<0.001 *vs.* respective baseline, $$ P<0.01, $$$ P<0.001 *vs* specified conditions, Bonferroni after significant mixed model repeated measures two-way ANOVA.

When the loading of the slices with ^{3}H-dopamine was made in the presence of desipramine (10 µM, to prevent norepinephrine terminal uptake and storage of ^{3}H-dopamine), MPH and ATX exert a significant increase of dopamine efflux but only when applied at the concentration of 100 µM (Fig. 4) and to a lower extent than what was previously observed in desipramine-free (normal) condition, respectively with 38% and 17% lower levels of dopamine efflux (*vs* baseline condition). This indicates that blocking the norepinephrine transporter during the loading of the slices significantly reduces, but does not prevent, the ability of MPH to induce dopamine efflux.

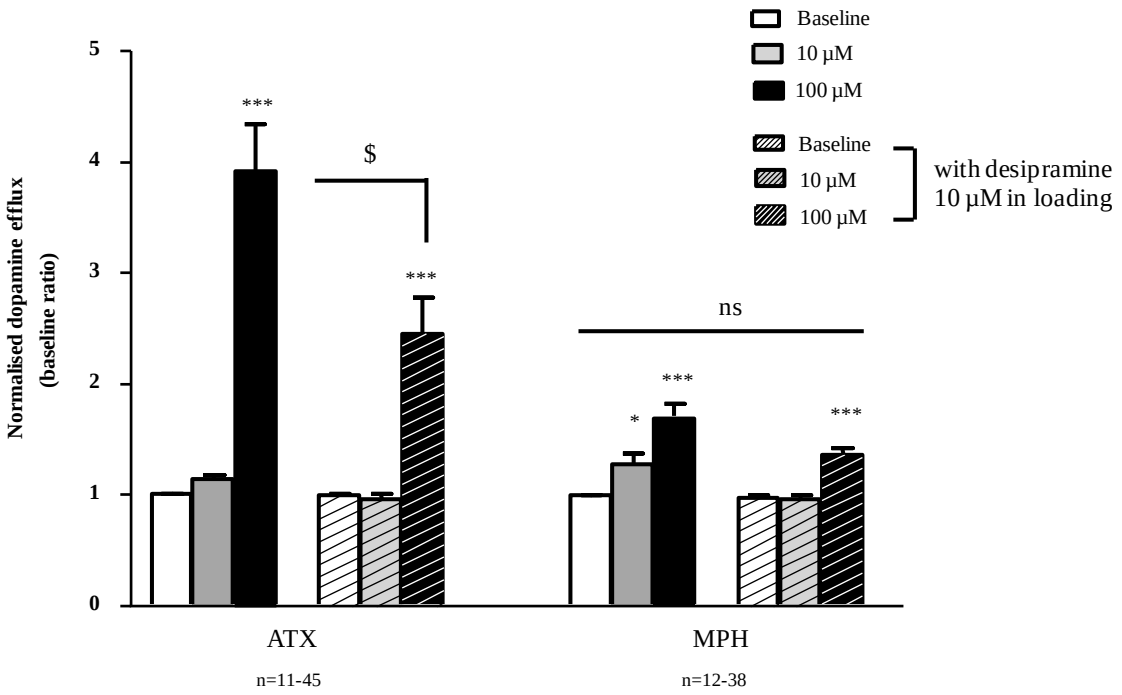


Figure 4: Role of norepinephrine terminals in ATX-induced dopamine efflux.
When prefrontal cortex tissues were loaded with tritiated dopamine in the presence of 10 µM of desipramine (NET inhibitor), 100 µM of ATX still promoted dopamine efflux, but to a level that is significantly lower than in desipramine-free conditions, indicating that ATX promotes dopamine efflux from both dopamine and noradrenergic terminals. ns: non-significant, *P<0.05, ***P<0.001 *vs.* respective baseline, $P<0.05 *vs.* specified condition, Neuman-Keuls after significant ANOVA (**A**).

I-3-A-2- In the striatum

As observed in the PFC, all drugs tested dose-dependently stimulated dopamine efflux from striatal slices (Fig. 5A). When applied at 10 µM, both GBR-12909 and MPH significantly increased dopamine efflux, but with different efficacies (15% and 108% over baseline, respectively, Fig. 5B). Interestingly, ATX, but not reboxetine or desipramine, two other potent NET inhibitors, also significantly increased the efflux of dopamine by 42% over baseline (Fig. 5A,B), when tested at the concentration of 10 µM ($p<0.001$, Neuman-Keuls test after ANOVA). Reboxetine and desipramine both have K_i values of over 10 µM for the dopamine transporter (Millan *et al.*, 2001; Zhou, 2004), which is consistent with our study (Fig. 5B). As expected, D-amph, applied at 10 µM, induced strong dopamine release (437% over baseline). When tested at higher concentrations (100 µM), ATX elicited an unexpected and robust efflux of dopamine, like in the PFC, increasing baseline levels by more than 680%, which is significantly higher than the efflux produced by the potent DAT blockers MPH

($p<0.001$, Neuman-Keuls test after ANOVA) and GBR-12909 ($p<0.01$, Neuman-Keuls test after ANOVA). As observed in the prefrontal cortex, in the striatum, ATX also induced dopamine efflux in a dose-response manner (Fig. 5C). On the other hand, we did not observe any saturation in the striatum. Very interestingly, the DAT blocker Modafinil (IC_{50} for dopamine reuptake of 4 µM) (Zolkowska *et al.*, 2009), induced dopamine efflux only at 1000 µM (Fig. 5D). This result may be explained by the ability of Modafinil to act as a partial dopamine D_2 receptor agonist (Seeman *et al.*, 2009) and by the higher concentration of drug perfusion needed in order to penetrate our tissue, compared to techniques using synaptosome assays. Both MPH and ATX perfusions significantly induce dopamine efflux in dose-dependent manners from 1 µM to 1 mM (Fig. 5E). These results suggest that ATX, and possibly the two other NET inhibitors desipramine and reboxetine, may interact with other targets in order to induce such strong dopamine efflux (7 folds over baseline), while other potent DAT blockers only increase striatal dopamine efflux by 3-4 folds and specific inhibitors such as GBR have very limited effects. Loading the striatal slices with ^{3}H-dopamine in the presence of desipramine (10 µM) did not alter at all the ability of ATX to induce ^{3}H-dopamine efflux at 100 µM, as it induced significantly greater efflux than under desipramine-free conditions ($p<0.001$, Neuman-Keuls after significant ANOVA, Fig. 6).

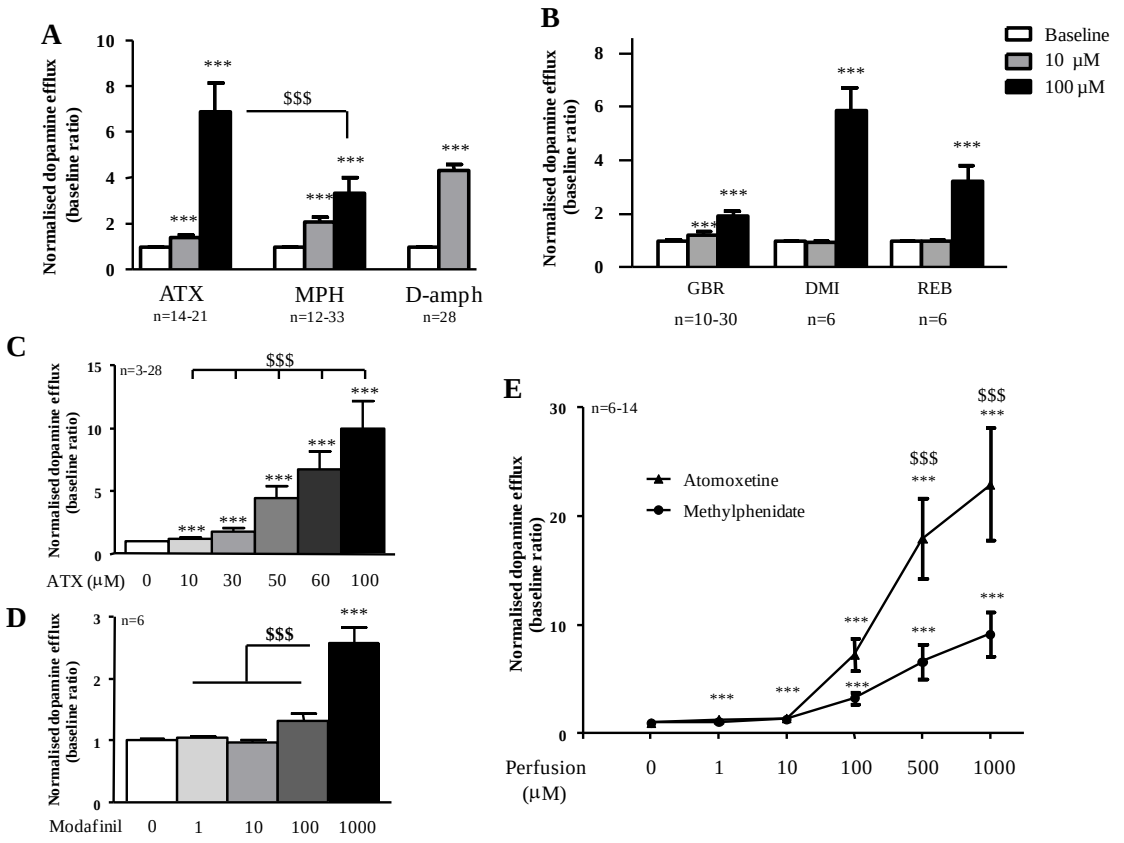


Figure 5: Dose-response of ADHD drugs and other selective uptake inhibitors on striatal ^{3}H-dopamine efflux.

(**A**): Superfusion of either ATX (10 and 100 µM), MPH (10 and 100 µM) or D-amph (10 µM) all significantly induced dopamine efflux with different level of efficacy. ATX promoted an unexpected massive dopamine efflux from striatal slices at concentrations higher than 60 µM. (**B**): GBR-12909, desipramine (DMI) and reboxetine (REB) all significantly induced dopamine efflux when applied at 100 µM. (**C**): ATX promotes striatal dopamine efflux in a dose-dependent manner from 10 µM to 100 µM. (**D**): Dose response of Modafinil, another drug used for ADHD, on striatal dopamine efflux. Here, Modafinil significantly induced dopamine efflux only if applied at concentrations above 100 µM. (**E**): In the striatum, ATX is more efficient than MPH in inducing dopamine efflux at high concentrations. ***P<0.001 *vs.* respective baseline, $$$P<0.001 *vs.* specified condition (**A**-**D**) or *vs.* MPH (**E**), Neuman-Keuls (**A**-**D**) or Bonferroni (**E**) after significant one-way or two-way ANOVA.

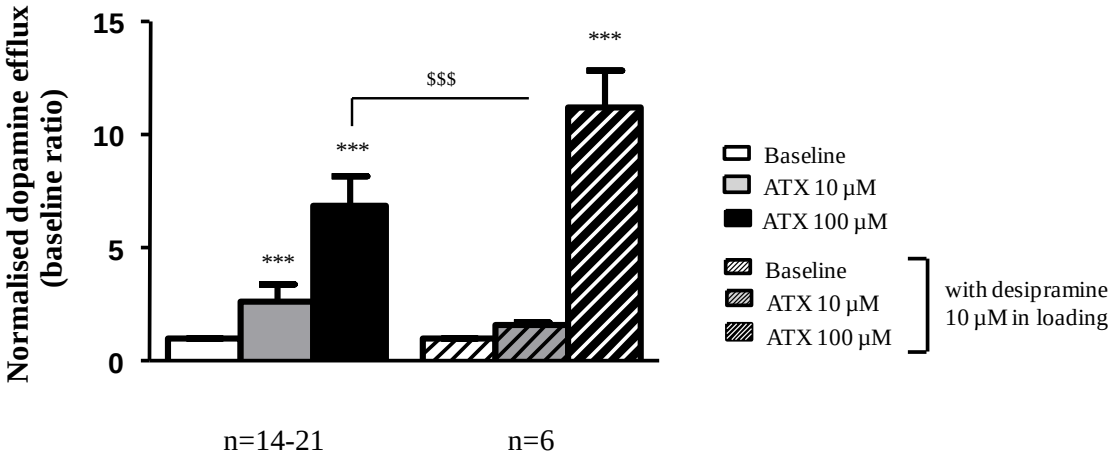


Figure 6: Atomoxetine induces striatal dopamine efflux from dopamine terminals, but not noradrenergic terminals.
Contrary to what was observed in the prefrontal cortex, when striatal slices were loaded with tritiated dopamine in the presence of 10 µM of desipramine, 100 µM of ATX still promoted dopamine efflux to higher levels than in desipramine-free conditions, indicating that ATX promotes dopamine efflux from dopamine terminals but not noradrenergic terminals. ***P<0.001 *vs*. respective baseline, $$$P<0.001 *vs*. specified condition, Neuman-Keuls after significant ANOVA.

I-3-A-3- Interaction between drugs

Pre-exposure of slices with ATX (100 µM), did prevent the releasing effect of MPH (100 µM) in the PFC, but not in the striatum, where efflux is stimulated with similar efficacy as in basal conditions (Fig. 7A). On the other hand, the pre-exposure of both PFC and striatal slices with MPH at 100 µM did not prevent the massive effect of ATX on dopamine efflux (Fig. 7A). This result suggests that ATX triggers dopamine efflux by a mechanism that is not dependent on the dopamine reuptake transporter, whilst MPH-induced dopamine efflux originates from the blockade of such a transporter. In both structures, D-amph-induced dopamine release was completely prevented when slices were previously perfused with MPH (Fig. 7B). Reciprocally, in both structures, MPH did not increase further the efflux of dopamine elicited by D-amph (10 µM). Dopamine efflux was even significantly decreased in striatal slices following MPH application (Fig. 7B). Interestingly, in the PFC, the outflow effects of ATX or D-amph were also partially reduced (in this case) by each other's pre-administration (Fig. 8), an effect not observed in the striatum (not shown). However, such a dampening may be due to ceiling effects and require therefore further experiments.

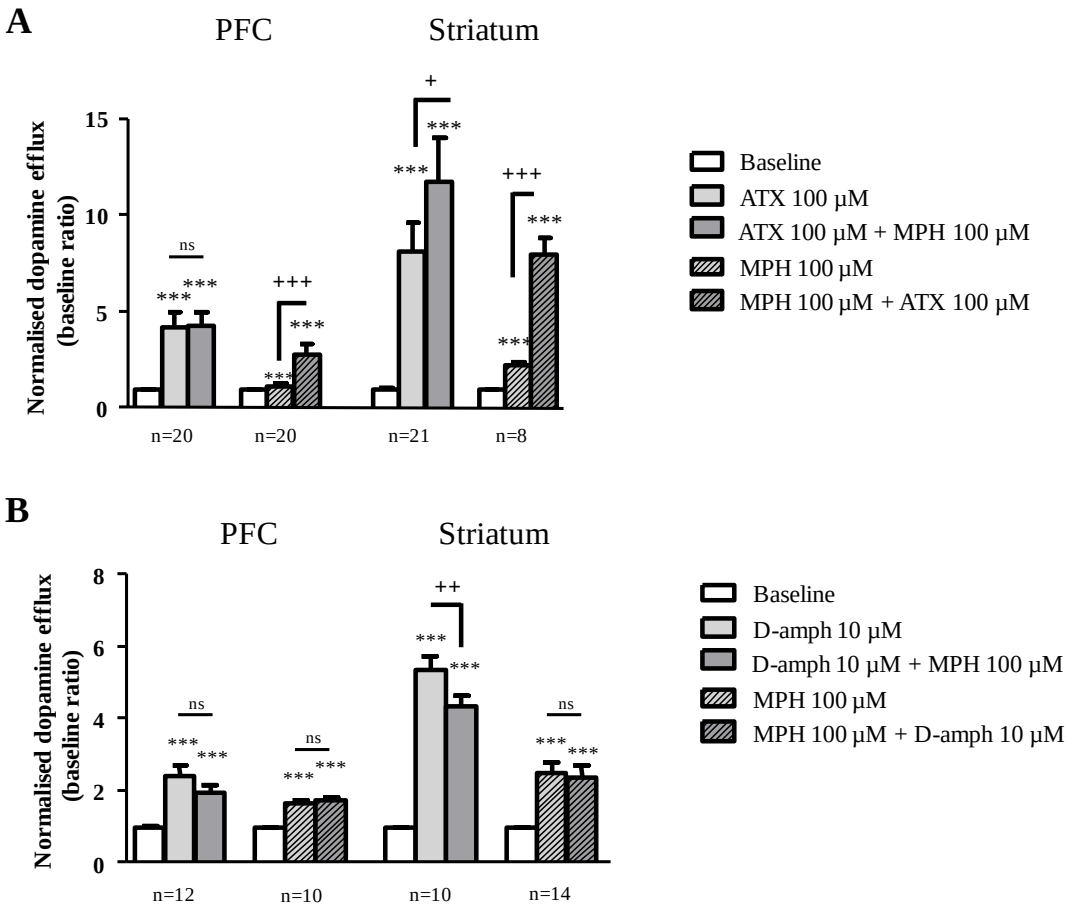


Figure 7: Interactions between ADHD drugs on prefrontal cortex and striatal ^{3}H-dopamine efflux.

(**A**): Pre-incubation with ATX (100 µM) prevented the outflow of dopamine induced by MPH (100 µM) in the prefrontal cortex, but not in the striatum. However, pre-incubation with MPH before ATX application did not reduce ATX effects in both regions. (**B**): In both regions, pre-exposure with MPH (100 µM) completely prevented the subsequent effects of D-amph. Similarly, pre-exposure with D-amph prevented or significantly reduced the effects of MPH (PFC and striatum, respectively). Here and in the following figures, co-perfusion of two drugs is represented by a + in the legends and denotes therefore addition of the last-mentioned drug into the buffer, already containing the first-mentioned drug. ns: non-significant, ***P<0.001 *vs.* respective baselines, +P<0.05, ++P<0.01, +++P<0.0001 *vs.* specified conditions. Newman-Keuls after significant ANOVA.

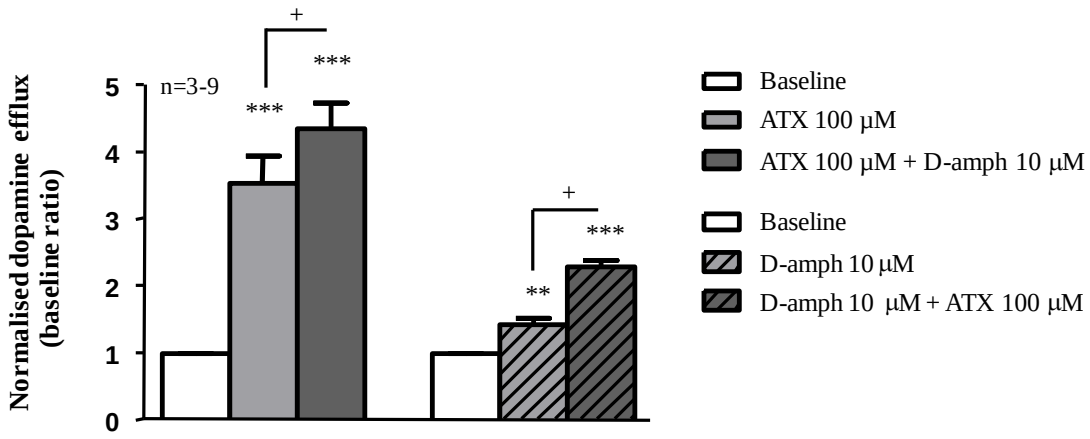


Figure 8: Interactions between atomoxetine and D-amphetamine on prefrontal cortex ^{3}H-dopamine efflux.

In the prefrontal cortex, pre-incubation with either ATX (100 µM) or D-amph (10 µM) did not prevent the dopamine efflux induced by subsequent applications of either drug. **P<0.01, ***P<0.001 *vs.* respective baselines, +P<0.05 *vs.* specified conditions, Newman-Keuls after significant ANOVA.

I-3-A-4- Dependency of dopamine efflux by ADHD drugs upon sodium, calcium, vesicle integrity and K_{IR} channels

Superfusion of striatal slices with an isotonic medium containing a low concentration of sodium (20 mEq instead of 125 mEq) increased significantly basal ^{3}H-dopamine efflux by nearly 380% (Fig. 9A) and completely prevented both MPH and ATX-induced dopamine efflux (Fig. 9B). The dopamine transporter strongly depends upon balanced intracellular and extracellular sodium concentrations (Itokawa *et al.*, 2002; Manepalli *et al.*, 2012; Pramod *et al.*, 2013; Vaughan *et al.*, 2013). Extracellular sodium depletion also significantly reduced D-amph-induced dopamine release. As expected, D-amph (10 µM), ATX (100 µM) and MPH (100 µM) all produced additional effects on KCl-induced dopamine efflux (*vs* buffer only, respectively p<0.001, p<0.01 and p<0.05, Newman-Keuls after significant ANOVA, Fig. 10). Interestingly, D-amph was the most efficient of the three drugs (Fig. 10B,E). Ouabain (100 µM), a potent Na^{+}/K^{+}-ATPase inhibitor, also increased basal dopamine efflux (Fig. 11A) and prevented dopamine efflux induced by ATX (Fig. 11B), indicating that ATX-induced dopamine efflux may be dependent upon active processes and/or physiological electrochemical gradients. However, such effects are most probably due to a ceiling effect by ouabain. Efflux processed in calcium-free buffer did

not change the ability of ATX to elicit ^{3}H-dopamine efflux from PFC or striatal slices (Fig. 12). On the other hand, the intracellular calcium chelator BAPTA-AM (50-100 µM), applied for 20 min before sample collection to the end of the experiment in combination with extracellular calcium depletion, significantly reduced ATX-induced ^{3}H-dopamine efflux in the striatum (Fig. 13) but not MPH-induced dopamine efflux. When the potent depleting agent of monoamine vesicles reserpine was applied at 1 µM, large dopamine efflux were observed from striatal slices and this effect was significantly more pronounced ($F_{(1,51)}$=173.7, p<0.01, two-way ANOVA) than on PFC slices (Fig 14). However, when the concentration of reserpine was increased to 10 µM, both regions produced stronger dopamine efflux, indicating a dose-response mechanism of reserpine on dopamine efflux. Reserpine, applied at 1 µM for 20 min before superfusion of PFC or striatal slices also significantly attenuated the effect of ATX on dopamine efflux (Fig. 15), but had no effect on MPH-induced striatal dopamine efflux (Fig. 16), indicating therefore that ATX, but not MPH, require catecholamine vesicle integrity in order to induce such a dopamine efflux.

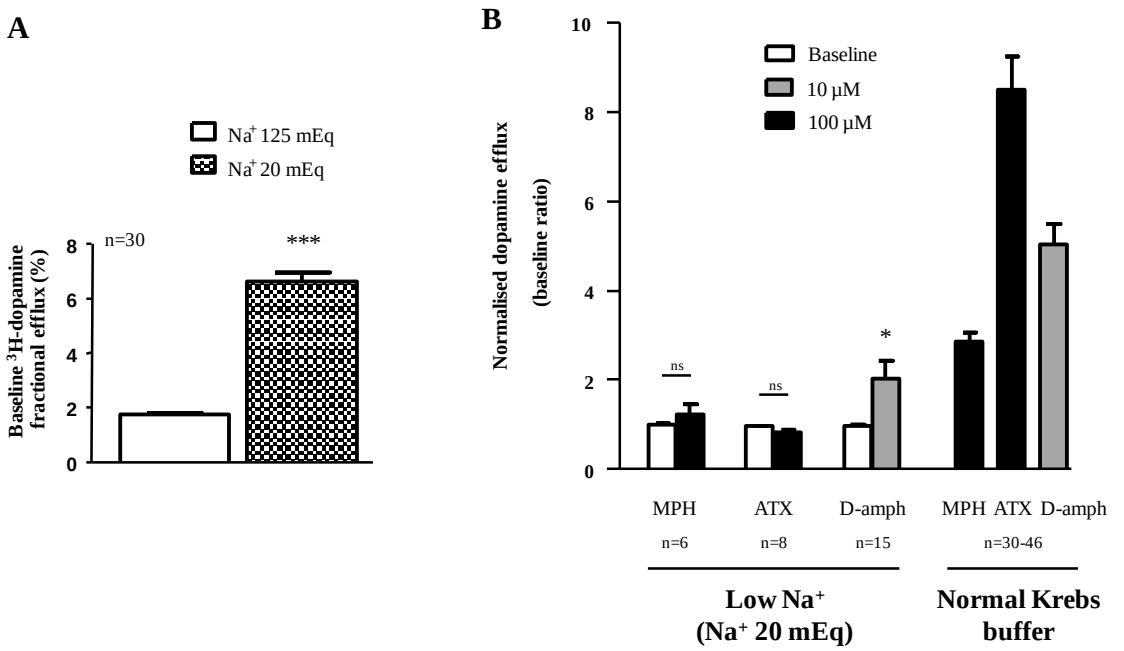


Figure 9: Sodium dependency of both methylphenidate and atomoxetine in inducing striatal tritiated dopamine efflux.
(**A**) Superfusion of a buffer containing a low sodium concentration (20 mEq of Na^{+}) significantly increased basal dopamine outflow. ***P<0.001 *vs.* respective baselines, unpaired Student's t-test. (**B**) Under these perfusion conditions, 100 µM of either ATX or MPH could not induce striatal dopamine efflux. However, 10 µM of D-amph significantly promoted dopamine efflux, but to a lower level than under physiological sodium concentration (125 mEq). ns: non-significant, *P<0.05 *vs.* respective baselines, unpaired Student's t-test.

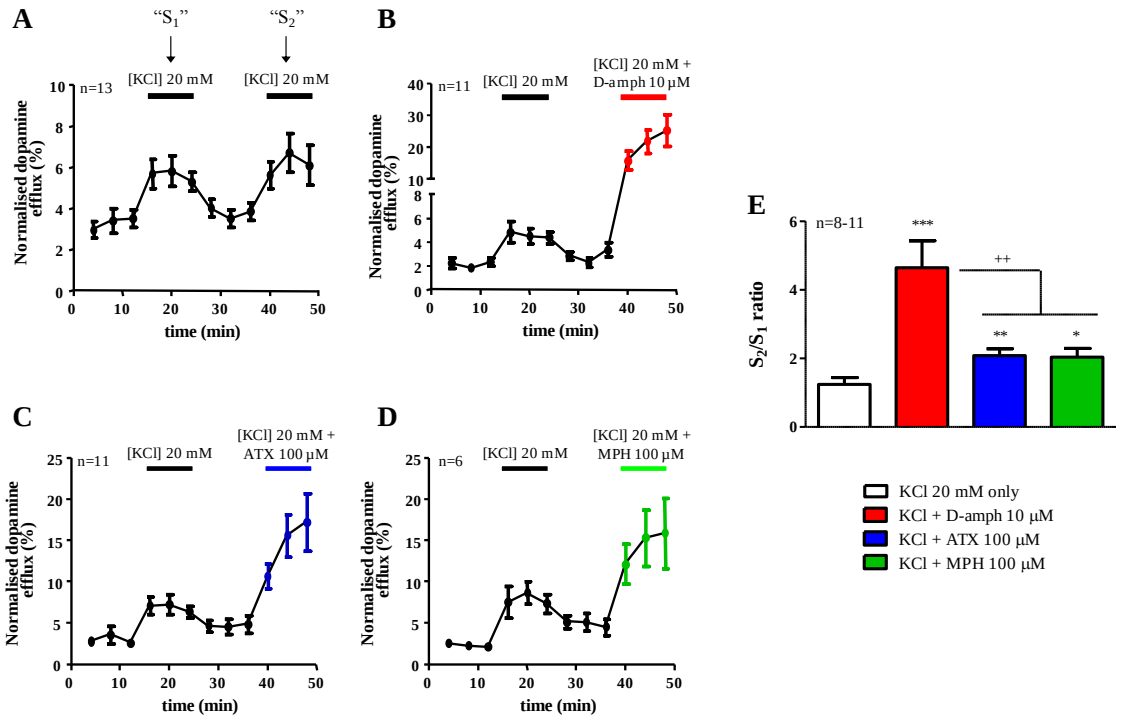


Figure 10: ADHD drugs produced additional effects on dopamine efflux from striatal slices when co-applied with a potassium-rich buffer.
(**A**) Superfusion of a buffer containing a high potassium concentration (20 mM of KCl) increased dopamine release in a reversible manner. The addition of 10 µM of D-amph (**B**), 100 µM of ATX (**C**) or 100 µM of MPH (**D**) significantly increased dopamine efflux when co-applied with KCl, assessed by increased S_2/S_1 ratios. D-amph was the most efficient of the three drugs used. Note the non-linear scale in **B**. *P<0.05, **P<0.01, ***P<0.001 *vs.* KCl 20 mM, ++P<0.01 *vs.* specified condition, Newman-Keuls after significant ANOVA.

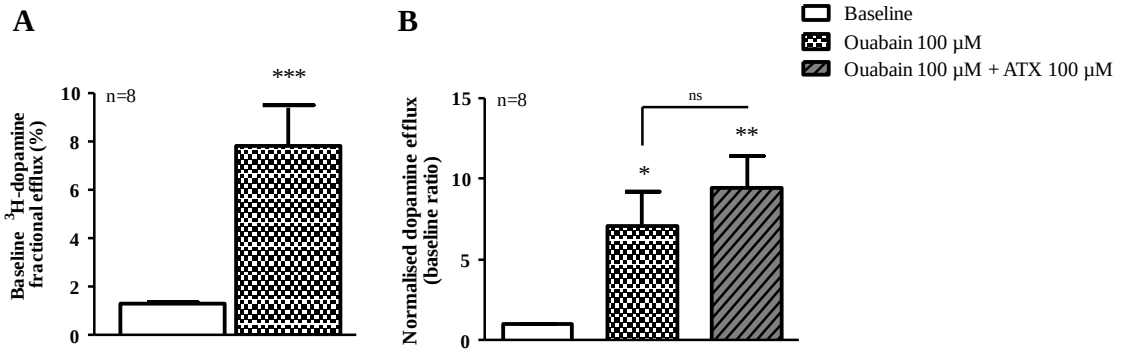


Figure 11: Atomoxetine induces striatal dopamine efflux by a gradient-dependent mechanism.
(**A**) Superfusion of a buffer containing 100 µM of ouabain, a potent Na^+/K^+-ATPase inhibitor, significantly (p<0.001, unpaired Student's t-test) increased basal dopamine outflow. (**B**) Under these perfusion conditions, 100 µM of ATX did not induce further striatal dopamine efflux, indicating that ATX may require physiological electrochemical gradients to induce dopamine outflow. ns: non-significant, most probably due to a ceiling effect, *P<0.05, **P<0.01 *vs.* respective baselines, Newman-Keuls after significant ANOVA.

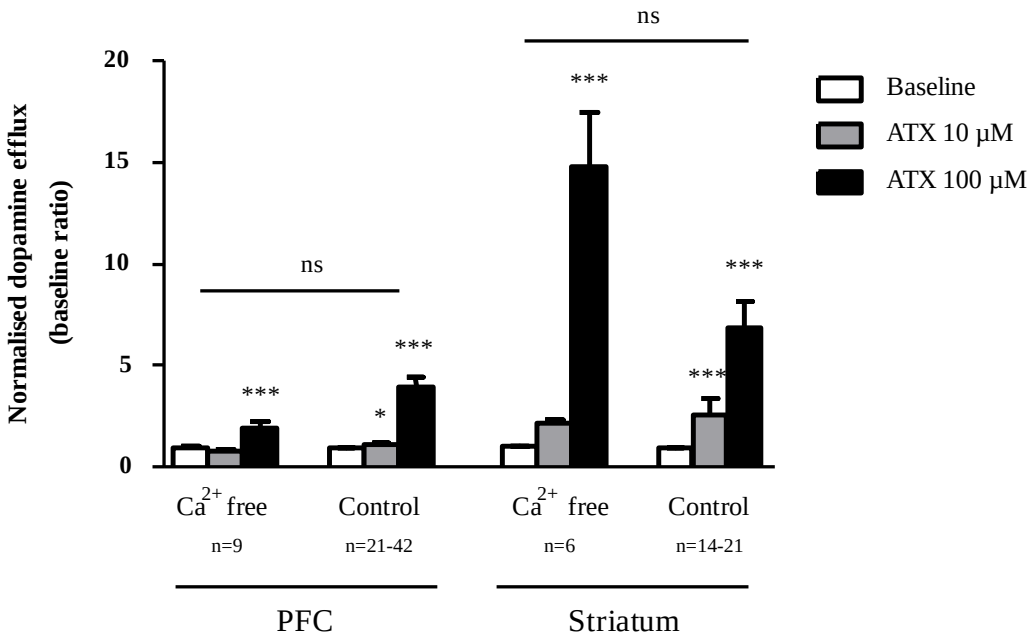


Figure 12: Atomoxetine-induced dopamine efflux is not dependent upon extracellular calcium concentrations.
In the PFC and the striatum, superfusion of a calcium-free buffer (0 mEq of Ca^{2+}) did not reduce the capacity of ATX to induce dopamine efflux. ns: non-significant, *P<0.05, ***P<0.001 *vs.* respective baselines, Newman-Keuls after significant ANOVA.

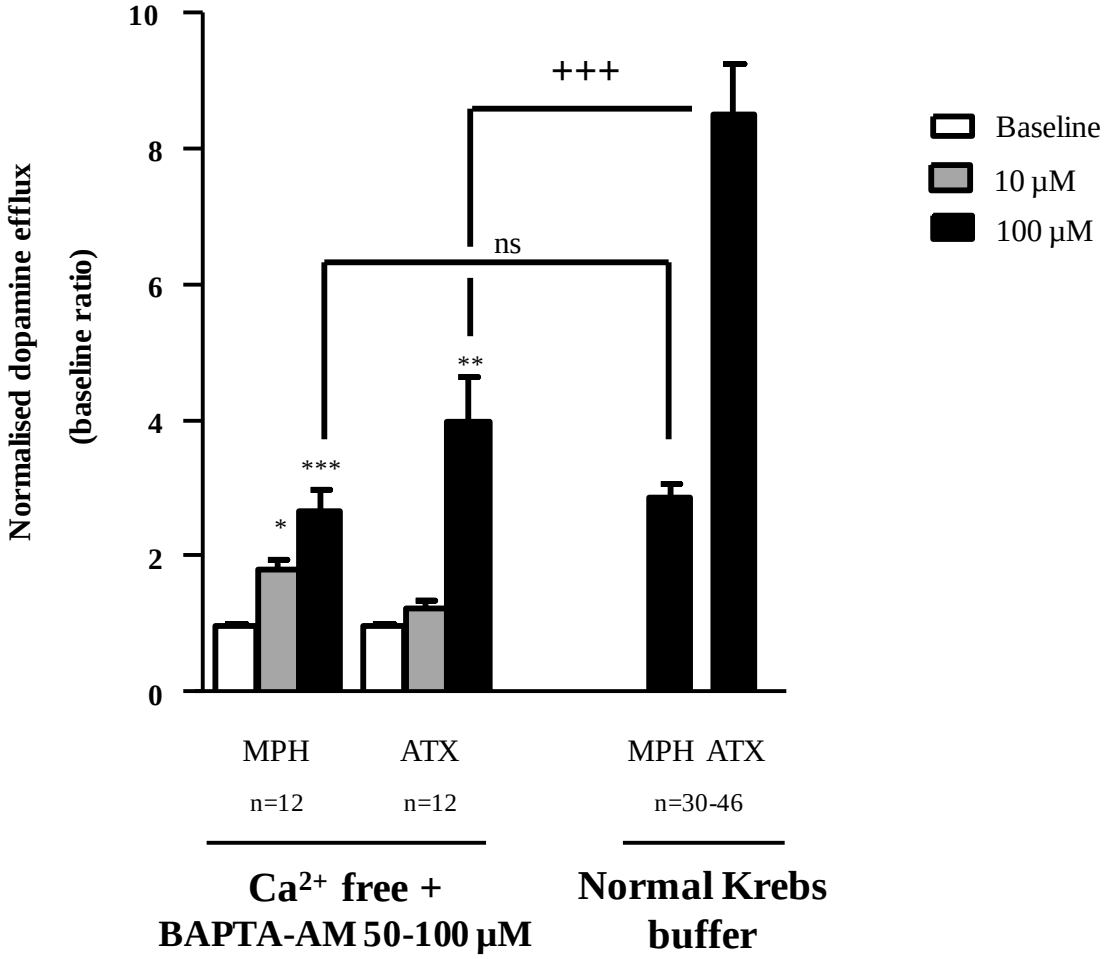


Figure 13: Atomoxetine-induced dopamine efflux, but not methylphenidate-induced dopamine efflux, is dependent upon intracellular calcium concentrations.
When intracellular calcium chelation was performed for 20 minutes before collection, using 50-100 µM of BAPTA-AM, together with the use of an extracellular calcium-free buffer, ATX-induced dopamine efflux was significantly reduced, an effect not observed with MPH.
BAPTA-AM: 1,3-bis(aminophenoxy)ethane-*N*-tetraacetic acid-acetoxymethyl ester, intracellular calcium chelator. ns: non-significant, *P<0.05, **P<0.01, ***P<0.001 *vs.* respective baselines, +++P<0.001 *vs.* control condition, Newman-Keuls after significant ANOVA.

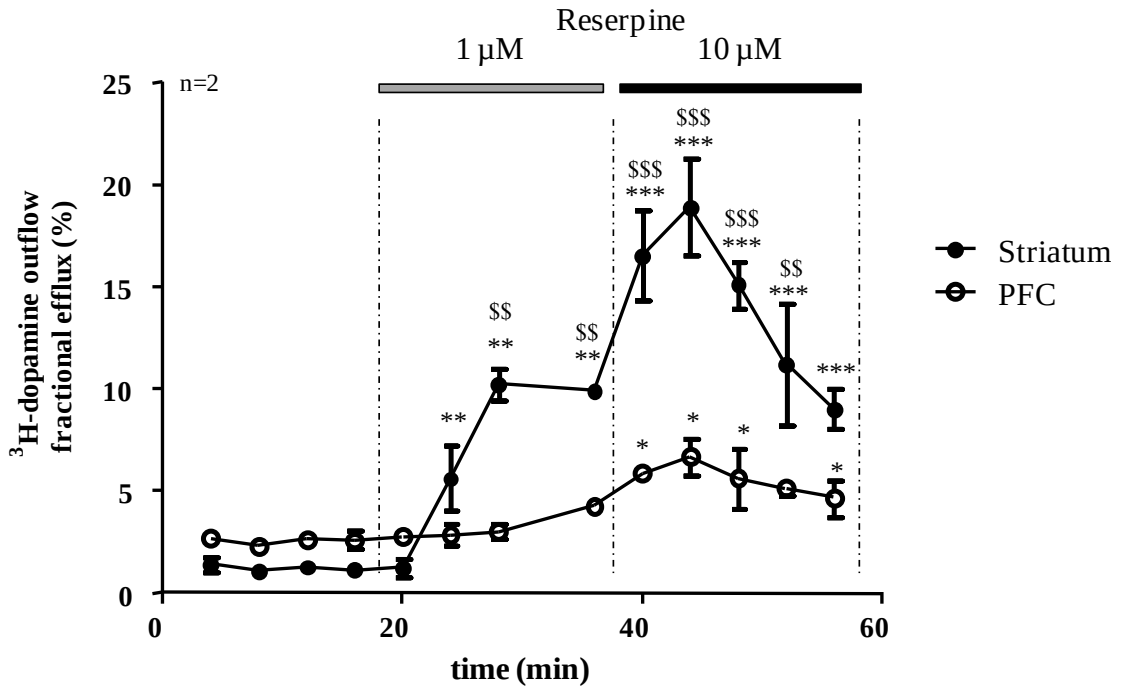


Figure 14: Superfusion of reserpine induces prefrontal and striatal dopamine efflux.

Superfusion with reserpine (1 and 10 µM), a powerful depleting agent of monoamine vesicles, produced significant dopamine efflux from prefrontal cortex and striatal slices. *P<0.05, **P<0.01, ***P<0.001 *vs.* baseline outflow, $$P<0.01, $$$P<0.001 *vs.* PFC, Bonferroni after significant repeated measures two-way ANOVA.

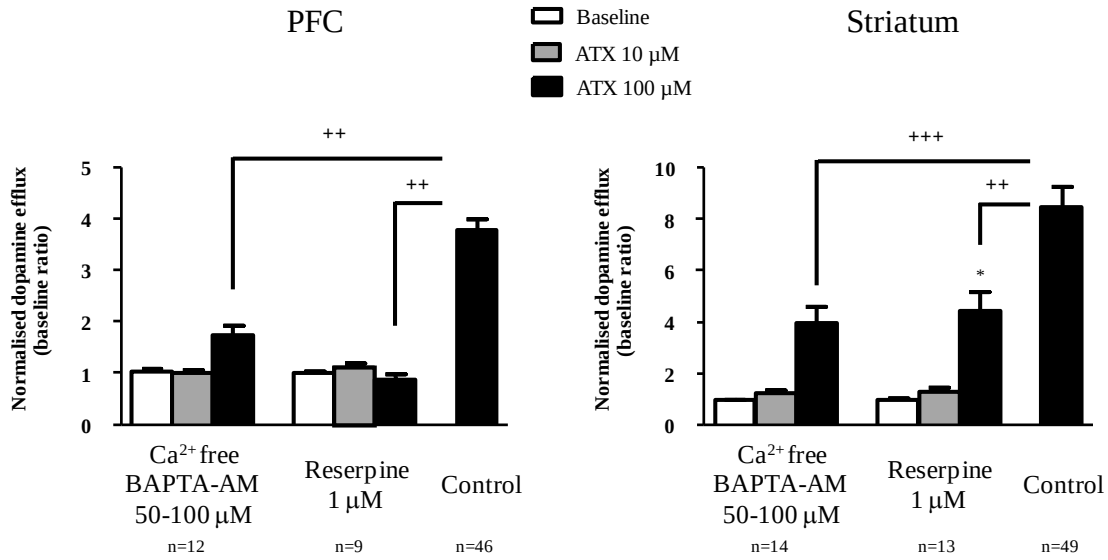


Figure 15: Atomoxetine-induced dopamine efflux is dependent upon vesicle integrity.

When monoamine vesicles were depleted (1 µM of reserpine) for 20 minutes before collection, subsequent application of ATX on prefrontal cortex and striatal slices induced significantly lower dopamine efflux than under control conditions. *P<0.05 *vs.* respective baselines, ++P<0.01, +++P<0.001 *vs.* specified conditions, Newman-Keuls after significant ANOVA.

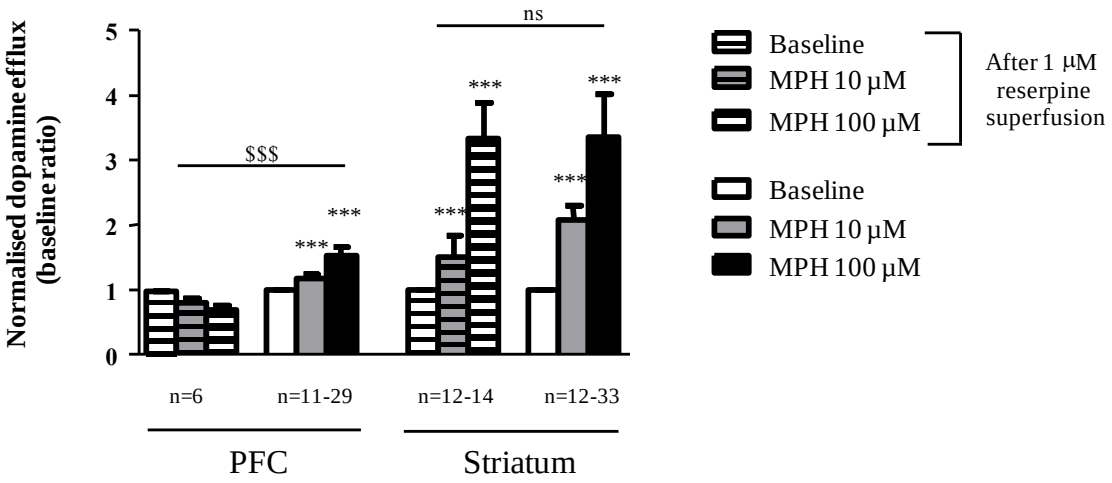


Figure 16: Dependency of methylphenidate on vesicle integrity to induce dopamine efflux.
In the prefrontal cortex, monoamine vesicle depletion prevented MPH-induced dopamine efflux, but not in the striatum, where MPH induced dopamine efflux to similar levels as under control conditions. ns: non-significant, ***P<0.001 *vs.* respective baselines, $$$P<0.001 *vs.* all other conditions, Newman-Keuls after significant ANOVA.

We then examined the possibility that ATX could induce dopamine efflux through the inwardly-rectifying K^+ channels (K_{IR}). Indeed, a previous study on frog oocytes has shown that ATX also interacts with the G_i-protein-activated K_{ir} channel with IC_{50} values in the 30-50 micromolar range (Table 1) (Kobayashi *et al.*, 2010). In our experiment, co-perfusion of 20 µM of SCH23390, a dopamine D_1 receptor antagonist as well as a K_{IR} blocker (IC_{50} of 268 nM) (Kuzhikandathil *et al.*, 2002), combined with 100 µM of ATX, failed to reduce ATX-induced dopamine efflux in a small population of samples (n=3, Fig. 17). SCH23390 did not have an effect on its own on baseline dopamine outflow. These observations indicate that ATX induces dopamine outflow through interaction with other surface receptors or intracellular targets, some of which may still be unknown.

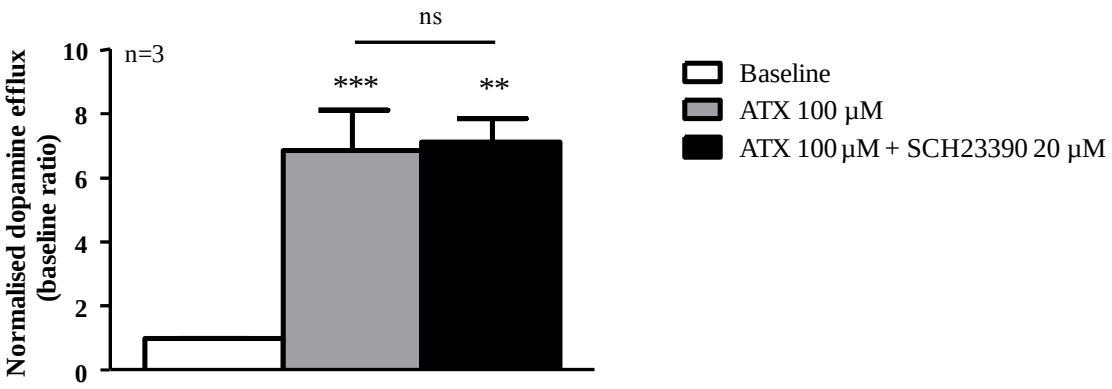


Figure 17: Atomoxetine-induced striatal dopamine efflux does not depend upon the inwardly rectifying potassium channels.
Specific blockade of K_{IR} channel, using 20 µM of SCH23390, did not prevent ATX-induced dopamine efflux in the striatum. ns: non-significant, **P<0.01, ***P<0.001 *vs.* baseline, Newman-Keuls after significant ANOVA.

I-3-B- ADHD drugs also induce PFC norepinephrine efflux

One hundred micromolars of ATX significantly induced prefrontal cortex norepinephrine efflux, (Fig. 18). However, the two other ADHD drugs, MPH and D-amph, did not induce norepinephrine efflux. Finally, we also tested whether ATX-induced norepinephrine efflux could be dependent upon alpha-2 receptors, as a previous study in monkeys has shown that ATX enhances memory tasks through indirect alpha-2 receptor stimulation (Gamo *et al.*, 2010). Blockade of α2 receptors did not potentiate the subsequent effects of ATX in our model (Fig. 19).

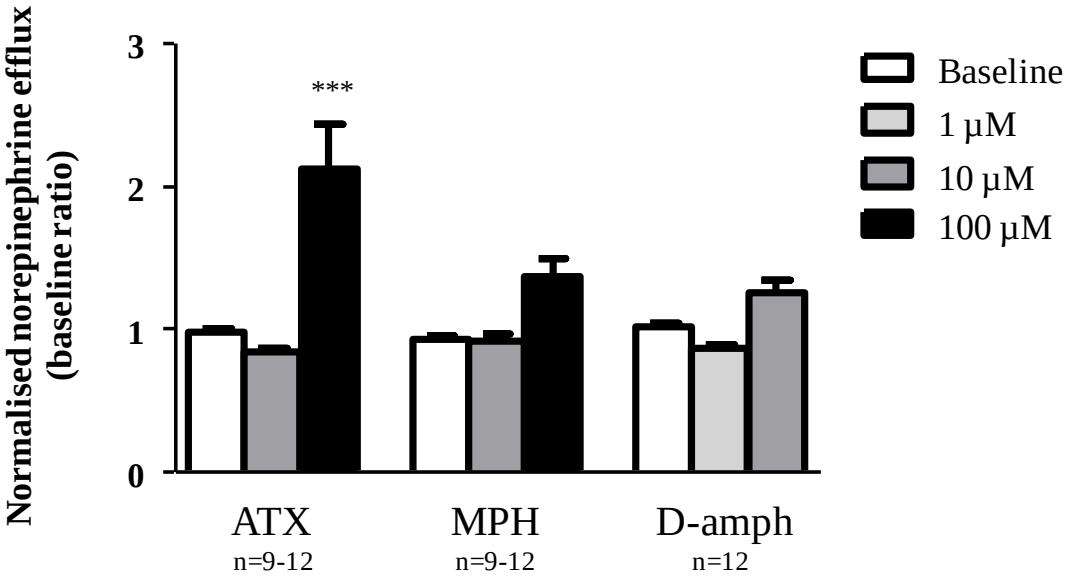


Figure 18: Atomoxetine also induces ^{3}H-norepinephrine efflux from prefrontal cortex slices.
Among the three ADHD drugs, only ATX (100 µM), significantly induced tritiated norepinephrine efflux from prefrontal cortex slices. ***P<0.001 *vs.* respective baselines, Newman-Keuls after significant ANOVA.

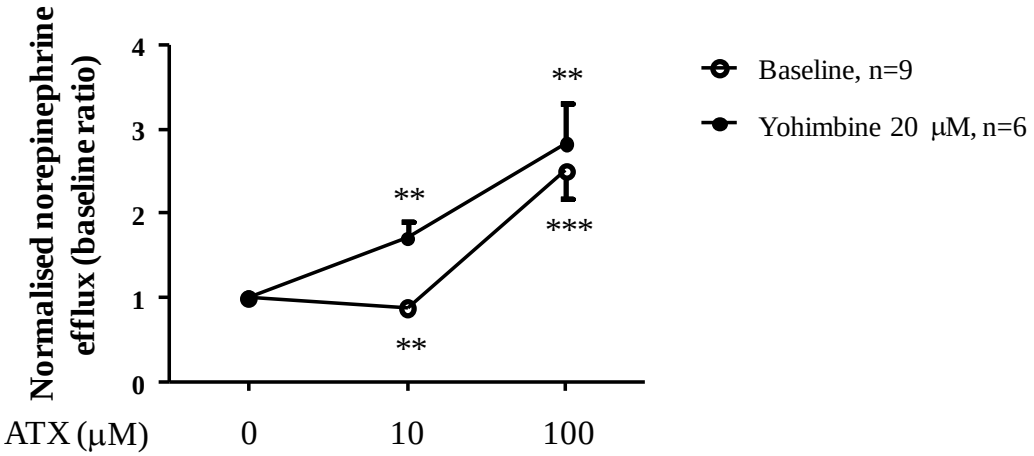


Figure 19: Role of adrenergic alpha-2 receptors on atomoxetine-induced norepinephrine efflux.
Blockade of adrenergic alpha-2 receptors, using 20 µM of yohimbine, did not affect ATX-induced (10 and 100 µM) norepinephrine efflux. **P<0.01, ***P<0.001 *vs.* ATX 0 µM, Bonferroni after significant repeated measures two-way ANOVA.

I-4- Discussion

Here, we have demonstrated that, compared to other drugs, D-amph is able to elicit the greatest amount of dopamine release in both striatum and PFC when applied at the lowest concentration (10 µM, Fig. 3A), a concentration in the same range as brain levels achieved following administration of therapeutic (0.5-2 mg/kg) D-amph doses (Wallace, 2012). D-amphetamine is known to exert its effect on dopamine efflux by initial competition with dopamine at the dopamine reuptake transporter, followed by inhibition of the vesicular monoamine transporter 2 ($VMAT_2$, IC_{50} of 3.3 µM, Table 1), required for dopamine reuptake from the cytosol into vesicles (Partilla *et al.*, 2006; Teng *et al.*, 1998). Blockade of $VMAT_2$ by D-amph and methamphetamine induces dopamine release from the neuron through dopamine transporter reversal (Brown *et al.*, 2000; Nickell *et al.*, 2014; Sulzer *et al.*, 1995). High intracellular dopamine concentrations induce cytotoxicity when D-amph is administered chronically, caused by apoptosis and oxidative stress (Steinkellner *et al.*, 2011; Stumm *et al.*, 1999). Other effects of D-amph on $VMAT_2$ include redistribution of the transporter from the vesicular membrane to the cytosol (Eiden *et al.*, 2011; Riddle *et al.*, 2007). These dysregulations of brain monoamine concentrations induce hypersensitivity to later drug intake (Robinson *et al.*, 1987) as well as hyperlocomotion (Antoniou *et al.*, 1998; Sallinen *et al.*, 1998) combined with stereotyped behaviour patterns (Sams-Dodd, 1998). Active derivatives of the metabolism of amphetamines, such as 4-hydroxyamphetamine and α-methyl-p-tyrosine, are known to accumulate in brain tissues following acute as well as chronic amphetamine administrations (Cho *et al.*, 1975; Dougan *et al.*, 1986a). These derivatives are known to respectively induce mydriasis (dilatation of the pupil) and inhibition of the production of catecholamines (Cho *et al.*, 1978; Dougan *et al.*, 1986b; Lepore, 1985). Interestingly, when 1 and 10 µM of D-amph were superfused onto striatal slices, dopamine release was significantly increased (Kantor *et al.*, 1999; Kantor *et al.*, 2002; Niddam *et al.*, 1985) and promoted DAT surface trafficking (Furman *et al.*, 2009). In non-human primates, chronic D-amph administration at 0.1-1 mg/kg twice a day for 1 to 4 weeks induced plasma D-amph concentration ranging from 0.4 to1.5 µM (Ricaurte *et al.*, 2005). Consistent with these findings, another study measured D-amph accumulation in rat brains following a single 3 mg/kg intraperitoneal injection and found peak plasma D-amph levels around 0.6 µM (Fuh *et al.*, 2004). Moreover, striatal perfusion through microdialysis of an ACSF

solution containing 30 µM of D-amphetamine induced significant dopamine release in rats (Loweth *et al.*, 2009). However, the use of the microdialysis technique is known to result in perfusate loss, up to 85% (Chefer *et al.*, 2009; Hillman *et al.*, 2005; Keeler *et al.*, 2014; MacVane *et al.*, 2014; Shippenberg *et al.*, 2001). In humans, a single 0.7 mg/kg dose of D-amph produced plasma D-amph levels peaking at 0.6 µM (Kirkpatrick *et al.*, 2012). In agreement with previous investigations (Jones *et al.*, 1998; Sulzer *et al.*, 1993), our findings suggest that D-amph enters the catecholamine terminals via a catecholamine uptake carrier system, given the preventive effect of MPH on D-amph-induced dopamine efflux (Fig. 5B) and the strict sodium dependency (Fig. 14B). Recent evidence suggests that uptake of D-amph, which competes very strongly with dopamine for the dopamine reuptake transporter (DAT), promotes sodium and to a lesser degree calcium influx inside the terminal to a level that is enough to promote reverse transport of dopamine (Robertson *et al.*, 2009). These observations corroborate our results, to some extent, because MPH significantly reduced D-amph-induced striatal dopamine efflux when subsequently perfused after D-amph, as MPH blocks the necessary transporter for D-amph to further penetrate inside the synapse and prevents dopamine from leaving the cell through reverse transport (Fig. 7B). Indeed, a previous study using mice lacking the dopamine transporter observed that perfusion of striatal slices with D-amphetamine fails to induce extracellular dopamine efflux, an effect not observed using wild-type mice (Jones *et al.*, 1998).

We have also demonstrated that pre-exposure with ATX prevents MPH-induced dopamine efflux in the PFC, but not in the striatum (Fig. 7A). This result suggests the involvement of the NET in MPH-dependent ^{3}H-dopamine efflux in the PFC but not in the striatum, where noradrenergic innervations as well as norepinephrine reuptake transporter levels are low (Berridge *et al.*, 1997; Moron *et al.*, 2002; Swanson *et al.*, 1975). Our data suggest that MPH, though a psychostimulant, may be a useful therapeutic tool to prevent D-amph from penetrating inside neuronal terminals and inducing persisting dysregulation of monoamine neurochemistry and behaviour (Fig. 7B). Besides, drugs that potently block dopamine transporters, including MPH, have been shown to prevent D-amph-induced dopamine release from HEK-293 cells expressing the human dopamine transporter (Simmler *et al.*, 2014), in agreement with our study on dopamine neuron terminals. There have been several recent clinical trials investigating the efficacy of DAT blockers in reducing severe abuse-related effects of

amphetamine-like compounds (Howell *et al.*, 2014). Indeed, the DAT blocker Modafinil was found to be efficient in reducing cocaine cravings and increasing the number of days without cocaine use (Anderson *et al.*, 2009; Dackis *et al.*, 2005; Dackis *et al.*, 2003; Hart *et al.*, 2008). Moreover, genetically-modified dopamine transporters can present decreased affinity for cocaine whilst still functioning efficiently for normal dopamine reuptake (Thomsen *et al.*, 2009), therefore reducing the dopamine releasing effect of cocaine whilst maintaining physiological dopamine transporter activity. In rhesus monkeys, strategies aiming to inhibit monoamine reuptake have proven efficient in reducing cocaine self-administration, with agents such as selective dopamine and non-selective monoamine reuptake inhibitors as well as ester derivatives of GBR 12909 (Glowa *et al.*, 1996; Negus *et al.*, 2009). In humans, chronic amphetamine maintenance, also called agonist replacement therapy, can significantly decrease cocaine self-administration in chronic cocaine users (Greenwald *et al.*, 2010; Rush *et al.*, 2010).

MPH, at the same concentration as D-amph (10 µM), more moderately stimulated dopamine efflux in the striatum and in the PFC. Modafinil, another DAT blocker, also induced dopamine efflux effect in the striatum (Fig. 5D), a result that corroborates, at least in part, previous *in vitro* observations on synaptosomes where inhibition of dopamine uptake was observed following 1 µM of Modafinil (Zolkowska *et al.*, 2009), which *in fine* produces extracellular dopamine concentration rises. Contrary to what was seen in the striatum, 100 µM of MPH does not increase further dopamine efflux in PFC slices previously exposed with 100 µM of ATX (Fig. 7A). Moreover, MPH-induced dopamine efflux is reduced in the PFC when the slices have been pre-loaded with the norepinephrine reuptake transporter (NET) inhibitor desipramine (Fig. 4). Therefore, our data supports previous assumptions that in the PFC, MPH elicits dopamine efflux mainly via an inhibition of the NET, suggesting that extracellular dopamine in the PFC originates not only from dopaminergic terminals but also from noradrenergic ones where it can act both as a precursor for norepinephrine and as a co-transmitter (Devoto *et al.*, 2006; Devoto *et al.*, 2003; Devoto *et al.*, 2001; Devoto *et al.*, 2004). Indeed, dopamine is transformed into norepinephrine by the dopamine beta-hydroxylase enzyme, requiring ascorbic acid and oxygen as co-factors (Kapoor *et al.*, 2011; Rush *et al.*, 1980). Some studies have suggested that both dopamine and norepinephrine are located within the same dense core vesicles in noradrenergic nerves (De Potter *et al.*, 1997; Ou *et al.*, 1998). Moreover, MPH has

similar *in vitro* affinity for the human NET (K_i 0.1 µM) as for the DAT (K_i 0.06 µM), whilst dopamine has an even higher affinity for the NET (K_m = 0.67 µM) than for the DAT (K_m = 2.54 µM, Table 1) (Giros *et al.*, 1994; Han *et al.*, 2006; Moron *et al.*, 2002). Previous microdialysis and neurochemical studies on DAT knockout mice, as well as on naive rats, have shown that selective NET inhibitors increase prefrontal, but not striatal, dopamine efflux (Bymaster *et al.*, 2002; Moron *et al.*, 2002), in line with previous published observation, showing that in the striatum, the NET is responsible for dopamine uptake only when DAT levels reach critically low levels, as observed in Parkinson's disease (Arai *et al.*, 2008; Chotibut *et al.*, 2012). This indicates the involvement of the NET in MPH-dependent ^{3}H-dopamine efflux in the PFC but not in the striatum, where noradrenergic innervations as well as norepinephrine reuptake transporter levels are low (Berridge *et al.*, 1997; Moron *et al.*, 2002; Swanson *et al.*, 1975). Taken together, these results suggest that MPH also induces dopamine efflux in the PFC through norepinephrine transporters blockade, but not in the striatum, where MPH rather targets dopamine transporters. We remain unable to evaluate the exact contribution of the dopamine transporter in the effects of MPH to induce dopamine efflux in the PFC in our experimental conditions. It probably contributes to increasing dopamine efflux as MPH can still exert its effects when the slices were previously loaded in the presence of desipramine. However, following the loading of the slices, redistributions of the tritiated dopamine within both dopamine and norepinephrine terminals could have occurred.

Microdialysis studies have demonstrated that MPH, administered at therapeutic doses (0.5-2 mg/kg), increased extracellular dopamine efflux in the PFC, but with limited or no effects in the striatum (Koda *et al.*, 2010). However, when the authors raised the MPH administration to 10 mg/kg, increases of dopamine efflux were also observed in the striatum. On the other hand, our *in vitro* study shows a more efficient effect of MPH in the striatum than in the PFC (respectively Fig. 5A, 5E vs Fig. 3A, 3D), which is compatible with the dense dopaminergic innervations found in striatal regions (Matsuda *et al.*, 2009). In the striatum, dopamine is cleared faster than in the PFC by the dopamine transporter because of higher DAT densities (Sesack *et al.*, 1998). Our results can be explained by the fact that other mechanisms contribute to extracellular dopamine clearance in the PFC, as emphasised before (Wayment *et al.*, 2001), while dopamine clearance in the striatum solely depends upon DAT activity. Moreover, *in vitro* DAT

blockade differs greatly from *in vivo* mechanisms, where compensatory mechanisms could occur, such as passive stabilisation, whereby adjacent neurons can uptake dopamine (Bergstrom *et al.*, 2001; Fujimori *et al.*, 1986; Woodward *et al.*, 1995).

Conversely, the efficacy of MPH and ATX in inducing dopamine efflux in the PFC under our conditions is much lower than under *in vivo* conditions using the microdialysis techniques. Systemic administration of low doses of MPH or ATX (1-2 mg/kg), probably reaching a concentration in the low micromolar range near the catecholamine synapse (Balcioglu *et al.*, 2009; Ding *et al.*, 2004; Gerasimov *et al.*, 2000), could stimulate dopamine efflux by more than 300% (Berridge *et al.*, 2006; Bymaster *et al.*, 2002). When administered on the intact brain, these drugs are likely to activate other neuronal circuitries potentiating further the release of dopamine in the PFC. Such activation may occur at local levels, as applications of MPH by reverse microdialysis in the PFC or the nucleus accumbens can still produce consistent large increase in dopamine efflux *in vivo* (Ahn *et al.*, 2013; Nomikos *et al.*, 1990; Schmeichel *et al.*, 2013).

However, at a higher concentration (100 µM), ATX produced a surprisingly massive efflux of dopamine in both brain regions (Fig. 3A,B, 5A,C,E), so did the other two NET inhibitors, reboxetine and desipramine (Fig. 3C, 5B). Such an effect does not seem to be related to the selective blockade of the NET, as noradrenergic innervations are practically absent in the dorsal striatum, but not in the nucleus accumbens (Berridge *et al.*, 1997; Ferrucci *et al.*, 2013; Saigusa *et al.*, 2012; Zhang *et al.*, 1999), and this effect is identical when striatal slices have been preloaded with ^{3}H-dopamine in the presence of the NET inhibitor desipramine (Fig. 6). In line with these results, previous studies found that the NET has a more limited impact in the reuptake of dopamine in the striatum than in the PFC (Berridge *et al.*, 1997; Moron *et al.*, 2002; Swanson *et al.*, 1975). Whether this effect would be relevant in some clinical situations is an open question. Therapeutic doses of ATX (1-2 mg/kg) are believed to reach brain concentrations of ATX in the low micromolar range, up to 5 µM, and possibly more, as the drug can slowly accumulate within the brain after chronic administration (Ludolph *et al.*, 2010). In our model, dose-responses of ATX in the striatum showed a massive effect on dopamine efflux starting at 30-50 µM (Fig. 5C). Atomoxetine is metabolized primarily by the polymorphically expressed enzyme cytochrome P450 CYP2D6

(Petroff, 2002). In children with poor metabolising status, who represent just under 10% of all ADHD children (ter Laak *et al.*, 2010), plasma ATX concentration can increase by 10 times the average therapeutic concentration (Loghin *et al.*, 2013). One can assume that, in these patients, concentration in the brain may reach levels likely to significantly affect dopamine release in the striatum, according to our *in vitro* investigation (as ATX can induce dopamine efflux in the striatum at concentration as low as 1-10 µM, Fig. 5E,C).

The mechanism by which ATX induces this massive efflux needs to be fully clarified. Our study demonstrates that it is dependent on sodium (Fig. 14B) and on the integrity of the catecholamine vesicles (Fig. 15). In our experimental design of sodium depletion, no osmolarity shock occurred due to the isotonic addition of choline chloride, preventing therefore astrocyte swelling (Lauderdale *et al.*, 2015; Schober *et al.*, 2015), as seen previously (Lehmann *et al.*, 1990). This sodium substitution is known to induce rapid membrane hyperpolarisation (Cvetkovic-Lopes *et al.*, 2010), which should decrease dopamine efflux. Our results are compatible with the sodium dependency of dopamine reuptake (Wheeler *et al.*, 1993). Indeed, under extracellular sodium depletion conditions, dopamine cannot be reuptaken by the DAT (Roitman *et al.*, 1999), as sodium gradients are known to be the driving forces of dopamine transport. Interestingly, extracellular calcium depletion on its own is not sufficient to reduce ATX-induced dopamine efflux (Fig. 12), although calcium is crucial for successful spike generation (Llinas *et al.*, 1981). However, intracellular calcium chelation, which has been found to successfully reduce neurotransmitter release (Adler *et al.*, 1991; Chavez *et al.*, 2006; Fredholm *et al.*, 1993; Hunt *et al.*, 1994), completely prevented ATX-induced dopamine efflux when combined with extracellular calcium depletion (Fig. 13 and 15). Taken together, these results indicate that ATX requires electrochemical gradients and intracellular calcium to induce dopamine efflux. *In vivo*, such outflows arise from vesicular fusion (Kasai, 1993; Simon *et al.*, 1985; Stanley, 1997). We can hypothesise that ATX may interfere with the efflux of catecholamine-containing vesicles by interacting with an unknown intracellular target after being transported inside the terminal through a sodium dependent process (as ATX-induced effects were found to be sodium dependent), but different from the NET/DAT. Atomoxetine may also bind extracellularly to ion channels or proteins located on the neuronal membrane. Atomoxetine, compared to MPH, has a non-negligible affinity (K_i of 3.4 µM, Table 1)

for the dopamine vesicle transporter (Easton *et al.*, 2007). Therefore ATX could be uptaken inside the dopaminergic terminals through a relatively low affinity transporter, but different from the DAT or NET, where it will then interact with the vesicular transporter to promote intracellular dopamine efflux, followed by extracellular dopamine efflux. However, the fact that we found that MPH can still increase dopamine efflux following ATX exposure indicates that dopamine does not leave the terminals *via* the dopamine transporter (unlike with D-amphetamine). Therefore, D-amph-like effects following ATX remains unlikely. To note, D-amph-induced monoamine release is also dependent on intracellular calcium concentration (Gnegy *et al.*, 2004; Kantor *et al.*, 2001). ATX has also been shown to interact with various ion channels with IC_{50} values in the micromolar range, including the G_i-protein-activated inwardly rectifying K^+ channel (K_{IR}), which has an important function in regulating neuronal excitability (Kobayashi *et al.*, 2010; Loghin *et al.*, 2013; Scherer *et al.*, 2009; Vandenberg *et al.*, 2012). However, we did not find any contribution of the potassium inward rectifying channel (K_{IR}) in triggering ATX-induced dopamine efflux, as the perfusion of SCH23390, an inhibitor of dopamine D_1 receptor as well as a K_{IR} blocker with an IC_{50} of 268 nM (Kobayashi *et al.*, 2010; Kuzhikandathil *et al.*, 2002), did not prevent ATX-induced massive dopamine efflux in the striatum (Fig. 17). These results are in line with a previous study where the authors found no involvement of K_{IR} channels in dopamine outflow (Martel *et al.*, 2011). One could put forward the hypothesis that ATX activates other intracellular target or membrane receptors, such as sodium channels. However, ATX may also interact with other potassium channels, different from the K_{IR} itself. Atomoxetine has been found to interact with NMDA channels, by exerting blockade of NMDA-induced currents in cortical neurons (IC_{50} = 3 µM) (Ludolph *et al.*, 2010). In the striatum, dopamine has been found to modulate NMDA currents *via* dopamine D_1 and D_2 receptors (Cepeda *et al.*, 1998; Flores-Hernandez *et al.*, 2002). However, more studies will be needed to understand the exact mechanism of ATX-induced dopamine efflux. Finally, in a preliminary study in our laboratory, superfusion of ATX did not induce any serotonin outflow from cortical slices.

In this chapter, we have demonstrated that the three ADHD drugs methylphenidate, D-amphetamine and atomoxetine can induce dopamine efflux from both cortical and striatal slices, with different efficacies. While both stimulants appeared to target the dopamine transporter system, ATX triggered an unexpected strong

dopamine efflux by other mechanisms. However, ATX requires vesicle integrity and physiological electrochemical gradients. When norepinephrine release was investigated, only ATX produced mild efflux, indicating that this effect is specific to dopaminergic terminals. The possible clinical implications of these unexpected effects induced by ATX should be investigated further.

Chapter II – Acute *in vivo* ADHD drug administration.

II-1- Introduction

Spike generation is closely linked to neuronal computation, which involves complex spatial and temporal summation (Tran-Van-Minh *et al.*, 2015). Although spike generation can be simplified *in silico* using mathematical models (Brown, 1999; Brown, 2000) adapted from previous observations (Hodgkin *et al.*, 1952; Llinas *et al.*, 1982; Morris *et al.*, 1981; Platkiewicz *et al.*, 2010; Sato *et al.*, 1974), *in vivo* electrical activities result from far more complex input/output dendrite currents. In this next chapter, we will investigate how ADHD drugs modulate *in vivo* neuronal electrical spike generation, which is closely linked to neurotransmitter release.

One of the main brain regions involved in the behavioural-calming and cognition-enhancing effects of ADHD drugs is the prefrontal cortex (PFC) (Gamo *et al.*, 2010). It plays a critical role in the control of higher cognitive functions such as vigilance, attention, impulsivity and behavioural inhibition (Kieling *et al.*, 2008), as well as on the activity of the basal ganglia which controls movement and emotional behaviour. Recent evidence supports the role played by the glutamatergic system in the pathology and treatment of ADHD (Chang *et al.*, 2014), particularly in the PFC, the main cellular constituents of which are glutamate pyramidal neurons. In the PFC, there is evidence for both functional and anatomical interaction between catecholamine and glutamate systems. Most prefrontal catecholamine innervations, originating from the midbrain, terminate onto pyramidal glutamate neurons and modulate various cortico-cortical circuits, as well as cortical excitatory descending pathways. This includes the massive prefrontal projections to the basal ganglia, known to regulate, as part of a series of complex feedback loops, executive, motor and affective functions, impaired in ADHD (Carli *et al.*, 2014). As a result, changes in PFC catecholamine levels could affect these excitatory drives and, in turn, impact on catecholamine neurotransmission in many subcortical structures (Sullivan *et al.*, 2003). Furthermore, there is evidence in

the PFC for colocalisation of glutamate and catecholamine receptors, which can interact with each other on common intracellular signalling pathways and modulate synaptic transmission (Tseng *et al.*, 2004; Urban *et al.*, 2013a). Imaging studies in drug-naive ADHD patients indicate dysregulations of glutamatergic fronto-striatal circuits, which are strongly modulated by dopamine and norepinephrine, as well as reduction in the thickness and hypofunction of some specific subregions of the PFC (Cubillo *et al.*, 2012). Attention has been drawn to the glutamate/N-Methyl-D-aspartate (NMDA) receptor. It has been well established that NMDA receptors have critical roles in excitatory synaptic transmission and plasticity underlying cognitive processes, short-term and long-term memory, long-term potentiation as well as motor function (Collingridge *et al.*, 2013; Hasan *et al.*, 2013; Henley *et al.*, 2013; Jurado, 2014; Luscher *et al.*, 2012; Rowland *et al.*, 2005). There is evidence for genetic polymorphism of both the NR_{2A} and the NR_{2B} subunits of the NMDA receptor in ADHD patients (Dorval *et al.*, 2007; Turic *et al.*, 2004). Although not a pharmacological model for ADHD, glutamate NMDA receptor antagonists induce hyperlocomotion and disrupt attention as well as impulsivity control (Amitai *et al.*, 2010). These NMDA antagonists also mitigate some of the neurochemical and behavioural effects of psychostimulants in animal models (Bristow *et al.*, 1994).

In this second chapter, we will investigate to what extent acute *in vivo* ADHD drug administration can modulate the spontaneous or NMDA-induced electrical activities of silent or tonically active neurons, located either in the prefrontal cortex or in the striatum. Moreover, we will assess the impact of local dopamine on NMDA neurotransmission in PFC pyramidal neurons and striatal medium spiny neurons, using microiontophoretic techniques. We will also study the impact of such treatments on NMDA receptor 2B protein expression using western blot techniques. Finally, we will perform some behavioural experiments on locomotor activity to examine whether we could correlate our electrophysiological results with such behavioural pattern.

II-2- Material and Methods

II-2-A- Subjects

Male Sprague-Dawley rats were purchased from Charles River, UK. Animals were housed in groups of 2-4 per cage, maintained at 20-22^{o}C with humidity rates above 40% under a 12:12 L/D cycle with lights ON at 07h00 AM. Food and water were provided *ad libitum*. Animals were allowed a 3-day acclimatisation period after delivery. All experiments were performed during the light phase and with permission from the UK Home Office and De Montfort University Ethics Committee under the Project Licence 60/4333 and with the Personal Licence 60/13750.

II-2-B- *In vivo* extracellular single unit electrophysiology

One hundred and seventy-five naïve rats weighing between 220 and 330 grams were used. Animals were initially deeply anaesthetized with urethane (1.2–1.7 g/kg, intraperitoneal, with additional doses administered if necessary), secured to a stereotaxic frame and maintained at 36–37 °C with a heating pad. A catheter was inserted into the lateral tail vein to perform systemic drug administration. An incision was made across the top of the head and the edges of the skin drawn back to reveal the cranium. Bregma was identified and a hole was drilled through the bone at the coordinates of the prefrontal cortex (PFC) or the striatum, according to the atlas of Paxinos and Watson (Paxinos *et al.*, 1997). Electrodes were manufactured in-house from borosilicate capillaries (1.5 mm, Harvard Apparatus Ltd., UK), pulled on a PP-830 vertical electrode puller (Narishige, Japan) and filled by hand with an electrolyte solution of NaCl 147 mM. The tip of the electrode was broken down under a microscope to an external diameter of 1-1.5 μm. Typical electrode resistance was in the range 4-8 MΩ. Single-unit recordings with iontophoresis drug application were made using five-barrel glass micropipettes (World Precision Instruments, UK). The central recording barrel was filled with NaCl 147 mM. The side barrels were filled with either: NaCl 147 mM, N-methyl-D-aspartate (NMDA, 30 mM, pH 7-8, Sigma, St. Louis, MO), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 5 mM, pH7-8, Abcam, UK), dopamine 20 mM, MPH 20 mM, D-amphetamine 20 mM, all dissolved into 147 mM of NaCl, or a combination of the above and NaCl 2 M for current

balancing, although we later found that current balancing was not necessary while using low currents (0-20 nA). NMDA or AMPA pulses were applied during 40-70 seconds onto prefrontal cortex (PFC) glutamatergic neurons at regular intervals (80-100 s). Outputs from the electrode were sent to a Neurolog AC pre-amplifier and amplifier (Digitimer, UK). If necessary, signal amplification was manually adjusted to record whole neuronal action potential amplitudes. Signals were filtered and sent to an audio amplifier, a Tektronix 2201 digital storage oscilloscope and a 1401 interface connected to a computer running Spike 2 (CED, UK) for data capture and analysis. Descent of the electrode was carried out using a hydraulic micromanipulator (Narishige, MO-103, Japan).

Coordinates for the prefrontal cortex were (Fig. 20A): anteroposterior 2.5-3.7 mm to Bregma, lateral 0.3-2 mm, dorsoventral 1-4.7 mm below cortical surface. Putative pyramidal glutamatergic neurons were identified according to previous electrophysiological criteria (Gronier, 2011; Wang *et al.*, 2011): a broad action potential (1 ms), with a biphasic or triphasic, large wave form, starting with a positive inflection, a relatively slow firing rate typically between 1 and 50 spikes/10 s and an irregular firing pattern. A burst activity of PFC pyramidal neuron is characterised as at least two bursts occurring within 45 ms or less and followed by a silence period of at least 90 ms. Compared to single spikes, bursts release greater quantities of neurotransmitters (Bean *et al*., 1991; Gonon, 1988; Oster *et al*., 2015), resulting in greater occupancies of post-synaptic receptors.

GABAergic medium spiny neurons (MSN) were found in the striatum (Fig. 20B) at the following coordinates: anteroposterior 2-3.7 mm to Bregma, lateral 0.2-2 mm, dorsoventral 5-9 mm below cortical surface. Presumed medium spiny neurons were identified according to previous electrophysiological criteria such as a very low level of spontaneous activity or even no spontaneous activity (Galvan *et al.*, 2012; Mallet *et al.*, 2005), in combination with a long-lasting action potential waveform, usually above 1 ms (McGinty *et al.*, 2008; Murray *et al.*, 2015). These neurons can be easily distinguished from both cholinergic and GABAergic interneurons, which respectively display spontaneous discharges and thinner action potentials (Lim *et al.*, 2014; McGinty *et al.*, 2008; Murray *et al.*, 2015; Wilson *et al.*, 1990).

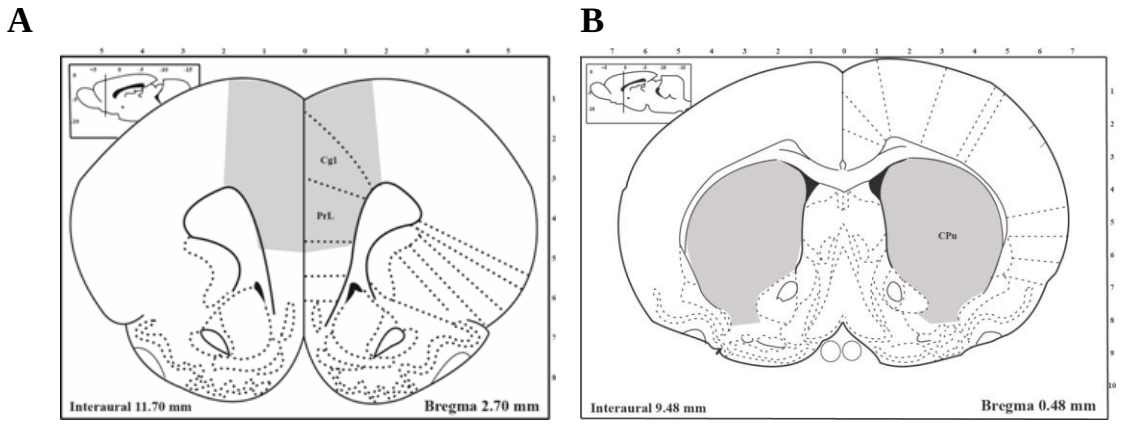


Figure 20: Areas of interest for *in vivo* single-cell extracellular electrophysiological recordings.
Shaded areas correspond to areas of interest for recordings of putative PFC glutamatergic pyramidal neurons (**A**) or putative GABAergic striatal medium spiny neurons (**B**). Scales represent distances (in mm) from the midline and the surface of the brain. Coronal slices adapted from Paxinos and Watson (1997). Cg1 cingulate cortex area, PrL prelimbic cortex, CPu caudate putamen.

GABAergic MSN receive strong GABA inputs from striatal GABA interneurons, also called feed-forward inhibition (Mallet *et al.*, 2006; Mallet *et al.*, 2005), explaining their very low or silent baseline firing activities. Such low spontaneous discharges are also the consequence of the inhibition of these medium spiny neurons by other medium spiny neurons, which represent lateral inhibition (Moyer *et al.*, 2014). In the rat striatum, MSN represent 95% of all neurons (Jiang *et al.*, 1991; Kemp *et al.*, 1971; Mallet *et al.*, 2006; Murray *et al.*, 2015), while cholinergic interneurons represent up to 5% of all neurons (Cachope *et al.*, 2012). Cholinergic interneurons strongly modulate the activity of MSN within the striatum (Zhou *et al.*, 2002). In our experiments, cholinergic interneurons were excluded from our recordings, as only medium spiny neurons (but not low-firing cholinergic interneurons) are silent and also display glutamate-induced (NMDA iontophoresis) firing rate excitation (Galati *et al.*, 2008; Sandstrom *et al.*, 2003). However, we cannot totally exclude that some extremely low-firing "tonically" active cholinergic interneurons might be included within this study, even if they only represent 1-2% of all striatal neurons (Bernacer *et al.*, 2007; Matamales *et al.*, 2016; Nelson *et al.*, 2014). Extensive literature now exists for the distinction between striatal GABAergic and cholinergic interneurons (Adler *et al.*, 2013; Chuhma *et al.*, 2011; Kawaguchi *et al.*, 1997; Stalnaker *et al.*, 2016).

II-2-C- Behaviour

Psychostimulants are known to alter locomotion (Amini *et al.*, 2004; Paulus *et al.*, 1991), activity (Fang *et al.*, 1998), grooming (Taylor *et al.*, 2010; Wooters *et al.*, 2009), scratching (Antoniou *et al.*, 1998; Malin *et al.*, 2009) and stereotypy (Bonasera *et al.*, 2008; Rebec *et al.*, 1997). Therefore, the consequences of psychostimulants on such behaviours were assessed.

Twelve naïve rats weighing 250 grams were housed individually at the beginning of the behavioural experiments. All drugs were dissolved into saline. Animals received a single intraperitoneal injection of either : 0.8 ml/kg of saline (NaCl 147 mM), 5 mg/kg of methylphenidate or 3 mg/kg of D-amphetamine. Animals were then scored for behavioural parameters during 15 minute time periods and up to a total of 60 minutes. Counting of well-defined behavioural traits such as rearing, scratching, grooming, jumping, running, climbing, catalepsy and stereotypical movements were done manually. At the end of the 60 minute observation period, animals were sacrificed by cervical dislocation and brains were quickly removed, dissected and immersed into liquid nitrogen. Brain regions were kept at -80^{o}C for further protein analysis using western blot techniques.

II-2-D- Western blot protein quantification

Following behavioural experiments, brain tissues were immediately dissected on an ice-cold platform and before being immersed in liquid nitrogen. Tissues were stored at -80^{o}C until further analysis. Brain tissue was homogenised in an ice-cold lysis buffer (radio-immuno-precipitation buffer, RIPA) containing: 150 mM NaCl, 1% Triton-X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, protease and phosphatase inhibitors, pH 8. Tissue homogenates were made using a glass potter and were then centrifuged at 12000 rpm at 4^{o}C for 20 minutes. Supernatants were kept for further analysis and total protein quantification was performed using bovine serum albumin (BSA) standards and the Bradford assay. Aliquots of protein lysates (20µg) were separated on 4-20% SDS gels (Biorad, UK) and transferred onto nitrocellulose membranes (GE Healthcare, USA) at 400 mA (constant current) for 1h. After transfer, membranes were stained with Ponceau red to help visualise correct protein transfer.

Membrane saturation was then performed for 1 hour at room temperature in a Tris buffer saline solution (20mM Tris- HCl, 150mM NaCl, pH 7.6) containing 5% of BSA and 0.1% of Tween 20 (TBS-T-BSA). The immuno-reaction was carried out overnight at $4^{o}C$ in TBS-T-BSA containing both $NMDA_{2B}$ and β-actin polyclonal primary antibodies, each diluted at 1:5000 (v/v). After being washed three times with TBS-T (5 min each), membranes were incubated with the corresponding secondary IgG-coupled horseradish peroxidase antibody diluted at 1:5000 in TBS-T-BSA (v/v) for 1h and at room temperature. The nitrocellulose sheets were rinsed three times in TBS-T (5min each) before developing the reaction with ECL (Biorad). Results were recorded on X-ray films (Kodak, USA).

II-2-E Data analysis

All data are expressed as the mean±standard error of the mean (SEM). Statistical analyses were performed using paired or unpaired Student's t-tests or one/two-way analysis of variance (ANOVA), followed, if appropriate, by Neuman–Keuls (one-way ANOVA) or Bonferroni tests (two-way ANOVA). Probabilities smaller than 0.05 were considered to be significant; n values refer to the number of cells tested or animals used.

For electrophysiology, the mean basal firing activity was evaluated after the neuron had attained a stable firing rate, generally after at least 5 min of recording. Pre-drug values of firing rate were obtained by averaging the firing rate over a period of at least 4 min immediately prior to the intravenous administration while post-drug values were obtained by averaging the firing over a period of 5 min following drug administration. Individual change in basal firing activity was considered significant following drug (or saline) administration when the post-drug value was significantly different from the pre-drug value (determined as indicated above, paired Student's t-test analysis). When neurons with very low spontaneous activity were recorded (baseline activity below 4 spikes per 10 seconds), a change of more than 5 spikes per 10 seconds was considered significant, or if the analysis of 100 spikes before and after drug administration led to significant unpaired Student's t-test for each neuron tested this way. These two methods of analysis did not differ in the results obtained. When appropriate, portions of recording involving iontophoretic application of excitatory

substances were excluded from these calculations. Proportions of a specific type of response in two different groups of animals were also compared using the Fisher's exact test (comparing proportions of responses and no response/opposite responses in two groups). The response of neurons to iontophoretic applications of NMDA or AMPA were assessed by subtracting the mean baseline activity measured immediately before the application (typically during the last 50 s before the application) of the drug from the mean activity obtained during the application of the drug (typically during the first 50 s of the application). Responses were expressed as the number of supplementary spikes (over baseline activity) per 10 nA of NMDA or per 5 nA of AMPA (as these currents were systematically administered to neurons). Effects of drugs/saline were examined by comparing the averaged response of at least three subsequent applications of NMDA/AMPA occurring immediately before or after the drug administration. For an individual neuron, a change in baseline, NMDA- or AMPA-induced firing rates (defined as the % difference between pre-drug and post-drug values) of more than 20% was considered significant, as observed in other electrophysiology studies (Conrad *et al.*, 2009; Hu *et al.*, 2011; Mulkey *et al.*, 2003; Roland *et al.*, 2011; Wang *et al.*, 1998). Although we acknowledge that this 20% cut-off value may seem arbitrary at first, one study reported that no more than 20% of the variability in firing of an individual neuron could be predicted from the activity of its neighbours (Shlens *et al.*, 2009). Therefore, we considered a drug to have a significant effect on the firing rate of an individual neuron if baseline firing varied by 20% or more (increase or decrease). All burst results are reported as the % of spikes in burst.

For behavioural experiments, the sum of each behavioural trait occurring during 15 minute interval periods was plotted against the type of drug administered. Results are expressed as the mean±standard error of the mean (SEM) of counts for each trait per group.

$NMDA_{2B}$ protein quantification was analysed using MCID software (GE Healthcare, USA) following densitometric analysis of Western blot autoradiograms. The expression of the NR_{2B} protein was normalized to the housekeeping protein β-actin. Data were expressed as a percentage of the control group (saline, mean±standard error of the mean).

II-3- Results

II-3-A- Neuronal population included in the present study

Only one neuron per rat (n=175 animals) was tested with each of the ADHD drugs, unless otherwise stated. All neurons included in the present data (n=230) were recorded in the dorsal part of the medial PFC (n=219) in subregions which include the cingulate and the prelimbic cortex and the medial part of the frontal cortex as well as in the striatum (n=11). Some recording sites were marked by ejection of pontamine blue (n=13, Fig. 21A) and were confirmed to be within the PFC at similar locations from what was determined from stereotaxic measurements. Moreover, pyramidal neurons were discriminated from other neurons (such as interneurons) according to their typical action potential shapes (Fig. 21B), such as a total action potential length of more than 1 ms, displaying a triphasic and large waveform, starting with a positive inflection (depolarisation) and presenting irregular slow firing rates typically between 1 and 50 spikes/10 s, sometimes with burst activity (two spikes occurring at an interval of 45 ms or less, followed by a silence period of at least 90 ms). The effects of ADHD drugs were investigated on neurons that were spontaneously active (n=103) and on neurons with low firing activity (n=45, firing rate <4 spikes/10 s, arbitral cut-off). These neurons were detected by recording neuronal activity using an iontophoretic electrode and applying small current pulses of NMDA or AMPA, which induced reversible neuronal activations. Spontaneously active neurons were usually recorded with single-barrel electrodes and low-firing neurons with multi-barrel ones. However, a few spontaneously active neurons (n=22) were also recorded with multi-barrel electrodes. Low-firing neurons and spontaneously active neurons did not differ in their action potential characteristics. When the vehicle was injected (saline, n=28, Fig. 21C), neurons did not display any significant change in firing, even during a long recording period (>2000 s), as already observed (Gronier, 2011).

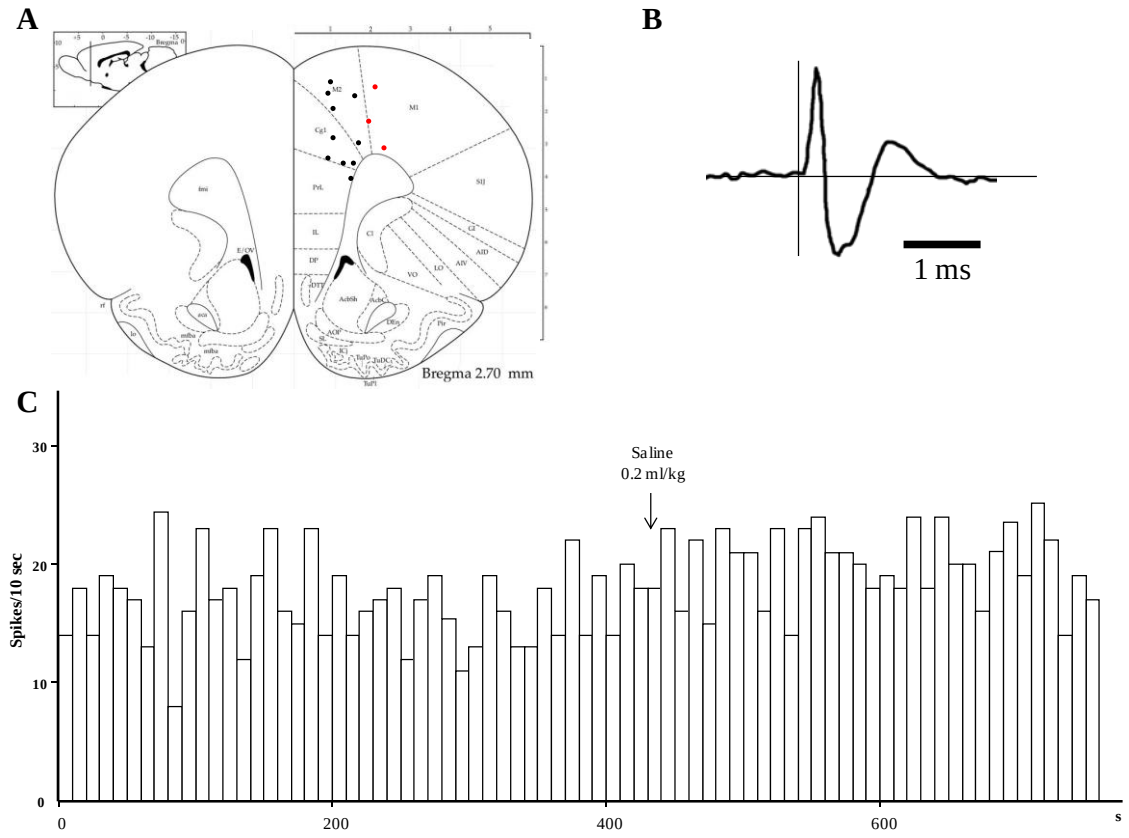


Figure 21: In vivo PFC pyramidal neuron recording example.
(**A**): Recording coordinates of 13 putative pyramidal neurons located in the prefrontal cortex. Ejection of pontamine blue (−400 nA, 20 min) was performed after some recordings to confirm stereotaxic coordinates. Each dot represents the location of one recording (n=13), determined using stereotaxic coordinates. This diagram is a stack of recordings ranging from +2 to +3.5 mm anteroposterior from Bregma. Scales represent distances (in mm) from the midline and the surface of the brain. Reproduced from Paxinos and Watson (1997). Recordings performed further than 1.9 mm from midline (arbitral cut-off, determined using stereotaxic coordinates) were excluded (n=3, red dots). (**B**): Typical action potential waveform (or spike) of a PFC glutamatergic pyramidal neuron. Note that the waveform can be divided into three phases (initial positive depolarisation, repolarisation and hyperpolarisation). Only neurons which displayed such features were recorded. The black bar represents a scale corresponding to 1 millisecond. (**C**): Typical electrophysiological recording of a PFC pyramidal neuron showing the firing rate (number of spikes generated per 10 seconds, represented by each vertical histogram) over a time course, in seconds (s). Please note that saline intravenous administration (0.2 ml/kg) did not alter the firing rate of this neuron.

II-3-B- Methylphenidate and D-amphetamine increase pyramidal neuron activity by a dopamine D_1 receptor dependent mechanism

We have found, in a small population of spontaneously active neurons (n=6), that 3 mg/kg of intravenous methylphenidate, a slightly higher dose to what is used in clinics, significantly increased the basal firing activity of PFC neurons ($p<0.05$, Student's paired t-test, Fig. 22A). In another population of low-firing neurons (n=7, firing activity below 4 spikes per 10 seconds, arbitral cut-off) 3 mg/kg of MPH fails to significantly increase neuronal firing activities (Fig. 22B), although significant firing rate activations (more than a 2-fold increase) were observed in 5 out of 7 neurons. We have also observed a small reduction of this excitatory effect following the administration of SCH23390, a dopamine D_1 receptor antagonist, in three out of four neurons tested this way (75%), in complete agreement with a previous study from our laboratory (Gronier, 2011). In our laboratory, the excitatory effect of MPH on the basal firing activity of PFC neurons has previously been demonstrated in a larger population (n=28) of PFC neurons and was found to be partially reversed (return of the firing rate to its baseline level) in 55% of neurons following the administration of SCH23390 (0.6 mg/kg), a drug that exerted no effects on its own on the basal firing activity of PFC neurons (Gronier, 2011).

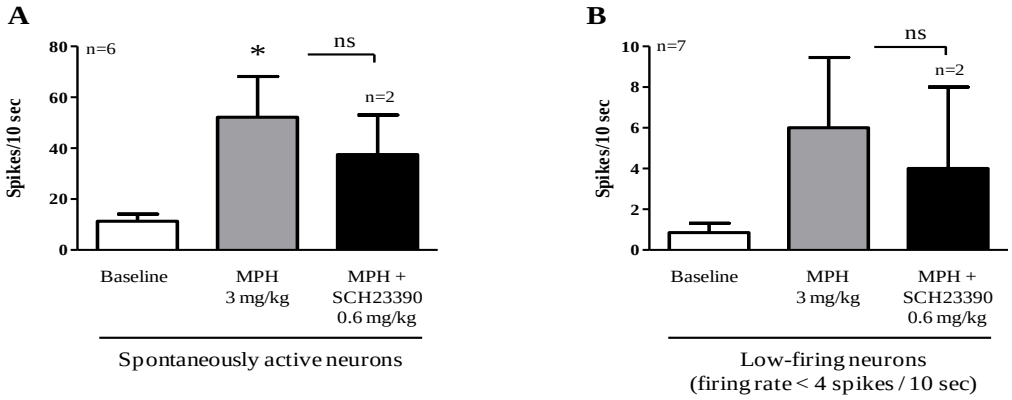


Figure 22: Methylphenidate preferentially increases the firing rate of the spontaneously active PFC neurons through a mechanism partially dependent on dopamine D_1 receptors.
Methylphenidate, administered intravenously at 3 mg/kg, significantly increased the firing rate of spontaneously active pyramidal neurons (**A**), but not of low-firing neurons (firing rate below 4 spikes per 10 seconds) (**B**). Administration of the D_1 receptor antagonist SCH 23390 (0.6 mg/kg) slightly decreased the activation of firing rate elicited by MPH on spontaneously active neurons. Here and in subsequent figures, the same neurons were studied in the complete sequence. ns: non-significant, *P< 0.05 *vs.* baseline, Newman–Keuls test after significant ANOVA.

In a group of 17 spontaneously active neurons, D-amphetamine was cumulatively administered up to a dose of 9 mg/kg (Fig. 23A), by consecutively injecting intravenous doses of 1, 2, 3 and 3 mg/kg. D-amph induced a significant increase of 34% and 207% in mean firing activity of PFC neurons, versus initial basal firing, at the cumulative dose of 3 and 6 mg/kg, respectively ($p<0.01$, compared to baseline levels for the 3 and 6 mg/kg doses, respectively, Newman-Keuls test after significant repeated measures ANOVA, n=17). Activity remained unchanged at the highest dose (9 mg/kg) compared to the 6 mg/kg dose. Three spontaneously active neurons (17%) remained insensitive to D-amph administration and only one neuron exhibited a progressive reduction in firing activity during the course of the cumulative administration of the drug. In another group of low-firing neurons (n=13, firing activity below 4 spikes per 10 seconds), 3 mg/kg of intravenous D-amphetamine significantly increased firing activities by 5 folds over baseline (Fig. 23B). While the majority of neurons (6/13, 46%) exhibited firing activity increases over the course of D-amph administration, 2 neurons (15%) responded by decreases of firing rate, while 5 neurons (39%) remained insensitive to any of the cumulative doses.

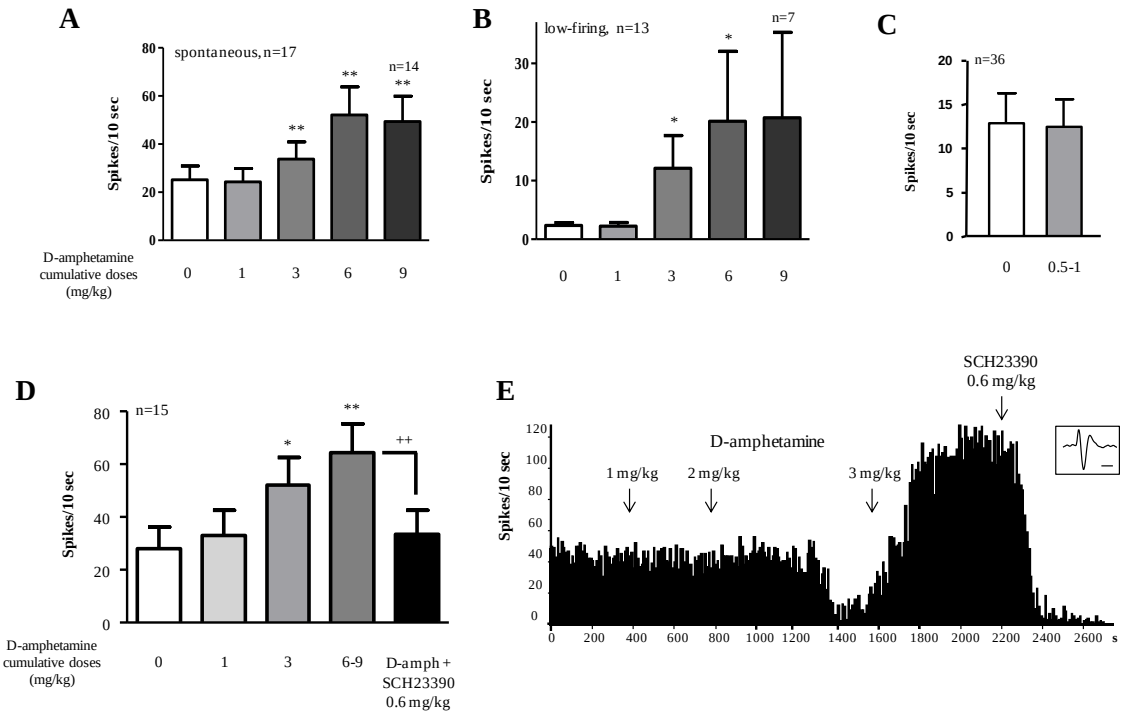


Figure 23: D-amphetamine dose-dependently increased the firing rate of PFC neurons through a dopamine D_1 receptor dependent mechanism.
Starting at 3 mg/kg, cumulative doses of D-amph significantly increased the firing rates of both spontaneously active (**A**) and silent neurons (**B**). However, in another neuronal population, doses of D-amph in the range of 0.5-1 mg/kg did not induce any significant change of basal firing rates (**C**). (**D**): Following cumulative administration of D-amph, selective blockade of dopamine D_1 receptors, using SCH23390 (0.6 mg/kg) significantly decreased the activation of firing rate elicited by D-amph. (**E**): Typical firing rate histogram of a prefrontal cortex neuron during D-amph and SCH23390 administrations. Boxed is shown a typical action potential shape of this pyramidal neuron. Here and in the following boxes, the horizontal black bars below the waveforms represent a 1 ms time interval. Note that in this neuron, a transient firing rate reduction was observed, as seen in few other recordings. Also, note that in this neuron, SCH23390 administration strongly reduced the firing rate following cumulative doses of D-amph (6 mg/kg). *P< 0.05, **P<0.01 *vs.* baseline, ++P<0.01 *vs.* specified condition, Newman–Keuls test after significant repeated-measures ANOVA.

When tested at lower doses (0.5–1 mg/kg) in a larger population of neurons (n=36), D-amph did not have an overall significant effect on basal activity (Fig. 23C), although 12/36 neurons (33%) had their firing activity significantly increased (individual firing activity increasing by at least 5 spikes/10 seconds or significant unpaired Student's t-test when 100 spikes were analysed before and after drug administration in each neuron recorded) compared to their respective baseline levels. Moreover, in this population of 36 neurons treated with low doses of D-amph, the proportion of neurons responding by an increase in firing activity is significantly higher

(33%) compared to neurons treated with saline, where only 3.5% of all neurons responded by an increase in firing rate following saline administration ($p<0.05$, Fisher's exact test). Similarly to what was observed with methylphenidate, Fig. 23D,E shows that the increase in firing activity elicited by cumulative doses of D-amph (6–9 mg/kg) is significantly attenuated by the subsequent administration of 0.6 mg/kg of the D_1 receptor antagonist SCH23390 ($p<0.01$ compared to levels obtained after 6-9 mg/kg of D-amph, Newman-Keuls test after significant repeated measures ANOVA). Complete reversal occurred in 9 out of 15 neurons tested this way (60%), while the firings of 6 neurons were not affected following SCH23390, despite D-amph was found to more than double their basal firing activities (Table 2). One should note that transient firing rate reduction is sometimes observed, as shown in Fig. 23E. Such temporary firing rate inhibitions are often recovered during the recording period, usually within 100-200 seconds, as also observed in Fig. 23E. In 2 out of 4 neurons, pre-administration of SCH23390 at 0.8 mg/kg successfully prevented D-amph-induced firing activation of pyramidal neurons, although there was more than a two-fold increase of firing activities in the 2 other neurons (Fig. 24A). Interestingly, blockade of both dopamine D_1 receptors and alpha-1 receptors (respectively with 0.8 mg/kg of SCH23390 and 0.6 mg/kg of prazosin) completely prevented the subsequent D-amph-induced firing activation, even with doses of D-amphetamine reaching 4 mg/kg (Fig. 24B).

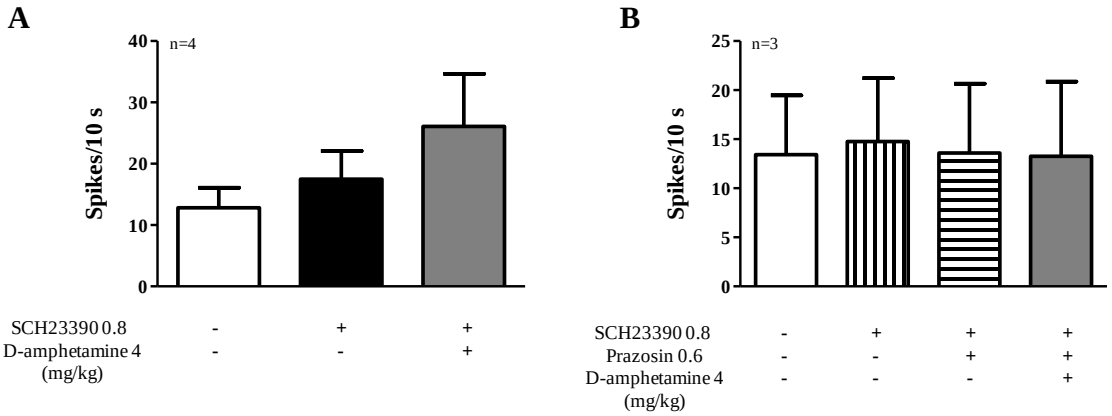


Figure 24: D-amphetamine requires functional dopamine D_1 and adrenergic alpha-1 receptors to induce firing rate increases.
Dopamine D_1 receptor blockade (SCH23390 0.8 mg/kg) alone (**A**) only partially prevented the subsequent D-amph-induced firing rate activation of PFC neurons, an effect totally prevented by both dopamine D_1 (SCH23390 0.8 mg/kg) and adrenergic alpha-1 receptor (prazosin 0.6 mg/kg) blockades (**B**). Note that SCH23390 and prazosin have no action on their own on firing rate levels.

II-3-C- Dose-dependent activating effects of ATX and effects of selective catecholamine antagonists on ATX-induced firing activation

Atomoxetine was cumulatively administered up to a dose of 9 mg/kg, by injecting sequential doses of 1, 2, 3 and 3 mg/kg. At clinically relevant doses (1 and 3 mg/kg) ATX did not increase significantly the basal activity of spontaneously active pyramidal neurons (Fig. 25A). A cumulative dose of 6 mg/kg significantly increased firing rate by 69% over baseline ($p<0.001$, Student's paired t-test). Ten neurons were further injected with a cumulative dose of 9 mg/kg of ATX, but this dose did not increase further the firing activities of spontaneously active neurons. When tested on silent neurons (n=7), a cumulative dose of ATX reaching 6 mg/kg also significantly increased firing activity very potently ($p<0.05$, Student's paired t-test, Fig. 25B). Interestingly, unlike methylphenidate or D-amphetamine, the same dose of ATX more potently increases firing activities of silent neurons than spontaneously active neurons. Indeed, 6 mg/kg of ATX increases baseline firing activity by 69% in spontaneously active neurons and induced massive firing activity enhancement in almost silent neurons (see paragraph below).

We have found previously that the different selective monoamine receptor antagonists SCH23390, prazosin and yohimbine exerted no effects on their own on the basal firing activity of PFC neurons (Gronier 2011). We then confirmed previous established data regarding neuronal insensitivity to yohimbine 1 mg/kg, SCH23390 0.6 mg/kg and prazosin 1 mg/kg (respectively n=5, n=5 and n=4). As observed before in our laboratory (Gronier 2011), these selective monoamine receptor antagonists exerted no effects on their own on the basal firing activity of PFC neurons compared to respective baseline levels (Fig. 24B, 25C, paired Student's t-test). In contrast to our previous findings with MPH and D-amph, administration of the D_1 dopamine receptor antagonist SCH23390 did not significantly reduce the mean excitatory effect produced by cumulative doses of ATX (Newman-Keuls test after significant repeated measures ANOVA, Fig. 25C, Table 1). However, in three out of seven neurons tested, a reduction of activation by more than 30% was observed. Similarly, administration of the α1 adrenoceptor antagonist prazosin, following cumulative doses of ATX, had no significant effect on the neuronal activation induced by ATX (Fig. 26A), although in three neurons out of seven tested, ATX-induced activation was reduced by more than 50% following prazosin administration. Fig. 26B represents the firing histogram of such a neuron whose firing activation induced by ATX was reduced by prazosin. In a group of seven neurons initially insensitive to ATX (cumulative doses reaching 3 mg/kg produced no significant change in firing activity), the α2 adrenergic antagonist yohimbine (1 mg/kg) promoted a significant activation of firing ($p<0.01$, compared to values obtained in baseline conditions or after ATX, Newman-Keuls test after significant repeated measures ANOVA, Fig. 26C). Moreover, a further administration of the dopamine D_1 antagonist SCH23390 reversed this stimulatory effect in two out of the four neurons were tested this way (Fig. 26D).

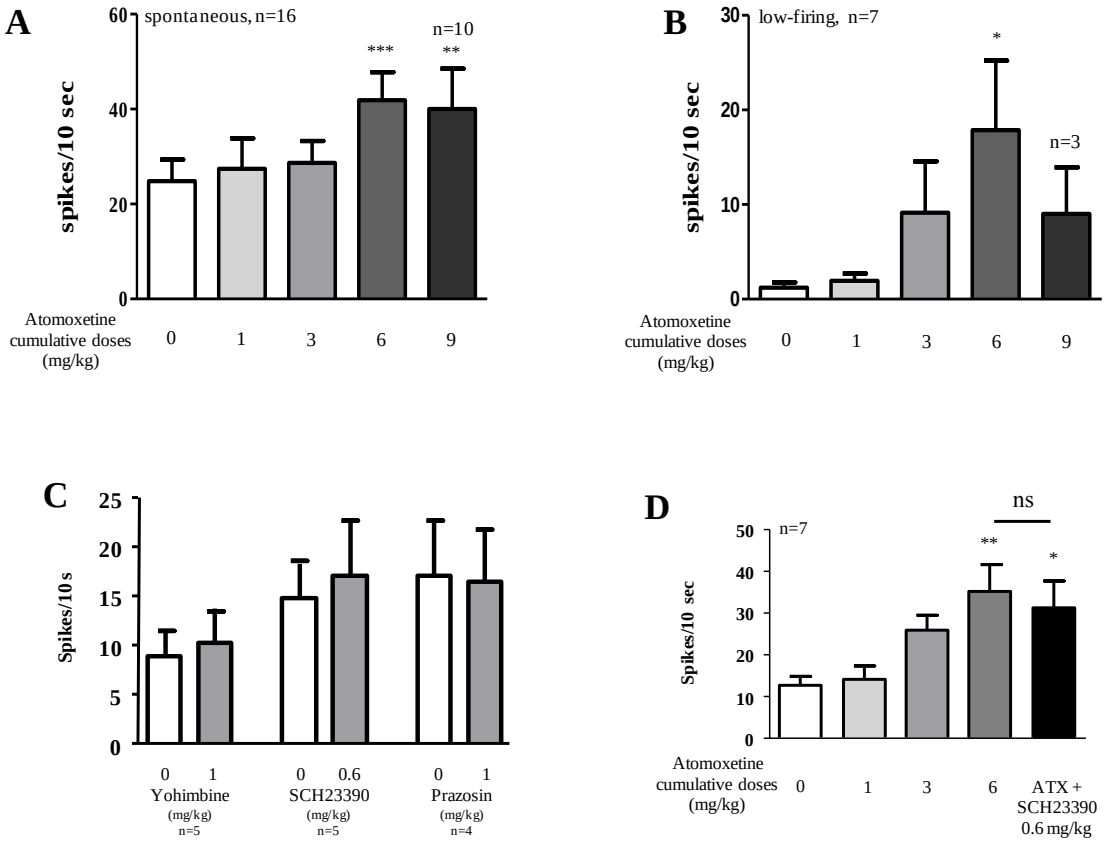


Figure 25: Effects of selective dopamine D_1 receptor antagonism on atomoxetine-induced activation of PFC neurons.

Administration of ATX, at a cumulative dose of 6 mg/kg, significantly increased the firing rates of spontaneously active (**A**) and silent neurons (**B**). (**C**): Neither yohimbine (1 mg/kg), SCH23390 (0.6 mg/kg) nor prazosin (1 mg/kg) could alter the firing rates of PFC pyramidal neurons. (**D**): Subsequent dopamine D_1 receptor blockade did not reverse ATX-induced firing rate activation, although 2 out of 4 neurons had their firing rates reversed following SCH23390 administration. *P< 0.05, **P<0.01, ***P<0.001 *vs.* 0 mg/kg, Newman–Keuls test after significant repeated-measures ANOVA.

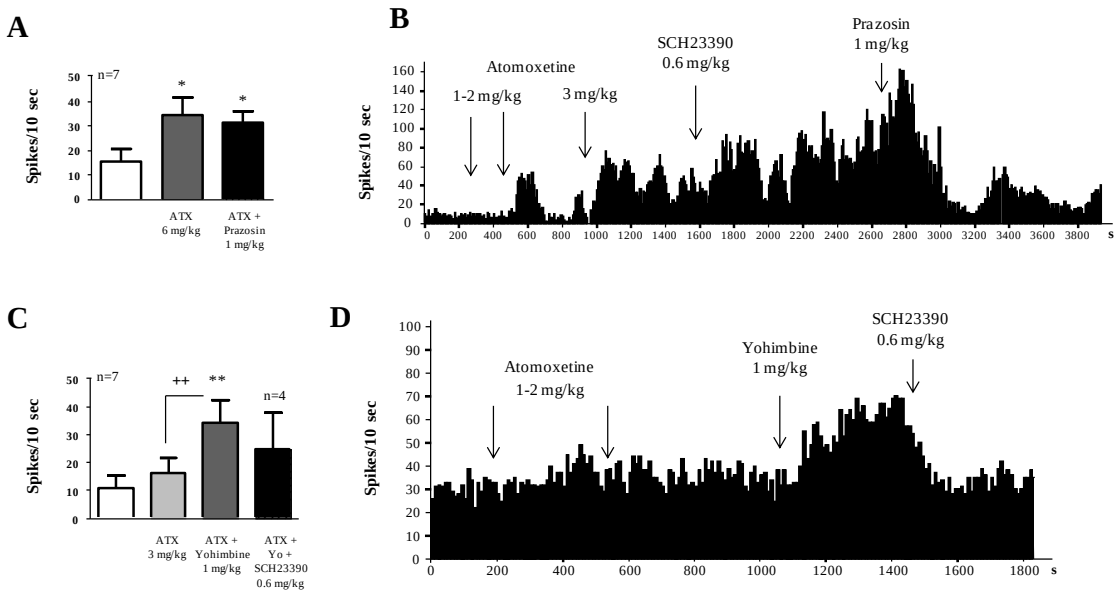


Figure 26: Effects of selective monoamine receptor antagonists on atomoxetine-induced activation of PFC neurons.
(**A**): Alpha-1 receptor antagonism (prazosin, 1 mg/kg) could not significantly reverse ATX-induced activation of PFC neurons. (**B**): However, in some neurons (3/7), prazosin strongly reduced ATX-induced firing rate activation. (**C**, **D**): The α2 receptor antagonist yohimbine further increased the activation of firing rate induced by a cumulative dose of 3 mg/kg of ATX. This activation is partially dependent on dopamine D_1 receptors. In two neurons out of 4 tested this way, shown in **D**, firing rate activities were dramatically increased following administrations of yohimbine, unlike other neurons tested this way. *P<0.05, **P<0.01 *vs.* baselines, ++P<0.01 vs. values obtained immediately before administration of yohimbine, Newman–Keuls test after significant repeated measures ANOVA.

II-3-D- ADHD drugs preferentially stimulate the firing activity of spontaneously active PFC neurons

Sixty-seven percent (16/24) of all neurons with low-firing (firing rate below 4 spikes per 10 seconds) or no baseline activity (silent neurons, see Fig. 27B) became spontaneously active or had their firing activity significantly increased, following the administration of the ADHD drugs, regardless of the type of drug tested. On the other hand, almost every spontaneously active neuron (36/39, 92%) was responsive to any of the ADHD drugs tested, which is significantly different from low-firing neurons ($p<0.05$, Chi-square test). Indeed, all spontaneously active neurons (6/6) tested with 3 mg/kg of methylphenidate increased their firing rate activities, while 5/7 low-firing neurons (firing < 4 spikes / 10 sec) had their firing activities increased. Similar results were observed with D-amphetamine, where 15/16 spontaneously active

neurons (94%) responded by an increase of firing rate following D-amph, while 6/9 low-firing neurons (67%) increased their firing rates. When atomoxetine was tested, 15/17 spontaneously active neurons (88%) responded by an increase in firing rate, while 5/7 low-firing neurons (71%) increased their firing rates. Therefore, ADHD drugs may act preferentially, or more efficiently, on spontaneously active neurons.

II-3-E- ADHD drugs potentiate NMDA-induced firing activation

Local application of NMDA (10 nA) onto PFC pyramidal neurons induced firing rate increases by a mechanism that is partially NR_{2B}-dependent. Indeed, when the NMDA receptor 2B antagonist Ifenprodil (Brittain *et al.*, 2012; Korinek *et al.*, 2011) was administered (2.5 mg/kg), local NMDA application produced significantly lower ($p<0.05$, Newman–Keuls test after significant one way ANOVA) firing rate increases (Fig. 27A,B). Although NMDA stimulation was strongly reduced following Ifenprodil intravenous administration, AMPA stimulations remained insensitive to cumulative doses of Ifenprodil (Fig. 27C).

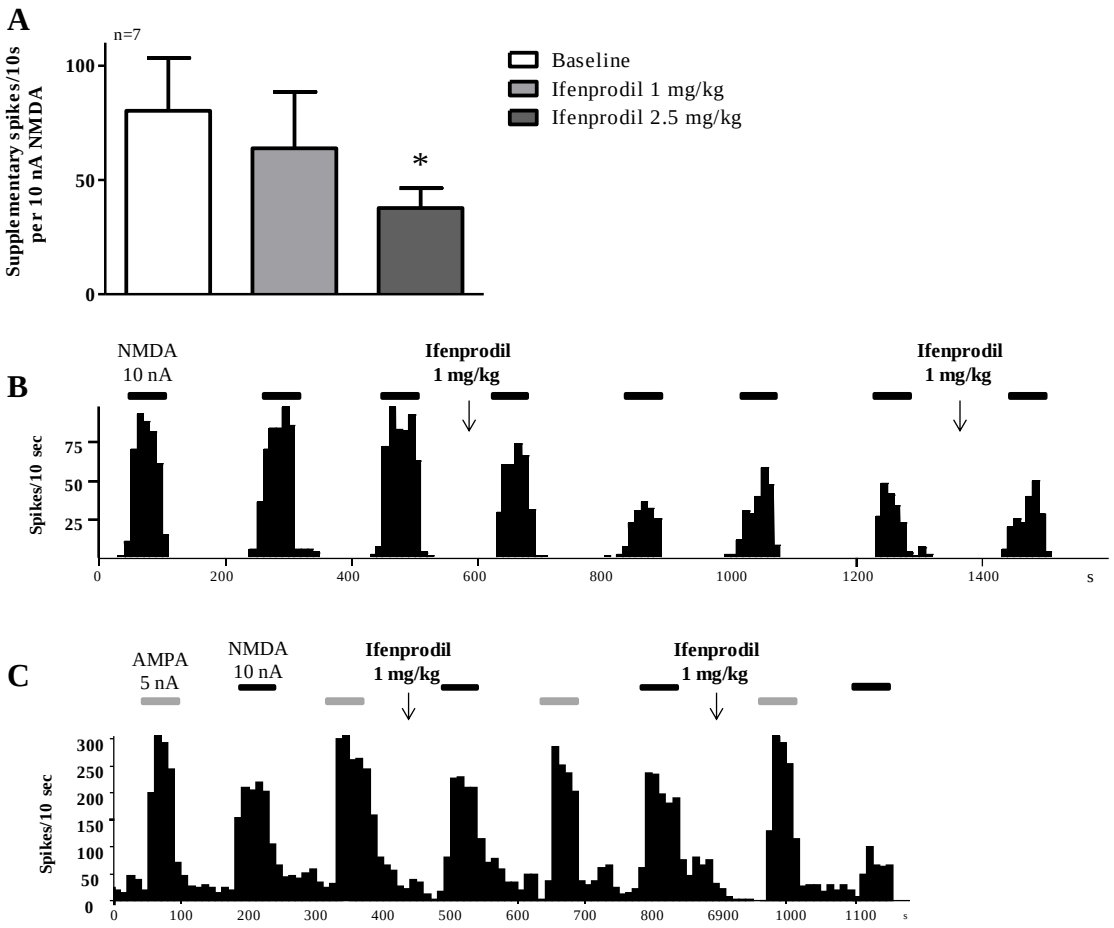


Figure 27: Local NMDA application induces transient firing rate activation of pyramidal neurons by a partial NMDA receptor 2B dependent mechanism.
(**A**): Administration of the specific NR_{2B} antagonist ifenprodil (2.5 mg/kg) significantly reduced NMDA-induced firing rate activation. (**B**, **C**): Typical recordings showing that NMDA-induced activations of firing rates partially decrease with cumulative administrations of ifenprodil, an effect not observed using AMPA microiontophoresis. Each top horizontal bar represents the pulsed current applied onto the neuron, therefore inducing transient neuronal activation (NMDA −10 nA, AMPA −5 nA). *P< 0.05 *vs.* baseline, Neuman-Keuls test after significant repeated measures ANOVA.

When applied using the same -10 nA current, NMDA and AMPA produced large increases in firing activity which typically increased baseline levels by 40-120 additional spikes per 10 s during the ejection period. Both NMDA and AMPA had a similar strong potential in inducing firing rate increases (Fig. 28A). Generally, we used lower AMPA (5 nA) than NMDA (10 nA) currents. Both NMDA and AMPA significantly increased burst activities ($p<0.001$, paired Student's t-test. Fig. 28B). However, NMDA was significantly more efficient than AMPA in increasing such burst

activities ($F_{(3,196)}$=6.38, p<0.001, Bonferroni test after significant two-way ANOVA, Fig. 28B).

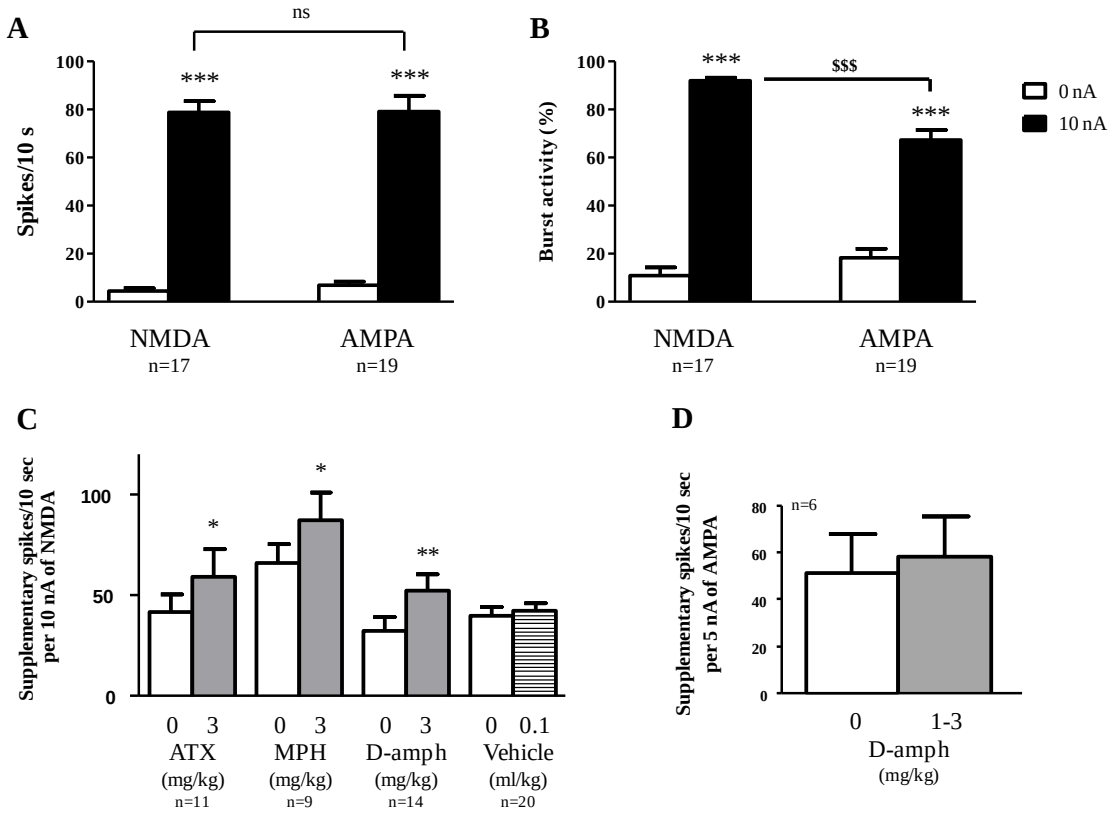


Figure 28: ADHD drugs increase NMDA-induced activation of PFC neurons.
Local applications of NMDA (10 nA) and AMPA (10 nA) significantly increased the firing rates (**A**) and burst activities (**B**) of PFC pyramidal neurons. NMDA was more potent than AMPA in inducing burst discharges, using the same 10 nA current. (**C**): All three ADHD drugs, administered intravenously at 3 mg/kg, significantly increased NMDA neurotransmission in PFC pyramidal neurons. (**D**): D-amphetamine injections (1-3 mg/kg) did not change the ability of AMPA (5 nA) to induce transient neuronal activations. ***P<0.001 *vs.* respective 0 nA currents, $$$P<0.001 *vs.* 10 nA currents, Bonferroni test after significant two-way ANOVA (**A**-**B**), *P< 0.05, **P<0.01 *vs.* respective controls (white bars), paired Student's t-test (**C**-**D**).

Only neurons on which NMDA applications produced consistent neuronal activations over time were selected. After at least four to seven consistent responses to NMDA, saline or ADHD drugs were administered at cumulative doses. The activation of firing induced by NMDA was significantly increased following the intravenous administration of MPH (3 mg/kg, p<0.04, n=9, paired Student's t-test), ATX (3 mg/kg, p<0.03, paired Student's t-test, n=11) and D-amph (3 mg/kg, p<0.01, paired Student's t-test, n=14) but remained unchanged following the administration of the vehicle (Fig. 28C). On the other hand, the response of neurons to AMPA application

(-5 nA) was also tested on a group of neurons treated with D-amph (n=6). No significant effect was observed, though two neurons exhibited an increase in both their NMDA and AMPA responses after D-amph administration (1-3 mg/kg) by more than 30% (Fig. 28D). Most of the neurons (14/18 in total) that were silent in basal conditions had their responses to NMDA significantly increased in response to the administration of the stimulant drugs but remained silent or with a very low baseline activity during the course of the experiment between two NMDA ejections (Fig. 29A-B). On the other hand, spontaneously active neurons generally showed an increase in both baseline and NMDA-induced firing (Fig. 29C-D). Lower doses of D-amph (0.5–1 mg/kg), closer to therapeutic ranges, did not change significantly the mean NMDA response (Fig. 30A). Although these low doses of D-amph failed to significantly change NMDA-induced firing activation in this population of 19 neurons, in 7 neurons (37%), such doses of D-amph still increased by more than 20% the mean NMDA response of these neurons (Fig. 30B, Table 2). Eleven out of fourteen neurons (79%) responded to the 3 mg/kg dose of D-amph (Table 2). In a group of 5 neurons, this effect was found to be dependent upon dopamine D_1 receptors (Fig. 30B-D), as a strong reduction of NMDA-induced firing activity was observed following SCH23390 administration in 4 neurons ($p<0.05$ when comparing values obtained immediately before and after administration of SCH23390, Newman–Keuls test after significant repeated measures ANOVA). On the other hand, in one other neuron tested (not included in the graph), the NMDA response was further increased following SCH23390. When neurons were pre-treated with SCH23390 (0.6 mg/kg), the administration of D-amph at 3 mg/kg did not change significantly the neuronal response to NMDA, though two neurons out of the five still had their response increased by 20% following D-amph (Fig. 31A-B). Table 2 summarises all data obtained in the present study, showing the proportion of neurons responding to the administration of the different drugs.

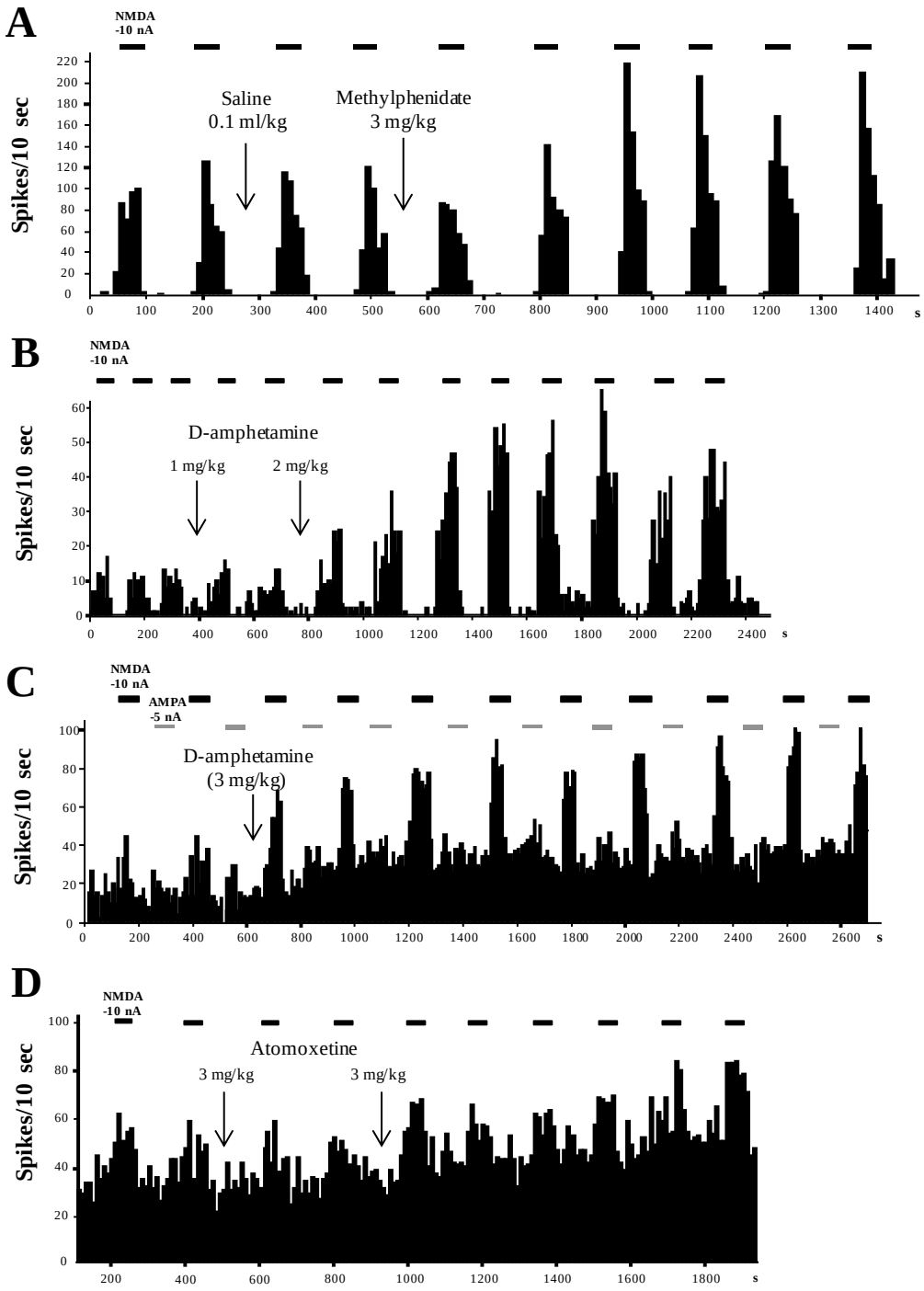


Figure 29: Individual neuronal responses to local NMDA application following ADHD drug intravenous administrations.
Illustrating recordings showing local NMDA neurotransmission increases following 3 mg/kg of MPH (**A**) and D-amph (**B**, **C**), while another neuron only mildly increased its NMDA-induced firing rate activation following 6 mg/kg of cumulative ATX doses, in conjunction with increases of baseline firing activity (**D**).

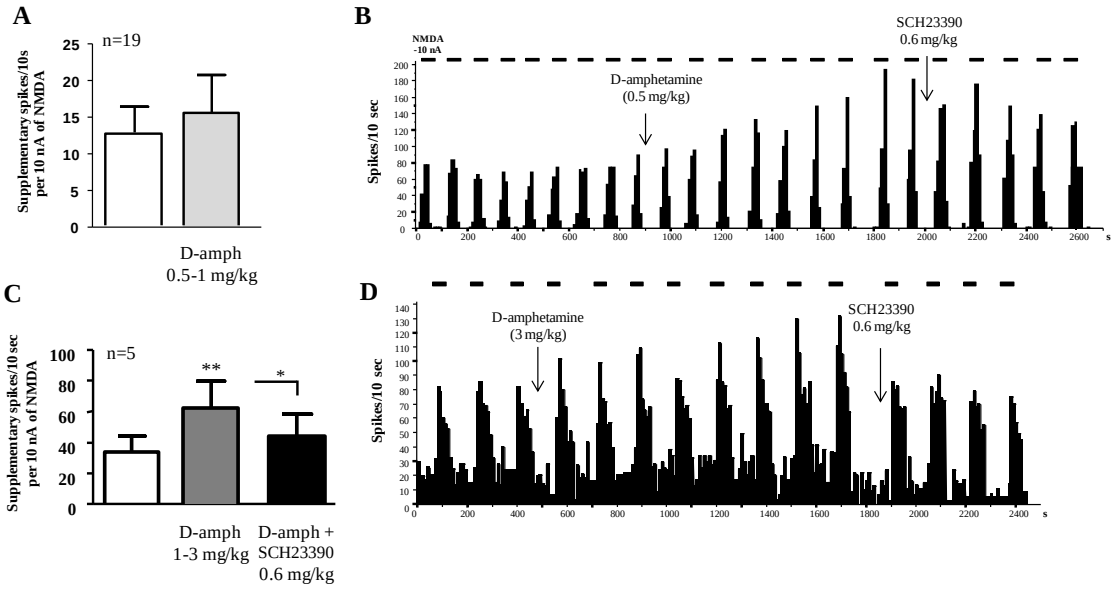


Figure 30: Effect of dopamine D_1 receptor blockade on NMDA-induced activation of PFC neurons induced by D-amphetamine.

Therapeutic doses of D-amph (0.5-1 mg/kg) did not change NMDA-induced firing rate activations in the population of 19 neurons recorded (**A**), although 7 neurons out of 19 had their NMDA-induced firing rates significantly increased by more than 20% (**B**). (**C**, **D**): Dopamine D_1 receptor blockade (SCH23390 0.6 mg/kg) significantly reversed the NMDA-induced firing rate activations induced by D-amph. The neurons in **B** and **C** showed an increase in their NMDA responses caused by D-amph, an effect partially reversed following dopamine D_1 receptor antagonism. The same neurons were recorded during the complete sequence. **P<0.01 *vs.* baseline, *P<0.05 *vs.* corresponding values obtained immediately before administration of SCH23390, Newman–Keuls test after significant repeated measures ANOVA.

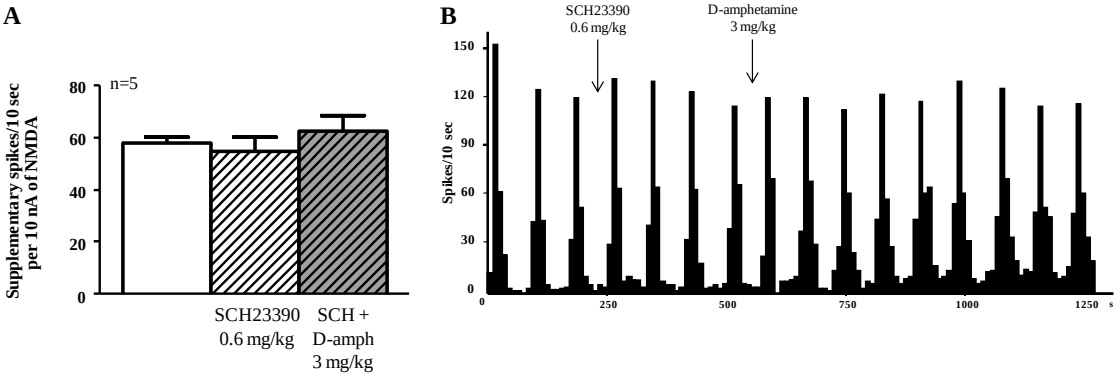
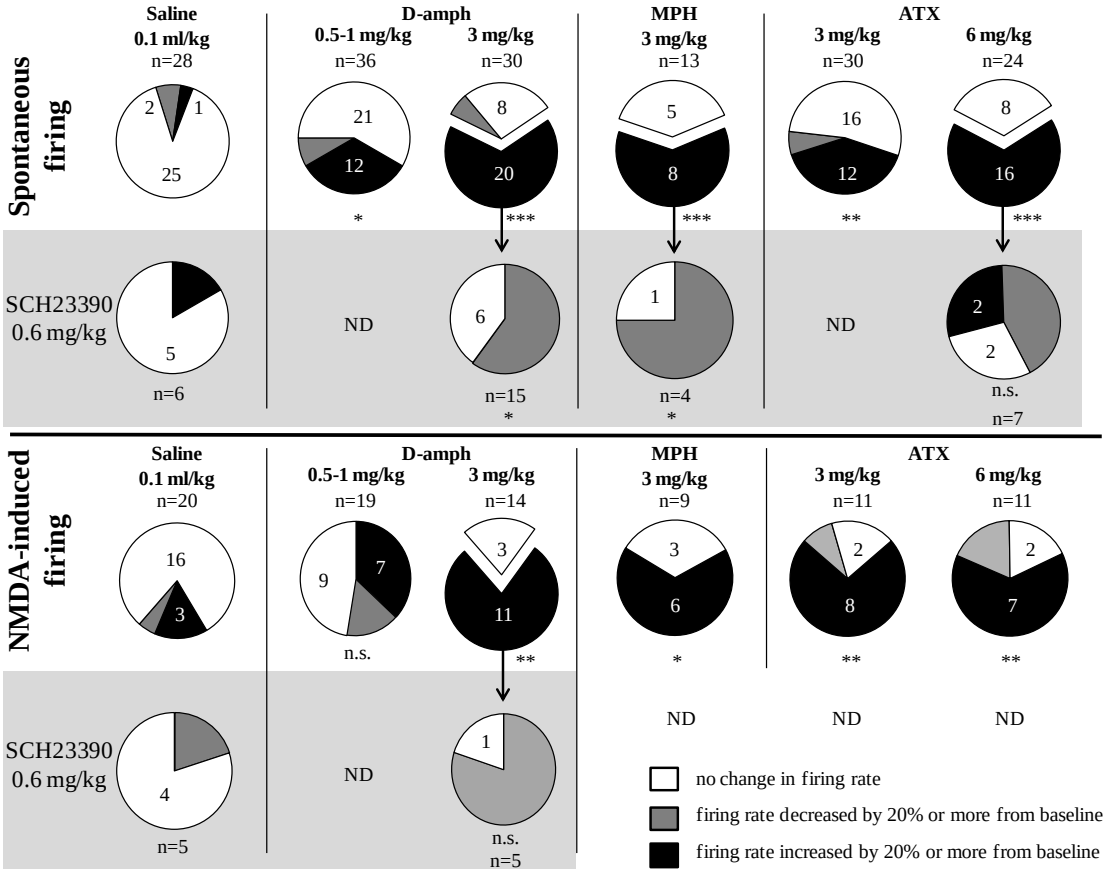


Figure 31: Effect of dopamine D_1 receptor blockade on subsequent NMDA-induced activation of PFC neurons induced by D-amph.

(**A**, **B**): Pre-administration of a dopamine D_1 antagonist (SCH23390, 0.6 mg/kg) successfully prevented subsequent increase of NMDA neurotransmission induced by 3 mg/kg of D-amph.

Table 2: Proportion of neurons showing significant or non-significant changes in basal firing rate and in their responses to the iontophoretic application of NMDA to the different ADHD drugs and to SCH23390.

Neuronal population responses to the three ADHD drugs were evaluated on spontaneous firing activity (top) and on NMDA-induced firing activity (bottom). The dopamine D_1 antagonist SCH23390 was subsequently injected when neurons had their firing activity increased by the different drugs. n.s.: not significant, ND: not determined *P<0.05, **P<0.01, ***P<0.001 versus corresponding control (saline) conditions, Fisher's exact test



II-3-F- Iontophoretic drug ejections induce spike amplitude variations without affecting spontaneous firing activity nor NMDA-induced responses

When drugs were locally applied onto neurons using iontophoresis, such currents induced strong action potential amplitude variations. Indeed, when saline (NaCl 130 mM) was applied by iontophoresis, positive ejection currents (+5 nA) resulted in significant spike amplitude increase (40% amplitude gain) whilst negative currents (-5 nA) induced spike amplitude decreases (22% amplitude loss, Fig. 32A). Ejection of NMDA using a 5 nA negative current also induced a 25% amplitude depolarisation, while ejection of 5 nA positive currents of D-amphetamine and

methylphenidate all induced significant action potential amplitude increases (55% and 95%, respectively). However, dopamine failed to induce any significant spike amplitude variation under such conditions. Specific examples of action potential amplitude variations under local drug delivery by iontophoresis are represented in Fig. 32B.

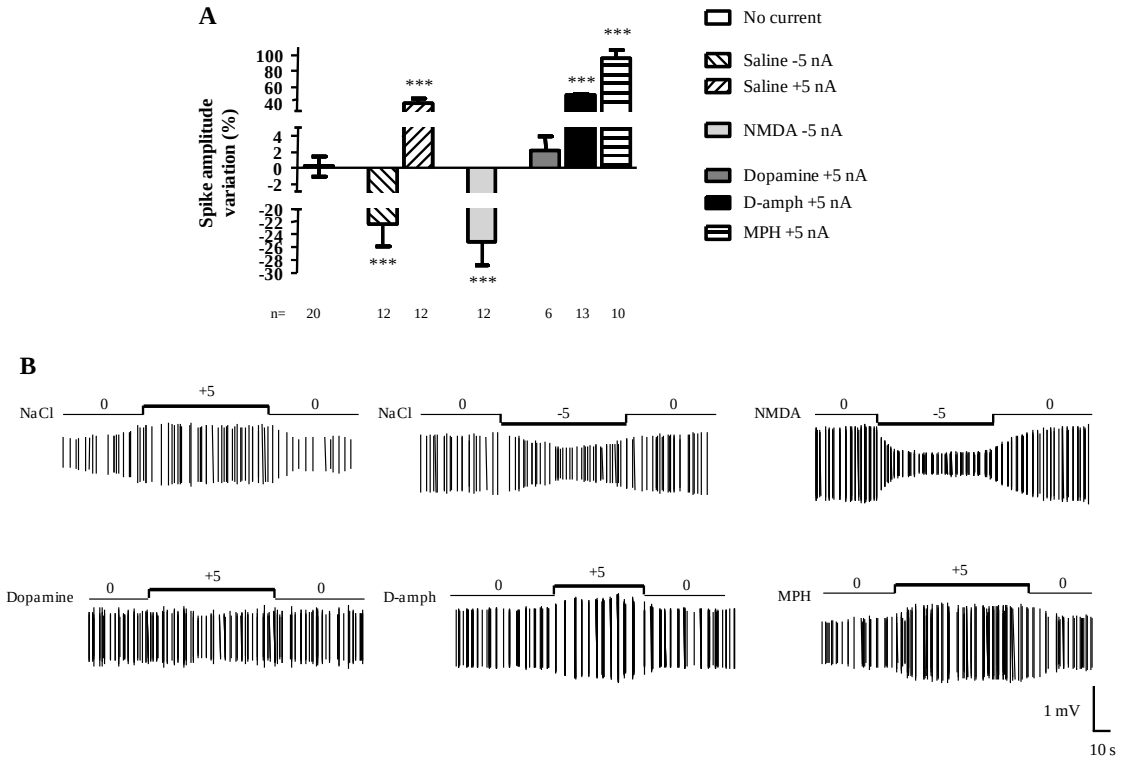


Figure 32: Effect of iontophoretically-applied molecules on spike amplitudes of PFC pyramidal neurons.
(**A**): Microiontophoresis of saline, NMDA and the 3 ADHD drugs MPH, D-amph and ATX induces electrical variations of spike amplitudes in pyramidal neurons. Here, positive currents increased spike amplitudes while negative currents decreased spike amplitudes. Please note that the ordinates are not linearly drawn. (**B**): Representative electrophysiological traces showing either increases or decreases in electrical spike amplitudes when molecules are iontophoretically applied. Note that NMDA induced strong amplitude variations compared to the other drugs.

Dopamine, MPH and D-amph did not change pyramidal neurons spontaneous firing activities when applied locally (Fig. 33A). When these drugs were applied locally in combination with NMDA pulses, no change was observed in the NMDA-induced firing activity of such neurons (Fig. 33B), although locally-applied dopamine had mixed effects (increase/decrease of NMDA responses in 50% of all recordings, Fig. 33B,D1,D2).

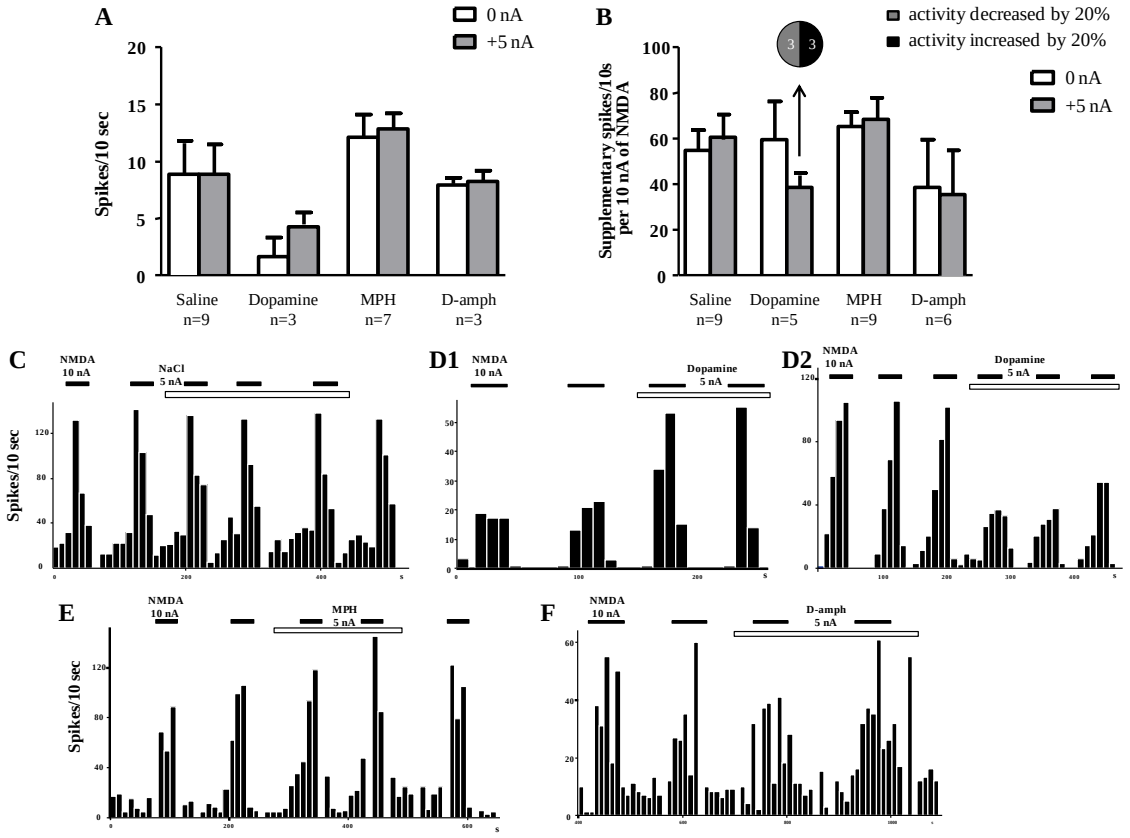


Figure 33: Effect of iontophoretically-applied dopamine, MPH and D-amph on electrical parameters of PFC pyramidal neurons.

Using currents of +5 nA, neither saline, dopamine, MPH or D-amph could alter the firing rates (**A**) or the burst activities (**B**) of pyramidal neurons. While co-application of saline and NMDA does not affect neuronal responses to NMDA (**C**), some neurons displayed either strong activation (3/6 neurons, **D1**) or reduction (3/6 neurons, **D2**) of their NMDA responses following co-application of both dopamine and NMDA. Both MPH (**E**) and D-amph (**F**) did not have an overall effect on the NMDA neurotransmission of PFC pyramidal neurons.

II-3-G- Influence of locally applied dopamine on NMDA-induced firing of striatal medium spiny neurons and acute MPH administration

In presumed striatal medium spiny neurons (MSN), local application of NMDA, using microiontophoresis, produced reversible firing rate activation (Fig. 34). Very interestingly, when dopamine was applied at 10 nA and together with NMDA, 6 neurons responded by a decrease in NMDA-induced firing rate activation, an effect that was recovered when ejection of dopamine was stopped (Fig. 34A,C). However, 4 MSN responded by an increase of NMDA-induced firing rate activation, an effect that was also recovered with dopamine retention (Fig. 34B,D). Such effects (potentiation/dampening) differ greatly from what was observed previously in the PFC, where locally-applied dopamine had only minimal effects on NMDA responses. These results highlight the importance of the strong regulation of NMDA neurotransmission by local dopamine in striatal medium spiny neurons, where it can either dampen and/or strengthen glutamate neurotransmission, as seen before on avian MSN (Ding *et al.*, 2002).

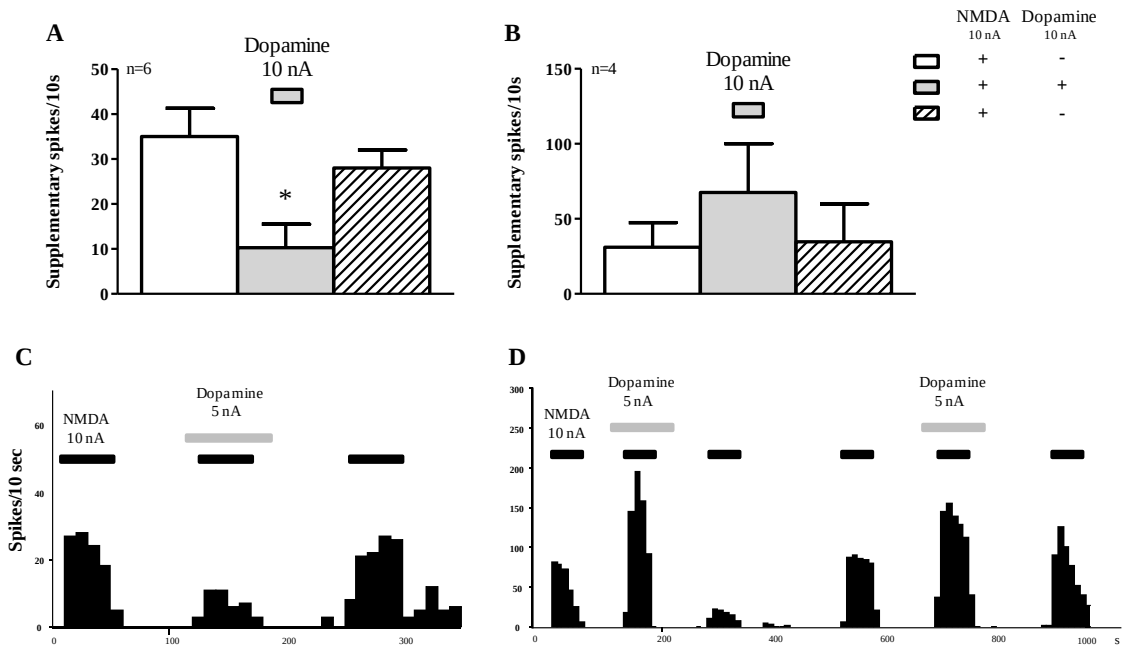


Figure 34: Modulation of the NMDA neurotransmission of striatal GABAergic medium spiny neurons by dopamine.
When dopamine (10 nA) was locally applied onto medium spiny neurons (MSN) simultaneously with NMDA (10 nA), two neuronal populations could be distinguished. Some neurons (6/10) displayed significant firing rate dampening (**A**, **C**) while others (4/10) displayed non-significant firing rate potentiation (**B**, **D**). When dopamine iontophoresis was no longer applied, NMDA-induced firing rates were recovered immediately. *P<0.05 vs. NMDA 10 nA, Newman–Keuls test after significant ANOVA.

Methylphenidate was acutely administered at a dose of 1 mg/kg and produced a significant increase ($p<0.027$, paired Student's t-test) of the NMDA-induced firing rate activation of medium spiny neurons (Fig. 35). A cumulative dose of 2 mg/kg of MPH further increased such effects, suggesting that glutamatergic neurotransmission of MSN neurons is altered following MPH exposure. Compared to what was found in the PFC, methylphenidate produced significant effects at lower doses, close to therapeutic doses, in the striatum.

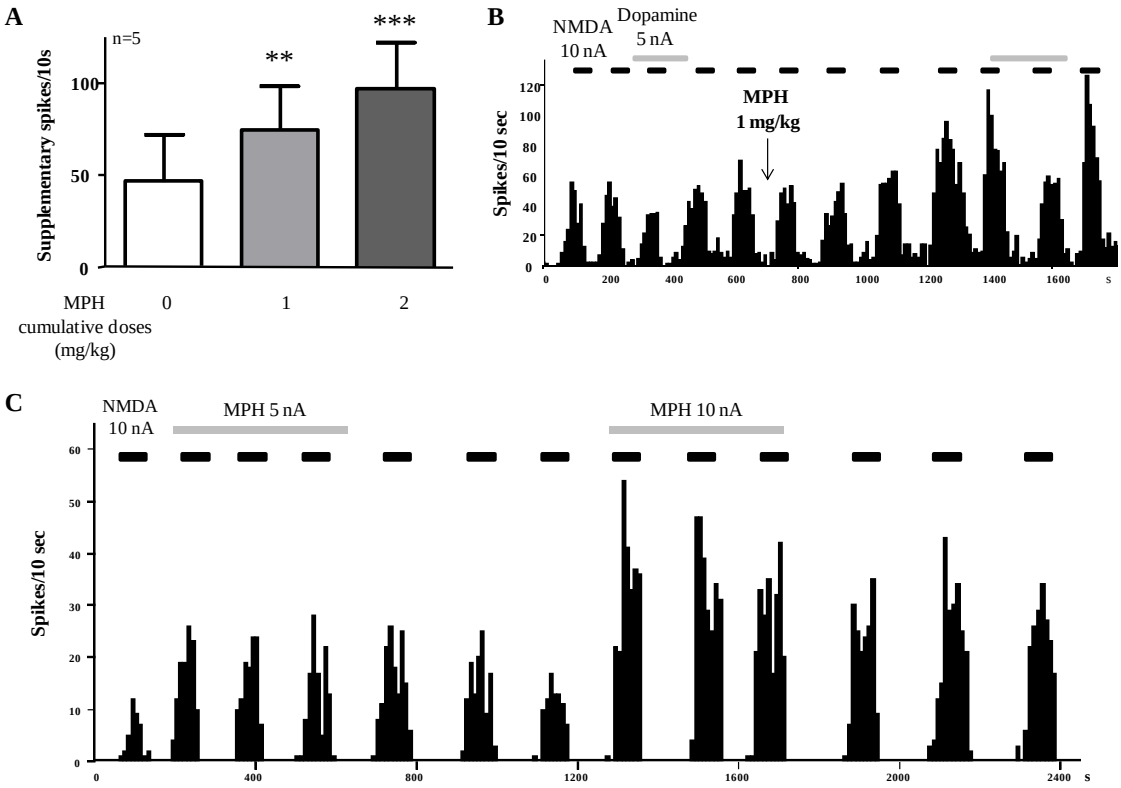


Figure 35: Modulation of the NMDA neurotransmission of striatal GABAergic medium spiny neurons by methylphenidate.
(**A**): Intravenous administration of 1-2 mg/kg of MPH significantly increased the firing rate activations observed during NMDA applications. (**B**): Example of one neuronal recording showing potentiation of NMDA neurotransmission by intravenous MPH (1 mg/kg). Here, local dopamine application following MPH exposure led to a strong reduction of the firing rate induced by NMDA applications. (**C**): Example of one out of five striatal medium spiny neurons where local applications of MPH (5 and 10 nA) dose-dependently potentiate NMDA responses. **P<0.01, ***P<0.001 *vs.* baseline, Newman–Keuls test after significant repeated measures ANOVA.

II-3-H- Acute psychostimulant administration enhances locomotor activity and $NMDAR_{2B}$ protein expression in the striatum

Behavioural traits such as rearing, grooming and scratching were assessed in single-housed rats during 60 minutes after a single D-amphetamine or methylphenidate administration. We observed that D-amphetamine, administered intraperitoneally at a single dose of 3 mg/kg, significantly decreased the grooming behaviour after the first 15 minutes and until the end of the observation period (Fig. 36A1,A2). D-amph did not change the scratching behaviour (Fig. 36B1,B2), but had an immediate effect in increasing the rearing activity (Fig. 36C1,C2). The D-amph-induced increased rearing activity remained 300% higher than the control condition throughout the observation period. In contrast, methylphenidate did not induce any significant changes in scratching or grooming activities when administered at 5 mg/kg (Fig. 36A3,B3). However, 5 mg/kg of MPH significantly increased rearing activity after 45 minutes (Fig. 36C3), but this effect was milder than the one induced by D-amph. We show here that acute psychostimulant exposure increased vertical locomotor activity, which could be one amongst many consequences of increased cortical excitability, as seen in previous paragraphs. Please note that these results were obtained from 4 animals in each group and that such a small sampling size could be considered too small.

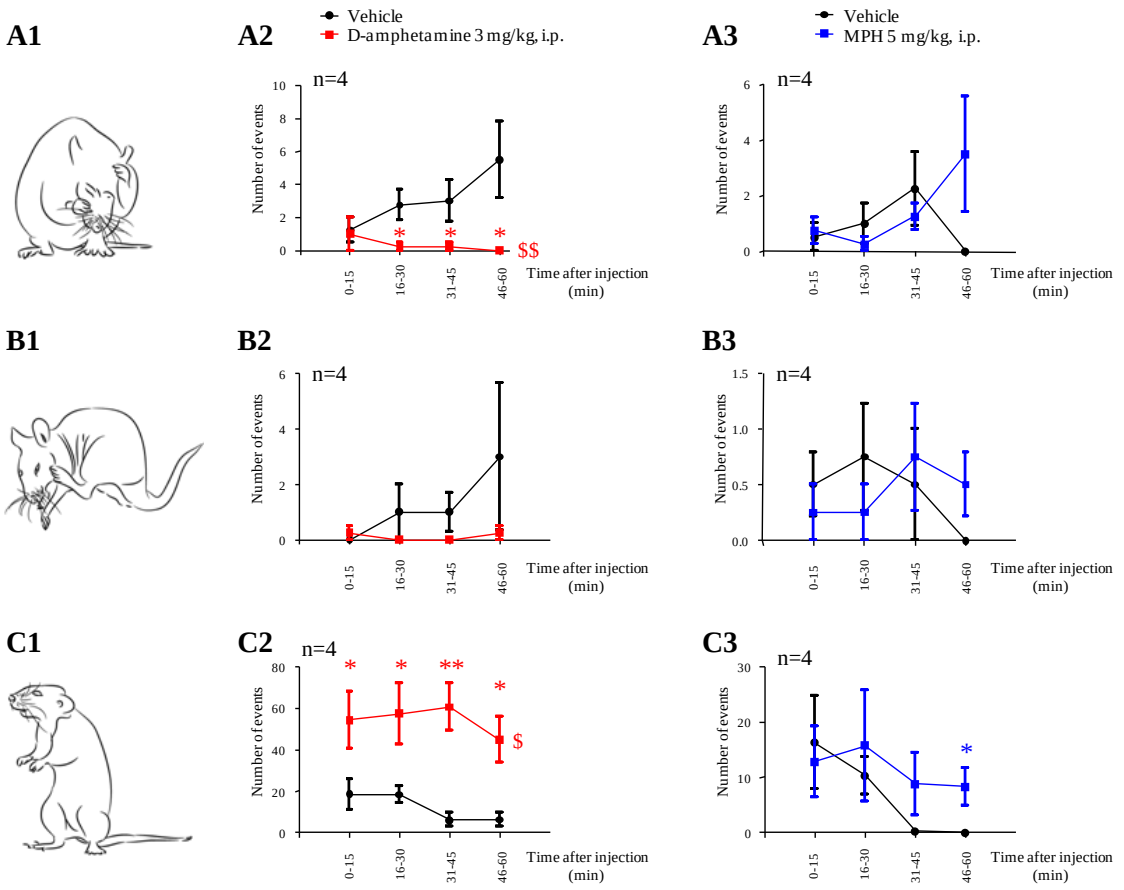


Figure 36: Behavioural effects of psychostimulant exposure using D-amphetamine and methylphenidate.
In this experiment, 3 different behavioural traits such as grooming (**A1**), scratching (**B1**) and rearing (**C1**) were analysed during the first 60 minutes following intraperitoneal drug exposure. (**A2, A3**): Exposure to D-amph (3 mg/kg), but not MPH (5 mg/kg), significantly decreased the cumulative number of grooming events during the entire observation period. Scratching events were not altered following either drug exposure (**B2**, **B3**). D-amph strongly increased the number of rearing events throughout the recording period (**C2**), an effect only seen during the last 15 minutes of the observation period (46-60 minute window) when MPH was used (**C3**). *P<0.05, **P<0.01 *vs.* vehicle, unpaired Student's t-test. $P<0.05, $$P<0.01 *vs.* vehicle, Bonferroni test after significant repeated measures two-way ANOVA.

We then examined the impact of such injections on the NMDA receptor 2B protein expression (NR_{2B}) using western blotting techniques in order to determine if a correlation between our electrophysiological results and NR_{2B} protein could exist. Once set up, the blotting techniques were qualitatively reliable and routinely used. As a matter of fact, consistent results were always observed. We observed a significant increase of the NR_{2B} protein expression in the PFC after D-amph administration (Fig. 37), but not following MPH administration. Interestingly, in the striatum, both

treatments led to significant increases of NR_{2B} expression. No change in this protein expression has been found in the hippocampus after any of the two treatments (Fig. 37).

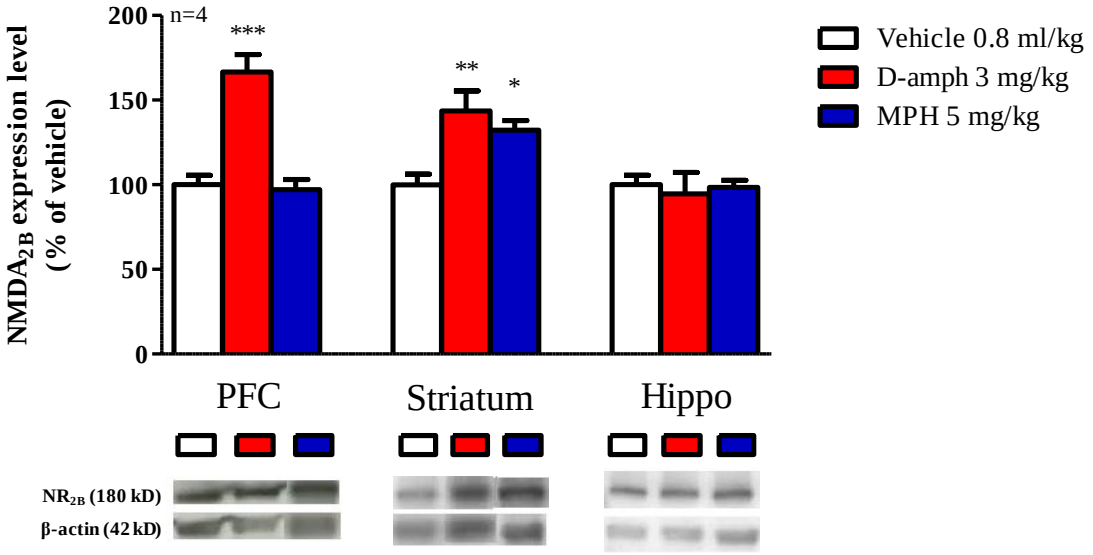


Figure 37: Psychostimulant exposure increases cortical and striatal NMDA receptor 2B protein expression.
Following behavioural experiments, NR_{2B} protein expression levels were quantified in different regions. In the prefrontal cortex, only D-amphetamine exposure (3 mg/kg) increased NR_{2B} protein expression. However, both psychostimulants (D-amph 3 mg/kg and MPH 5 mg/kg) increased NR_{2B} protein expression in the striatum, but not in the hippocampus. Bottom are displayed representative immunoblots with protein sizes in kilodaltons (kD). *P<0.05, **P<0.01, ***P<0.001 *vs.* respective vehicle, Newman–Keuls test after significant ANOVA.

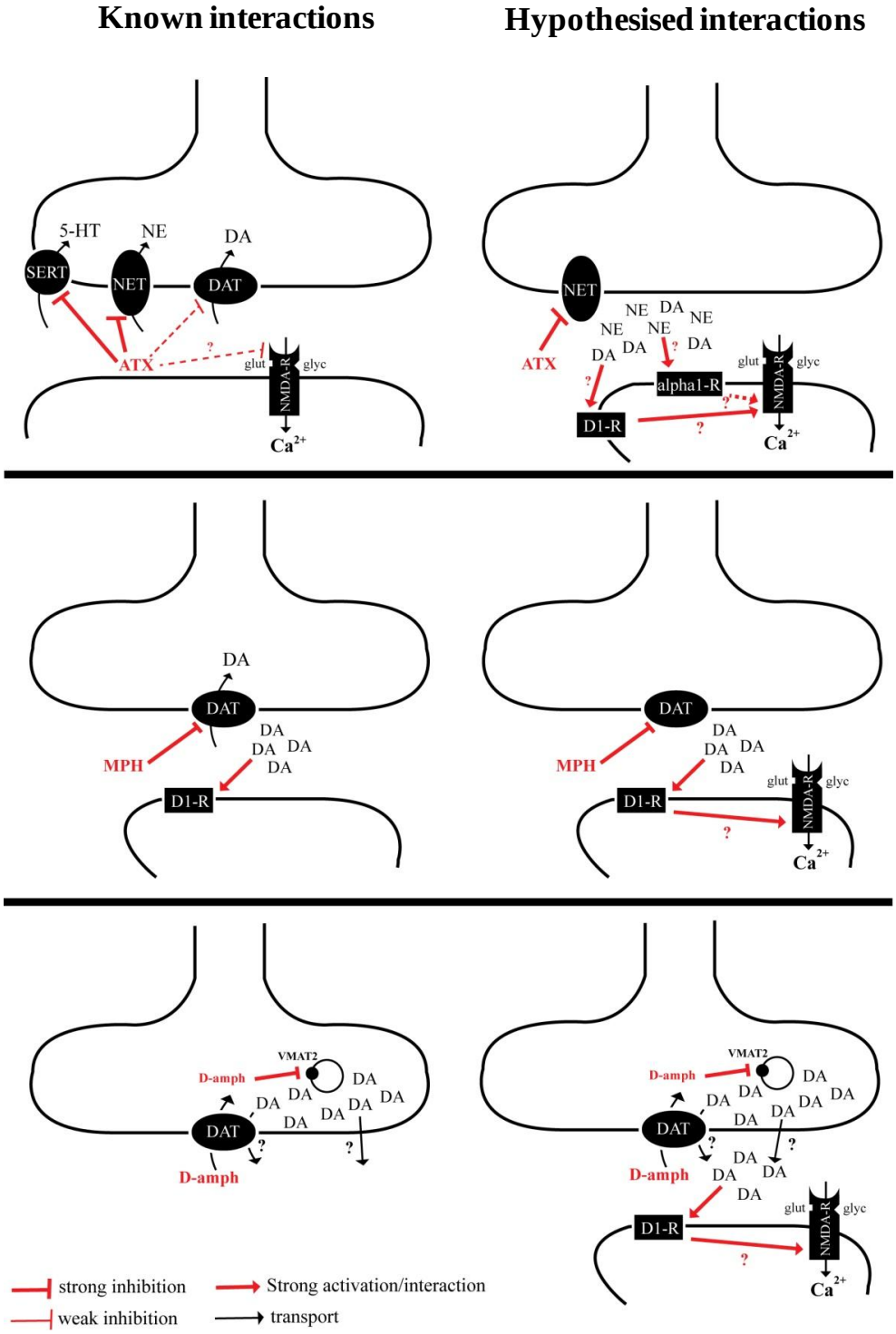
These results indicate a partial relationship between increased cortical/striatal excitability, locomotor activity and NR_{2B} expression.

II-4- Discussion

The present chapter demonstrates that the ADHD drugs MPH, D-amph and ATX modulate the firing activity and glutamate excitability of PFC pyramidal neurons. Table 3 summarised the proposed interactions within this study.

Table 3: Summary of known and proposed interactions between the molecules under study.

This table summarises our main findings within this chapter. Please refer to in-text citations. ATX: atomoxetine, MPH: methylphenidate, D-amph: D-amphetamine, DAT: dopamine transporter, DA: dopamine, NET: norepinephrine transporter, NE: norepinephrine, SERT: serotonin transporter, 5-HT: serotonin, NMDA-R: N-methyl-D-aspartate receptors, glut: glutamate, glyc: glycine, $VMAT_2$: vesicular monoamine transporter 2, alpha1-R: adrenergic α1 receptors, D1-R: dopamine D_1 receptor.



As already demonstrated in our laboratory (Gronier, 2011), we confirmed that MPH excitatory effects on PFC neurons are largely dependent on dopamine D_1 receptors (Fig. 22). Indeed, MPH administration led to increases in the firing activities of spontaneously active neurons as well as silent neurons. MPH was able to elicit large firing activations, as observed before (Claussen *et al.*, 2014b), some of which were reversed by subsequent dopamine D_1 receptor antagonism, in line with a previous study showing that MPH enhances cortical excitability through D_1 receptors (Gronier, 2011). However, a few other studies found the MPH-induced effects to be dependent upon alpha-2 receptors (Andrews *et al.*, 2006; Berridge, 2006). Interestingly, according to Andrews' study, the alpha-2 dependency of MPH to induce such activation of cortical pyramidal neurons might be mediated by stimulation of alpha-2 receptors on interneurons rather than direct pyramidal cell activation (Andrews *et al.*, 2006). Indeed, alpha-2 receptors are majorly G_i protein coupled but some receptor subtypes can also interact with G_s or G_{q11} proteins (Aantaa *et al.*, 1995). When activated, they produce hyperpolarisation (Andrade *et al.*, 1985; Egan *et al.*, 1983) by increasing potassium conductance (Williams *et al.*, 1985). Therefore, it was stipulated that MPH is likely to induce disinhibition of pyramidal neurons *via* hyperpolarisation of cortical interneurons (Andrews *et al.*, 2006). Nonetheless, the present study is the first to demonstrate that D-amph also stimulated PFC firing activity in a dose-dependent manner which is also partially dependent on the stimulation of dopamine D_1 receptors (Fig. 23D,E). This is compatible with the fact that both D-amph and MPH, by interacting with synaptic catecholamine transporters and increasing dopamine efflux (Arnold *et al.*, 2001), act as indirect dopamine D_1 agonists and that D_1 receptor activation can generate increases in neuronal excitability (Gronier *et al.*, 2013; Tseng *et al.*, 2007; Wang *et al.*, 2011). As a matter of fact, a recent study from our laboratory found that the systemic administration of D_1 selective agonists, and to a lesser extent their local application, can stimulate the firing activity of PFC neurons *in vivo* (Gronier *et al.*, 2013).

The magnitude of the increase in firing activity elicited by both psychostimulants, MPH and D-amph, does not seem directly proportional to the amount of dopamine release that these drugs can induce. According to microdialysis studies, D-amph is far more potent than MPH in inducing dopamine release (Kuczenski *et al.*, 1997) but does not produce a more dramatic increase in firing activity (Fig 22-23). Indeed, we have found that D-amph and MPH produce similar firing activity

activations. This result indicates that D_1-dependent activation of pyramidal neurons, produced by increases in extracellular dopamine levels, is likely to be at its maximum level, when the two drugs are already administered at intermediate doses (3-6 mg/kg).

However, the present study also shows that these large activations occur at doses that slightly exceed therapeutic levels. Lower doses of MPH and D-amph closer to the therapeutic range (0.5–1 mg/kg for both drugs) produced milder electrical activation (Table 2) only in a subset of PFC neurons (Gronier, 2011; Gronier *et al.*, 2010). Although it is difficult to compare doses in humans and rodents, which have a much more powerful metabolism capacity (Demetrius, 2005; Martignoni *et al.*, 2006; Mraz *et al.*, 1989), it can be expected that, in humans, therapeutic doses of D-amph and MPH are likely to produce a moderate dopamine D_1-dependent excitability increase in a subset of pyramidal PFC neurons. Larger activation of PFC neurons occurring at higher doses is likely to be associated with the well-known motor and cognitive impairments induced by these drugs (Swanson *et al.*, 2003). Indeed, psychostimulants can induce a sensation of being "high". As an example, such sensations appear almost immediately (1-3 minutes) when acute intravenous exposure to 0.37-0.5 mg/kg of methylphenidate or cocaine occurs (Morton *et al.*, 2000; Volkow *et al.*, 1999b; Volkow *et al.*, 1996b). These doses induce a dopamine transporter occupancy ranging from 80-93%, but only occupancies above 80% are considered to be necessary to induce a feeling of a "high", although some patients with DAT occupancies higher than 80% do not experience the "high" but display clear behavioural effects (Volkow *et al.*, 1996a) such as a need to move and anxiety as well as cardiovascular side effects (on heart rate, diastolic and systolic blood pressures). Very interestingly, in patients, cocaine and methylphenidate result in similar patterns for dopamine transporter occupancy. Both drugs display similar pharmacokinetic properties, except for their clearance profiles (Volkow *et al.*, 1995). The dopamine D_1 receptor is known to exert a biphasic effect on PFC functions, with moderate activation producing cognition improvement and sharpening synaptic transmission, while excessive stimulation impairs cognitive function and neuronal network activity (Goldman-Rakic *et al.*, 2000; Seamans *et al.*, 2004). However, dopamine D_1 receptor stimulation is probably not the only catecholamine receptor involved in psychostimulant-induced activation of PFC neurons. As a matter of fact, only 60-65% of the neurons activated by MPH or D-amph had their activation significantly reversed by D_1 receptor antagonist and, in some of these D_1-sensitive

neurons, the effect of the antagonist was only partial. Therefore, the contribution of other catecholamine receptors is also probable. It should be noted that we found in a previous study that adrenergic β1 and dopamine D_2 receptors could contribute to MPH-induced activation of PFC neurons in a subset of neurons. Nevertheless, in the present study, D_1 receptor blockade alone or combined with alpha-1 receptor blockade were the only two pharmacological manipulations that could significantly prevent the excitatory effects of psychostimulants (Fig. 24).

On the other hand, ATX-induced neuronal activation of PFC neurons did not seem to be primarily dependent on D_1 receptors (Fig. 25C). Whether this can be associated with the lack of immediate effect of ATX, over psychostimulants, in ADHD patients is an interesting question to address. However, ATX is known to promote, with similar potency as MPH, large amounts of dopamine release in the PFC under *in vivo* conditions (Bymaster *et al.*, 2002), most likely via its action on the NET, which plays a key role in the clearance of dopamine in the PFC (Devoto *et al.*, 2006). The ATX-induced effect may result from a more complex combination of the activation of different catecholamine receptors. The contribution of the α1 adrenoceptor has been demonstrated in our study in a fraction of the neurons tested (Fig. 26A-B). While one study acknowledges the role of alpha-1 receptor in ATX-induced cortical acetylcholine release (Tzavara *et al.*, 2006), others failed to find any contribution of apha-1 receptors in ATX-induced risk taking (Yang *et al.*, 2016), which is a PFC-controlled behavioural trait. Although acting as an indirect alpha-1 receptor agonist, ATX has also been shown to bind to alpha-1 receptors with low affinity (Bymaster *et al.*, 2002). Furthermore, subsequent blockade of α2 receptors by yohimbine (Fig. 26C-D) was found to potentiate the effect of ATX on firing activity, at least in a subset of neurons in our experiments. These results are in agreement with a previous investigation in monkeys, where ATX and MPH indirectly improved working memory *via* alpha-2 and dopamine D_1 receptor dependent mechanisms (Gamo *et al.*, 2010). ATX, by potently blocking the NET, may initially indirectly activate inhibitory α2 receptors on the pyramidal cell and therefore attenuate possible excitatory effects mediated by other catecholamine receptors, including the D_1 receptors, as demonstrated in some neurons tested in the present study (Fig. 26C). Therefore, in this way, ATX may not be able to promote the same dopamine D_1 receptor-dependent excessive activation of PFC neurons which may be associated with the well-described behavioural disturbances elicited by psychostimulants when

administered at high doses (Spiller *et al.*, 2013). However, in our study, we found similar potencies for D-amph and ATX to induce neuronal excitations (Fig. 23 and 25, respectively).

Nevertheless, the fact that a significant proportion of neurons are activated by therapeutic doses of ADHD drugs (compared to saline-treated neurons) may help to improve ADHD symptoms. Moderate enhancement of PFC neuronal excitability may compensate for the well-documented cortical hypofunction observed in ADHD, as evidenced by reduced cortical thickness and metabolic activity in some subregions of the PFC in drug-free patients (Fernandez-Jaen *et al.*, 2014; McLaughlin *et al.*, 2014).

In parallel with some of our latest observations using D_1 receptor agonists (Gronier *et al.*, 2013), we found that ADHD drugs preferentially increased the basal activity of spontaneously active PFC pyramidal neurons over silent neurons. This is compatible with the fact that dopamine D_1 receptor may not directly stimulate the activity of PFC pyramidal neurons but may rather strengthen pre-existing tonic excitatory regulations of the firing activity of PFC neurons. Such tonic excitatory input is likely to be mainly maintained by glutamatergic neurotransmission, as the spontaneous activity of PFC neurons has been shown to be mediated by mutual excitation between glutamatergic pyramidal neurons (Kritzer *et al.*, 1995). Moreover, we found that ADHD drugs powerfully stimulate the excitability of PFC neurons mediated by glutamate receptor activation (Fig. 27C).

Indeed, the present study, for the first time, demonstrates that ADHD drugs promote an *in vivo* potentiation of the excitatory response of PFC neurons to the glutamate agonist NMDA, an effect not associated with changes in basal firing activity (Fig. 29-30). Ionotropic glutamate receptors in the PFC are essential for attention processes, impulse control and for efficient memory function (Nakanishi, 1992). In the PFC, local NMDA application by microiontophoresis produces transient neuronal activation (El Iskandrani *et al.*, 2015; Gobbi *et al.*, 2006), which is strongly decreased when glycine B site antagonists such as HA-966 and MRZ 2/576 are co-applied (Chen *et al.*, 2003a; Christoph *et al.*, 2006). In rodents and humans, NR_{2B} and NR_{2A} subunits predominate in prefrontal regions (Cull-Candy *et al.*, 2001; Dingledine *et al.*, 1999; Zhuo, 2009) and always form heterogeneous complexes with NR_1 receptors. NR_{2B}

subunits are major actors for synaptic potentiation, which is crucial for learning and plasticity (Zhuo, 2009). Moreover, administration of the specific NR_{2B} antagonist ifenprodil, before a learning paradigm, is known to impair normal learning, indicating that $NMDAR_{2B}$ are required for synaptic potentiation (Zhuo, 2009). Our study also demonstrated the role of NMDA receptor 2B in the responses to local NMDA application, as ifenprodil (2.5 mg/kg) strongly reduced NMDA-induced firing rate activation (Fig. 28). Antagonism of NMDA receptor has been shown to prevent some of the acute and chronic neurochemical and behavioural effects of psychostimulants (Bristow *et al.*, 1994; Gaytan *et al.*, 2000; Hemrick-Luecke *et al.*, 1992; Yang *et al.*, 2000), indicating that NMDA receptors may play a crucial role in stimulant-induced effects. Moreover, recent behavioural evidence shows that administration of NMDA antagonists directly in the PFC induced a profound deficit in attention and increased impulsivity in rodents (Carli *et al.*, 2014).

Imaging studies have shown that children with ADHD exhibit hypoactivity in prefrontal region, as well as anatomical and functional dysfunction in fronto-striatal circuits, associated with white matter loss (Cubillo *et al.*, 2012; Liston *et al.*, 2011). Patients suffering from ADHD exhibit regional abnormalities such as decreased cortical thickness in regions located beneath the cortex such as the right caudate, the insula and thalamus (Ivanov *et al.*, 2010; Lopez-Larson *et al.*, 2012; Rubia *et al.*, 2014; Valera *et al.*, 2007). These abnormalities can also be related to brain function. Indeed, hypo-activation during sustained attention tasks has been observed in ADHD patients (Cubillo *et al.*, 2012). Finally prefrontal and striatal connectivity impairments are also found in ADHD subjects (Liston *et al.*, 2011; Tomasi *et al.*, 2012). The elevated NMDA receptor function induced by ADHD drugs (Fig. 27C) may lead to an increase in the excitability of PFC neurons that could be essential in regulating glutamatergic cortico-striatal transmission and strengthening synaptic transmission at these synapses. This NMDA receptor activation may trigger cortical as well as striatal synaptic plasticity and could therefore exert beneficial effects on various aspects of cognitive and executive functions, both impaired in ADHD. As a matter of fact, glutamate neurotransmission in ventral and dorsal striatum, originating mainly from glutamate fronto-striatal projections, plays a major role in controlling cognitive flexibility, required for normal attention and cognition (Ding *et al.*, 2014). Moreover, NMDA receptor activation is known to induce synaptic plasticity, assessed by membrane receptor insertion and

long/short-term potentiation/depression (Lu *et al.*, 2001; Malenka *et al.*, 1993). ADHD drugs, by enhancing NMDA tone on PFC pyramidal neurons, may therefore restore adequate neurotransmission and plasticity in ADHD patients, as observed in animal models (Gandal *et al.*, 2012; Sasaki *et al.*, 2015). Moreover, we found that methylphenidate increased NMDA-induced responses of striatal medium spiny neurons (Fig. 35), an effect that may strengthen functional cortico-striatal connectivity.

The question arises as to whether pharmacological manipulation that can enhance NMDA receptor function can be of therapeutic benefit for ADHD patients. For instance, one can wonder if cognition enhancers such as direct or indirect glycine B agonists of the NMDA receptor (e.g., Glycine transporter uptake inhibitors, $GlyT_1$) could be of any clinical use (Cheng *et al.*, 2014). Such agents have been shown to improve cognitive flexibility and working memory (Bado *et al.*, 2011; Kuriyama *et al.*, 2011; Nikiforuk *et al.*, 2011), as well as attention deficits caused by NMDA receptor blockade (Chang *et al.*, 2014). Recently, glycine uptake inhibitors were proven to potentiate NMDA-dependent long-term potentiation in hippocampal CA1 pyramidal neurons (Alberati *et al.*, 2012) and could restore MK801-induced LTP impairment (Manahan-Vaughan *et al.*, 2008). Finally, $GlyT_1$ antagonists increase *in vitro* and *in vivo* post-synaptic NMDA currents in neurons (Bergeron *et al.*, 1998; Chen *et al.*, 2003a; Martina *et al.*, 2004). However, such agents may be able to positively modulate NMDA channels only under precise conditions (Sur *et al.*, 2007), such as under AMPA-dependent release of the magnesium block. Despite the fact that these drugs have not yet been proven to have a clearly beneficial therapeutical effect on negative deficits in schizophrenia (Chue, 2013; Schoemaker *et al.*, 2014), one cannot rule out that they can help to alleviate some of the symptoms of ADHD. Moreover, aberrant glutamate neurotransmission has been found in ADHD patients (Lesch *et al.*, 2013). This recent study reinforces previous studies where ADHD was positively associated with polymorphisms in different glutamate receptor subtypes such as $GluR_7$, NR_{2A} and NR_{2B} (Akutagava-Martins *et al.*, 2014; Dorval *et al.*, 2007; Elia *et al.*, 2012; Park *et al.*, 2013; Park *et al.*, 2014; Turic *et al.*, 2004), although one study did not find any relationship between ADHD and NR_{2A} (Adams *et al.*, 2004). Nevertheless, an increase in NMDA receptor function may only be one component of the complex mechanisms that can lead to therapeutic responses and it is now necessary to establish whether this effect can persist after the chronic administration of ADHD drugs. Our data confirms, in part, a

recent *in vitro* electrophysiological study showing that an acute low dose administration of MPH can increase NMDA-dependent excitatory post-synaptic potentials in PFC slices (Cheng *et al.*, 2014). However, the authors did not demonstrate any contribution of the D_1 receptor in this modulation but suggested rather the involvement of alpha-1/2 adrenoceptors.

Interestingly, we found that, for D-amph, this increase in NMDA neurotransmission may occur, at least partially, via an interaction with dopamine D_1 receptors as we found in a significant number of neurons tested (but not all) that the selective D_1 antagonist SCH23390 reversed or prevented the potentiation of NMDA response elicited by D-amph. Dopamine is known to facilitate glutamatergic transmission onto PFC neurons via interactions with both AMPA and NMDA receptors. Intracellular electrophysiological studies have shown that NMDA receptor currents are potentiated by dopamine D_1 receptor activation (Sarantis *et al.*, 2009), while D_1 receptor stimulation may promote AMPA receptor trafficking in the PFC (Sun *et al.*, 2005). Postsynaptic dopamine D_1 and some NMDA receptors colocalise in the PFC and have been shown to interact synergically (Goldman-Rakic *et al.*, 2000; Kruse *et al.*, 2009; Wang *et al.*, 2001). It is generally believed that dopamine promotes NMDA receptor trafficking through phosphorylation induced by a D_1 receptor-like signalling cascade (Hu *et al.*, 2010; Trepanier *et al.*, 2012; Urban *et al.*, 2013b; Yang *et al.*, 2005). Nevertheless, the role of dopamine on NMDA receptor function can be very complex in the PFC, with differential modulation depending on receptor subtypes (Urban *et al.*, 2013b). In addition, not all neurons tested were responsive to the dopamine D_1 receptor antagonist after potentiation of their NMDA response by D-amph or MPH. While dopamine has a small dual effect on the NMDA-induced firing activation of PFC neurons (strengthening or dampening NMDA neurotransmission, in 50% of all neurons, Fig. 33), it strongly affects NMDA neurotransmission in striatal medium spiny neurons (also strengthening or dampening). Therefore, other mechanisms may also explain the increase in glutamate/NMDA excitability generated by ADHD drugs (Zhang *et al.*, 2012a).

We have also found that acute psychostimulant administrations induce changes in local NMDA receptor 2B protein expression. The NMDA receptor 2B is responsible for neuronal excitations, which are increased following acute ADHD drug

administration (Fig. 27C). NR_{2B} functions range greatly, from learning to pain and memory processing as well as feeding habits (Loftis *et al.*, 2003). Both MPH and D-amph induced strong NR_{2B} protein expression elevation in the striatum, while only D-amphetamine significantly increases NR_{2B} protein expression in the prefrontal cortex (Fig. 37). Such increases in NR_{2B} receptor expression can arise from either newly-synthesised receptors or from membrane insertion of internally-stored receptors. One can assume that ADHD drugs may increase NR_{2B} protein expression in specific brain regions, which, in turn, promotes neuronal excitation. These results are also in line with previous findings where the amount of NR_{2B} protein level at the membrane surface increased following a single intraperitoneal 0.5 mg/kg MPH exposure, inducing increases in NMDA receptor-mediated excitatory post-synaptic current (Cheng *et al.*, 2014). The NR_{2B} subunit of the NMDA receptor complex is responsible for long-term potentiation (Yoshimura *et al.*, 2003), which is crucial for the development of plasticity as well as memory and learning (Loftis *et al.*, 2003). And yet, a 1 mg/kg dose of MPH produced opposite effects on NR_{2B} expression after 60 minutes (Urban *et al.*, 2013b). NR_1 protein, however, was found to be decreased in the frontal cortex following acute methamphetamine administration, while both NR_{2A} and $GluR_2$ proteins were increased (Simoes *et al.*, 2008). Moreover, *in vitro* cocaine exposure (1 µM) induced increased total NR_{2B} expression in the midbrain following 10 minutes of cocaine exposure (Schilstrom *et al.*, 2006). Because such an increase of NR_{2B} expression was abolished when slices were pre-treated with a protein inhibitor (anisomycin 20 µM), such a result can only be attributed to newly synthesised NR_{2B} receptors (Schilstrom *et al.*, 2006). Similar results were observed following bath application of a dopamine D_1 receptor agonist (Hu *et al.*, 2010) in the prefrontal cortex (SKF-81297, 10 µM). Chronic exposure to amphetamine (4 mg/kg) led to significant decreases of NR_{2B} levels in the striatum, consistently with our study on chronic MPH exposure (see Chapter IV), while NR_1, NR_{2A}, NR_{3A}, NR_{3B} levels were not changed following chronic amphetamine exposure (Mao *et al.*, 2009). However, in the prefrontal cortex, NR_{2B} protein expression was not altered by such treatment in Mao's study, contrary to what we have found. In the striatum, *in vivo* interactions between the dopamine D_2 receptor and the $NMDA_{2B}$ receptor have been observed (Liu *et al.*, 2006). Moreover, such functional interactions are responsible for the behavioural responses to cocaine. Therefore, NR_{2B} protein may be linked to exposures with stimulants (Brunk *et al.*, 2010; Gutierrez-Arenas *et al.*, 2014; Pascoli *et al.*, 2011) or alcohol (Kash *et al.*, 2008; Nagy, 2004; Wang *et al.*,

2007a; Wang *et al.*, 2010a). In our experiments, NR_{2B} protein expression levels increased following acute stimulant exposure in some brain regions, which may reflect immediate synapse reorganisation and plasticity. The time allocated to our blotting project having run out, we were unable to elaborate our results, although we were considering studying phosphorylated NR_{2B} receptors as well as distinguishing different cellular fractions, such as membrane-bound receptors or internally-stored receptors.

Our results, showing that ATX also enhances NMDA-mediated response on PFC neurons, stand in contrast with a recent *in vitro* electrophysiological study, demonstrating that ATX, at a relatively low concentration (5 μM), inhibits NMDA-induced current when studied on PFC slices, via an open-channel blocking mechanism (Ludolph *et al.*, 2010). Similar results were observed with D-amph which could act as a low affinity inhibitor of the NMDA receptor complex (Yeh *et al.*, 2002). However, ATX, like D-amph, enhances dopamine efflux in PFC according to microdialysis studies. Even if D_1 receptor stimulation helps to moderately enhance the basal activity of PFC neurons in ATX-treated animals, ATX may generate enough D_1 receptor activation to interact with NMDA receptors and compensate for the weak inhibitory effects of this drug on NMDA receptors.

Our data also differs from two other electrophysiological investigations carried out in non-anaesthetised rats, using different recording techniques, showing that D-amph (2 mg/kg) and MPH (0.25–15 mg/kg) do not elicit firing activation of PFC neurons but rather, in the case of D-amph, tend to have suppressant effects (Devilbiss *et al.*, 2008; Wood *et al.*, 2012). Such divergence might be associated with differences in the recording techniques, in particular regarding the use of probes with different sensitivity levels for the recording of electrical signals. Nevertheless, the study by Devilbiss in 2008 shows that a low dose of MPH (0.5 mg/kg) increased the evoked excitatory responses of PFC neurons following hippocampus stimulation. Such a stimulatory effect, which is likely to involve glutamate-dependent processes, is not in contradiction with our study.

We have also reported that local drug delivery, through iontophoresis, induces strong spike amplitude variations (Fig. 32). While positive currents tend to induce spike amplitude gain, negative currents tend to induce loss of amplitude. These results are in

agreement with numerous electrophysiological investigations in humans (Cepeda *et al.*, 1991), rodents (Kiyatkin *et al.*, 1998; Stratton *et al.*, 2012) and other species (Rose *et al.*, 2013; Russo *et al.*, 1992). Recent *in vitro* studies on subthalamic nucleus neurons by Shen have shown that NMDA application (10 µM) is responsible for the activation of ATP-sensitive outwards potassium currents (Shen *et al.*, 2010; Shen *et al.*, 2013). In another 2014 study, Shen also observed that such mechanisms are regulated by the adenosine monophosphate (AMP) kinase (Shen *et al.*, 2014). These studies may explain the membrane hyperpolarisation that we observed in our results after termination of local NMDA application (Fig. 32) and for the post-NMDA firing rate reduction observed in spontaneously active neurons, as seen in Fig. 30D. Because none of the drugs applied iontophoretically induced a significant spike amplitude variation compared to saline iontophoresis, we can assume that such spike amplitude variations are not likely to change spontaneous spike generation (Fig. 33A) or NMDA-induced firing (Fig. 33B) and are rather the consequences of local current delivery in close vicinity to the neuronal membrane. Very interestingly, such electric artefacts have been observed before (Belle *et al.*, 2013; Herr *et al.*, 2010; Wang *et al.*, 1977).

Our results also show that acute MPH (at therapeutical doses) significantly increases NMDA neurotransmission in medium spiny neurons located in the striatum (Fig. 35). Interestingly, another study has shown that chronic MPH administration (daily 15 mg/kg for 2 weeks) leads to increased dendritic spine densities of such neurons (Kim *et al.*, 2009). Moreover, ionotropic glutamate receptors (NMDA and AMPA receptors) are strongly expressed in dendritic spines of excitatory neurons (Alvarez *et al.*, 2007; Bellot *et al.*, 2014; Hasbani *et al.*, 2001) and play key roles in synaptic plasticity (Fischer *et al.*, 2000). Taken together, these results may link MPH exposure to increased glutamatergic neurotransmission of striatal MSN as well as improved cortico-striatal functional connectivity.

Finally, we have positively correlated acute MPH and D-amph administrations with increases in the total amount of rearing activities (Fig. 36C), a consequence of increased vertical exploration behaviour (al-Khatib *et al.*, 1995; Alves *et al.*, 2005; Brown *et al.*, 2008; El Yacoubi *et al.*, 2000). Although the rearing activity is not similar to the locomotor activity, some consider rearing events to be the vertical components of the locomotor activity (al-Khatib *et al.*, 1995; El Yacoubi *et al.*, 2000). None of the two

drugs changed the animals' scratching parameters while only D-amphetamine significantly reduced the total number of grooming events, 45 minutes after the injection (Fig. 36A). In previous studies, 1-3 mg/kg doses of D-amph (Aguilar-Rivera *et al.*, 2015; Backstrom *et al.*, 2011; Kubera *et al.*, 2002; Leza *et al.*, 1991; McNamara *et al.*, 2006) or 2.5-20 mg/kg of MPH (Amini *et al.*, 2004; Cheng *et al.*, 2014; Claussen *et al.*, 2014b; Marsteller *et al.*, 2002; Penner *et al.*, 2001) all significantly increased locomotor activity in rodents, in consistence with our study, where rearing activities were significantly increased following stimulant exposure. In addition, these locomotor activities solely depend upon blockade of the DAT, as DAT knockout mice do not display any hyper-locomotion following 1 mg/kg of D-amph (Spielewoy *et al.*, 2001). In children, only a few studies have reported that MPH, given to children, can induce abnormal movements such as motor tics or dyskinesia (Balazs *et al.*, 2011; Lipkin *et al.*, 1994; Wulbert *et al.*, 1977). Acute administrations did not alter stereotypical events in our animals (not shown), as observed in another study (Claussen *et al.*, 2014b). However, several studies have found opposite results, where MPH induced strong stereotypical events following high doses (15-50 mg/kg) of methylphenidate (Bell *et al.*, 1982; Davis *et al.*, 1978; Mueller, 1993).

In conclusion, our electrophysiological studies indicate that all three ADHD drugs equally stimulate the excitability of PFC pyramidal neurons, in basal and NMDA-evoked conditions, when administered acutely. While the electrophysiological effects elicited by psychostimulants may be primarily dependent on dopamine D_1 receptor activation, those induced by ATX may also rely on other mechanisms. When applied locally on prefrontal cortex pyramidal neurons, methylphenidate, D-amphetamine and dopamine induce spike amplitude variations, but without affecting spontaneous and NMDA-induced firing activities. However, in the striatum, locally-applied dopamine produced more striking effects, showing two very distinct neuronal populations. In these striatal medium spiny neurons, low doses of MPH (1 mg/kg) produced significant effects on NMDA-induced firing rate activations. Striatal NR_{2B} protein expression increased following acute exposure to MPH (5 mg/kg) and D-amph (3 mg/kg), while prefrontal cortex NR_{2B} protein expression only increased following D-amph exposure. Finally, acute psychostimulant injections increased vertical locomotor activity in our rodent model.

Chapter III – Excitatory glutamate components involved in the electrophysiological response of ventral tegmental area dopamine neurons to acute methylphenidate.

III-1- Introduction

The ventral tegmental area (VTA) plays a central role in the response to psychostimulants (Kalivas *et al.*, 1993). Indeed, VTA neuronal excitability has been found to be modulated by psychostimulants such as cocaine (Lee *et al.*, 1999; Nimitvilai *et al.*, 2012; Steffensen *et al.*, 2008; Zhou *et al.*, 2006), D-amphetamine (Shi *et al.*, 2000b; Xu *et al.*, 2001), methamphetamine (Shi *et al.*, 2004), methylphenidate (Jones *et al.*, 2013), nicotine (Eddine *et al.*, 2015; Erhardt *et al.*, 2002; Zhang *et al.*, 2012b) as well as cannabinoids (French *et al.*, 1997). Addiction may first settle within the VTA (Adinoff, 2004; Koob *et al.*, 2010; Taylor *et al.*, 2013), whether drug-related or not, such as addiction to food, video games, pornography and gambling (Baik, 2013; Gearhardt *et al.*, 2011; Han *et al.*, 2011; Hilton *et al.*, 2011; Love *et al.*, 2015; Potenza *et al.*, 2002; Shriner *et al.*, 2014; Zhang *et al.*, 2015).

Midbrain dopamine neurons strongly modify their firing and burst activities following psychostimulant administration, but not necessarily by firing rate activation (Bunney *et al.*, 1973; Einhorn *et al.*, 1988; Shi *et al.*, 2004). *In vivo* administration of 0.25 mg/kg of D-amphetamine produced midbrain dopamine neuron silencing in 20% of all recordings (Bunney *et al.*, 1973). In addition, this study also calculated an average IC_{50} of 1.6 mg/kg. However, this effect was specific to D-amph, as it was not observed following L-amph exposure, even with doses up to 25 mg/kg. When cocaine was administered intravenously (0.5 mg/kg), VTA GABAergic neurons increased their firing rates by 160%, an effect not observed with lidocaine (Steffensen *et al.*, 2008). Cocaine administration (0.06-16 mg/kg, i.v.) results in dose-dependent inhibition of

VTA dopamine neurons (Hinerth *et al.*, 2000). However, another study observed a proportion of VTA dopamine neurons (50% of all neurons recorded) that responded by an increase in the firing rate following cocaine administration (0.25 mg/kg) (Mejias-Aponte *et al.*, 2015). The study by Jones in 2014 on freely behaving animals observed dose-dependent responses of such neurons to 0.6, 2.5 and 10 mg/kg of acute intravenous methylphenidate, with increases in firing rates in 30%, 66% and 57% of all recordings, respectively (Jones *et al.*, 2014). Other addictive substances, such as the two opioids morphine and heroin, can induce modifications of firing rates of midbrain dopamine neurons. Indeed, intra-VTA morphine infusions (60 nl of a 1 mg/ml morphine solution), increased baseline firing rates by 47%, while intravenous morphine administrations (1 mg/kg) increased baseline firing rates by 25% (Jalabert *et al.*, 2011). Finally, heroin (1 µM), applied in baths onto midbrain slices, produced strong firing rate increases (4-fold) of dopamine neurons (de Guglielmo *et al.*, 2015).

Because local dopamine transporter blockade will result in dopamine concentration increases within the synaptic cleft, psychostimulants will also trigger neuronal reorganisation (plasticity) as well as behavioural responses (Hughes, 1972; Russo *et al.*, 2010; Sproson *et al.*, 2001; Zehle *et al.*, 2007). These modifications are now believed to be glutamate-related (Cheng *et al.*, 2014; Guillem *et al.*, 2015; Schmitz *et al.*, 2015; Warton *et al.*, 2009), because MPH treatment seems to normalise some of the glutamatergic abnormalities observed in ADHD patients (Hammerness *et al.*, 2012). Several studies, using proton magnetic resonance spectroscopy, observed that drug-free ADHD patients present abnormal measurements of glutamate metabolites in the prefrontal cortex, the cerebellum, the basal ganglia and the striatum (Carrey *et al.*, 2007; Courvoisie *et al.*, 2004; Ferreira *et al.*, 2009; MacMaster *et al.*, 2003; Maltezos *et al.*, 2014; Perlov *et al.*, 2010). In his 2012 study, Hammerness observed higher glutamate/myo-inositol ratio with ADHD patients under MPH treatment, while both untreated and control patients display lower ratios. In ADHD children, low levels of GABA and high levels of glutamate were detected (Purkayastha *et al.*, 2015; Schuch *et al.*, 2015).

The relationship between glutamate and dopamine is central to the regulation of the excitability of prefrontal cortex pyramidal neurons (Tseng *et al.*, 2004). Dopamine is known to protect neurons against glutamate excitotoxicity (Vaarmann *et al.*, 2013).

Moreover, dopamine also prevents calcium dysregulations in midbrain dopamine neurons (Vaarmann *et al.*, 2013), which can be triggered by over activation of ionotropic glutamate receptors. In an animal study on SHR rats, it has been suggested that the glutamate neurotransmission terminating onto dopamine-rich areas, such as the striatum, is hyperfunctional (Miller *et al.*, 2014). Indeed, Miller's study has shown significantly increased evoked glutamate release within the striatum from SHR (ADHD) rats compared to Wistar Kyoto (control) rats.

In this third chapter, we will study the consequences of acute methylphenidate administration on the electrical activities of ventral tegmental area neurons and the implication of glutamate in such activities. Besides, using iontophoresis, we will also determine the role of glutamate neurotransmission, as well as the influence of local catecholamines, in the regulation of the electrical activities of midbrain dopamine neurons.

III-2- Material and Methods

III-2-A- Subjects

Please refer to paragraph II-2-A. Within this chapter, 85 animals were used, from which 188 neurons were recorded.

III-2-B- *In vivo* extracellular single unit electrophysiology

Please refer to paragraph II-2-B. Putative midbrain dopamine neurons were identified according to electrophysiological criteria summarised by Ungless and Grace in 2012 (Ungless *et al.*, 2012). Only presumed dopaminergic neurons presenting a notch in the rising phase, a prominent negative compound and a time greater than 1 ms from the start of the depolarisation to the end of the repolarisation were selected in our study (Chenu *et al.*, 2013; Grace *et al.*, 1984; Grace *et al.*, 1983; Ungless *et al.*, 2004; Valenti *et al.*, 2011). A burst activity in such neurons is defined as two spikes occurring at an interval of 80 ms or less, followed by a silence period of at least 160 ms (Grace *et al.*, 1984; Overton *et al.*, 1997; Paladini *et al.*, 2014). Using these identification criteria, we may exclude PFC-projecting and amygdala-projecting

midbrain dopamine neurons, as action potential duration does not always accurately determine dopamine-containing neurons (Chieng *et al.*, 2011; Ford *et al.*, 2006; Hnasko *et al.*, 2012; Lammel *et al.*, 2008; Margolis *et al.*, 2006; Margolis *et al.*, 2008; Marinelli *et al.*, 2014; Zhang *et al.*, 2010b). However, our strict criteria allow for the recording of a rather homogenous population of midbrain dopamine neurons. Coordinates for the ventral tegmental area (Fig. 38A) were: anteroposterior -4.5 to -5.5 mm to Bregma, lateral 0.3-1.2 mm, dorsoventral 7.2-9.5 mm below cortical surface. For microiontophoresis, multibarrel pipettes were filled with either: NMDA, 30 mM, dopamine 20 mM, MPH 20 mM, norepinephrine 20 mM, HA-966 20 mM, 7-chloro-kinurenic acid 20 mM, all at pH 7-8 (dissolved into NaCl 147 mM), or a combination of the above and NaCl 2 M for current balancing. The central recording channel was filled with saline (NaCl 147 mM).

III-2-C- Prefrontal cortex inhibition

Adapted from a previous study in rhesus monkeys (Tehovnik *et al.*, 1997), local prefrontal cortex inhibition was achieved by locally perfusing lidocaine (2% w/v in saline, pH 7) at a rate of 2 µl/min immediately prior to intravenous MPH challenge. Two and a half microliters of total volume was injected at each injection site. Two injection sites in total were made per hemisphere at the following coordinates (in mm from Bregma): anteroposterior/lateral: +3/+2 and +2/+1. Two depths per injection site were chosen: 1.5 and 3 mm below cortical surface (Fig. 38B).

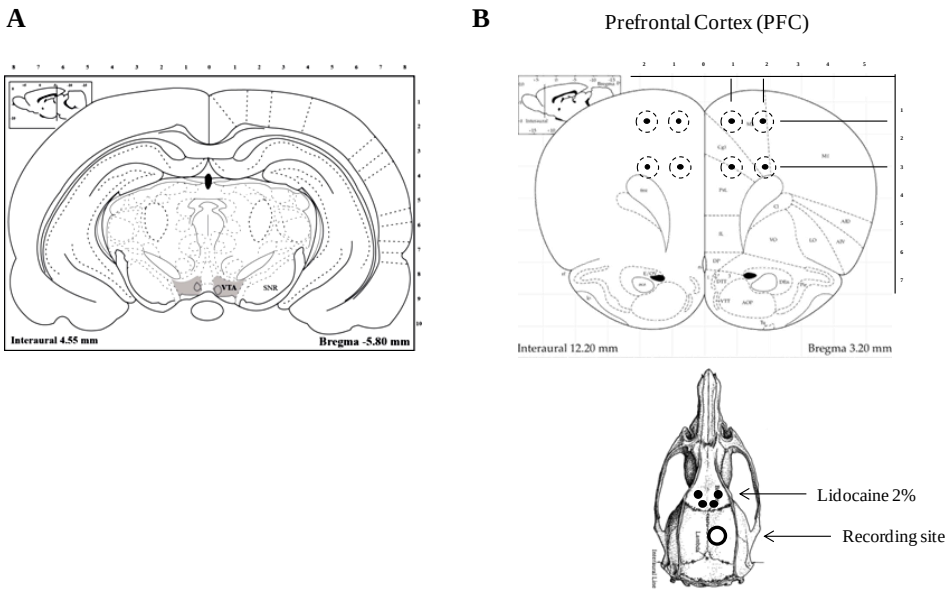


Figure 38: Locations of the ventral tegmental area and local PFC lidocaine perfusions.
(**A**): The small shaded area located ventrally corresponds to the location of the ventral tegmental area (VTA). (**B**): Representation of local intra-PFC lidocaine perfusions (2% in saline, w/v), performed immediately before intravenous MPH challenge. This diagram is a stack of two coronal slices with anteroposterior coordinates of +3 and +2 mm from Bregma. Four injection sites were made per anteroposterior location. Black dots indicate microperfusion locations, while dotted circles indicate mean diffusion areas adapted from experiments in the rhesus monkey (Tehovnik *et al.*, 1997). The open dot indicates the location of the VTA recording site, seen from the above. Adapted from Paxinos and Watson (1997). Cg1 cingulate cortex area, PrL prelimbic cortex, VTA ventral tegmental area, SNR substantia nigra reticular.

III-2-D- Data analysis

Please refer to the electrophysiology analysis in paragraph II-2-E.

III-3- Results

III-3-A- Methylphenidate, but not atomoxetine, decreases firing and burst activities of VTA dopamine neurons in a dose-dependent manner

As expected, vehicle intravenous administration did not change either the firing (Fig. 39A) or the burst activity (Fig. 39B) of ventral tegmental area dopamine neurons. Methylphenidate, injected intravenously at 2 mg/kg, significantly reduced the firing and burst activity of midbrain dopamine neurons by 48% and 59%, respectively

(Fig. 40A,C). The vast majority (20/24) of neurons had their firing activity reduced by more than 20% after 2 mg/kg of MPH (Fig. 40B), but only a few neurons (4/24) remained insensitive to this dose, despite fulfilling all the assessment criteria used for midbrain dopamine neuron characterisation (Fig. 40D). On a smaller population of neurons (n=8), MPH was administered by 2 mg/kg increment doses up to a cumulative dose of 4 mg/kg, which further reduced firing and burst activities of ventral tegmental area dopamine neuron (Fig. 41A,C).

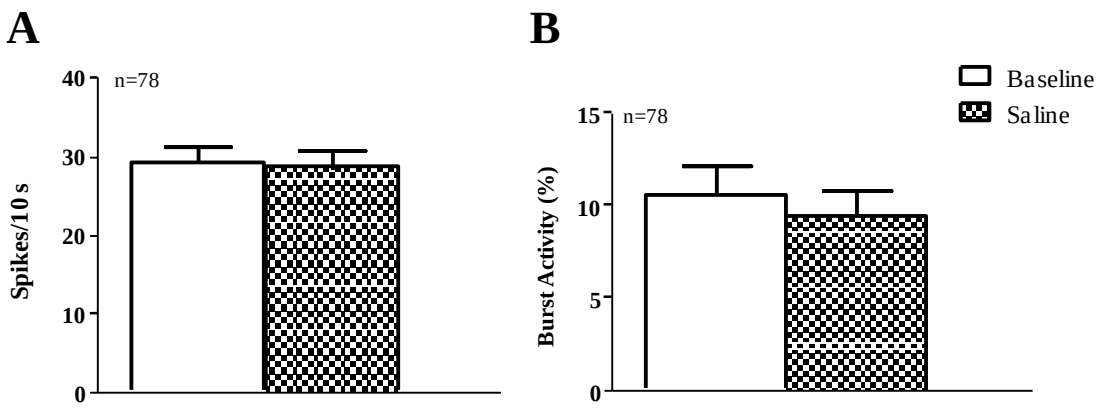


Figure 39: Vehicle administration does not alter the electrophysiological activity of midbrain dopamine neurons.
Neither the firing rate (**A**) nor the burst activity (**B**) of ventral tegmental area (VTA) dopaminergic neurons were significantly affected by intravenous vehicle administrations (saline, 0.2 ml/kg).

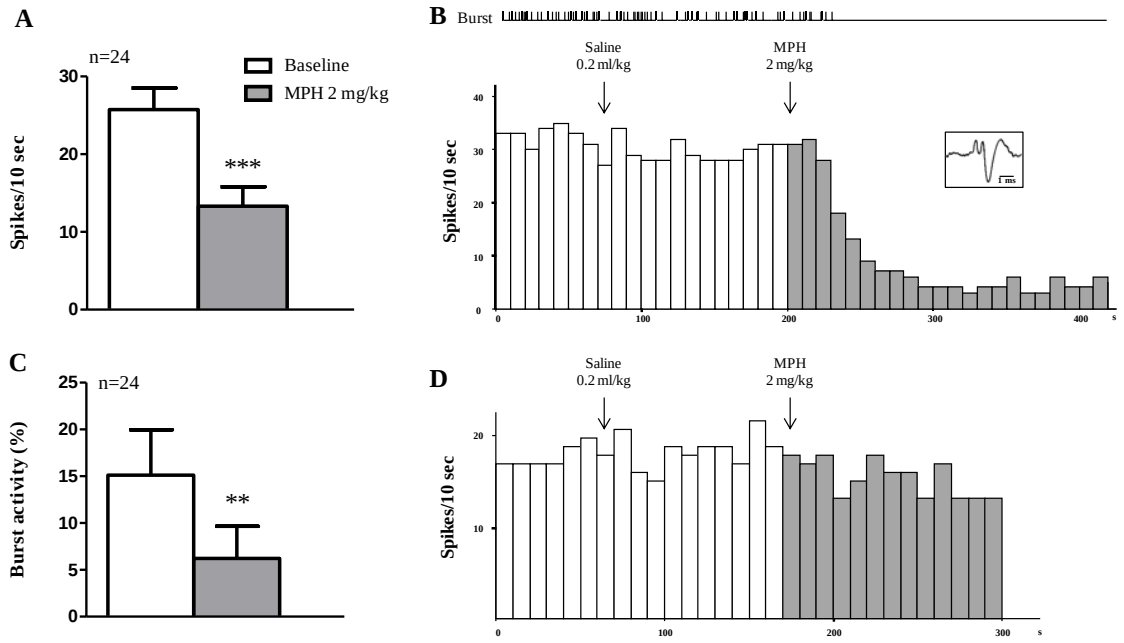


Figure 40: Methylphenidate decreases the firing and burst activities of midbrain dopamine neurons.

Intravenous administration of a 2 mg/kg single dose of methylphenidate significantly reduced the firing rate (**A**) and burst activity (**C**) of 20 out of 24 VTA dopamine neurons. (**B**): Representative recording examples of such firing rate reduction following MPH exposure, although a few neurons (4/24) remained insensitive to this dose of MPH (**D**). Boxed is shown a typical action potential waveform of the VTA dopamine neuron recorded in **B**, which presents the typical notch during the initial positive inflexion. **P<0.01, ***P<0.001 *vs.* baseline, paired Student's t-test.

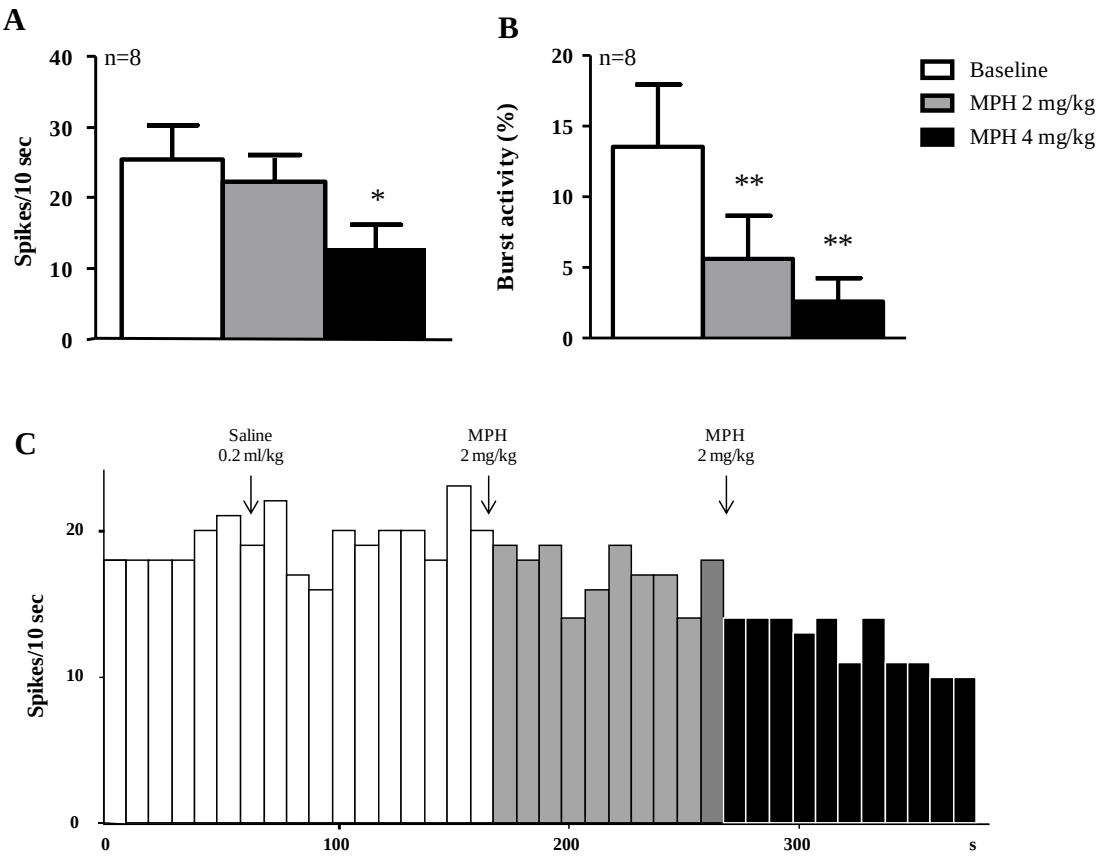


Figure 41: Methylphenidate decreases the firing and burst activities of midbrain dopamine neurons in a dose-dependent manner.
In another population of neurons, a cumulative dose of 4 mg/kg of MPH significantly reduced the firing rate (**A**) and burst activity (**B**) of VTA dopamine neurons. (**C**): Representative recording example of such progressive firing rate reduction following 4 mg/kg intravenous MPH exposure. *P<0.05 *vs.* baseline, Neuman-Keuls test after significant repeated measures ANOVA.

In contrast to what was observed with MPH, cumulative administrations of atomoxetine by 2 mg/kg increments and up to 10 mg/kg did not change significantly the firing and burst activity of dopaminergic neurons (Fig. 42), although 6 mg/kg of ATX resulted in an almost significant but mild decrease (p=0.06, baseline *vs* ATX 6 mg/kg, paired Student's t-test) in baseline burst activity (Fig. 42B).

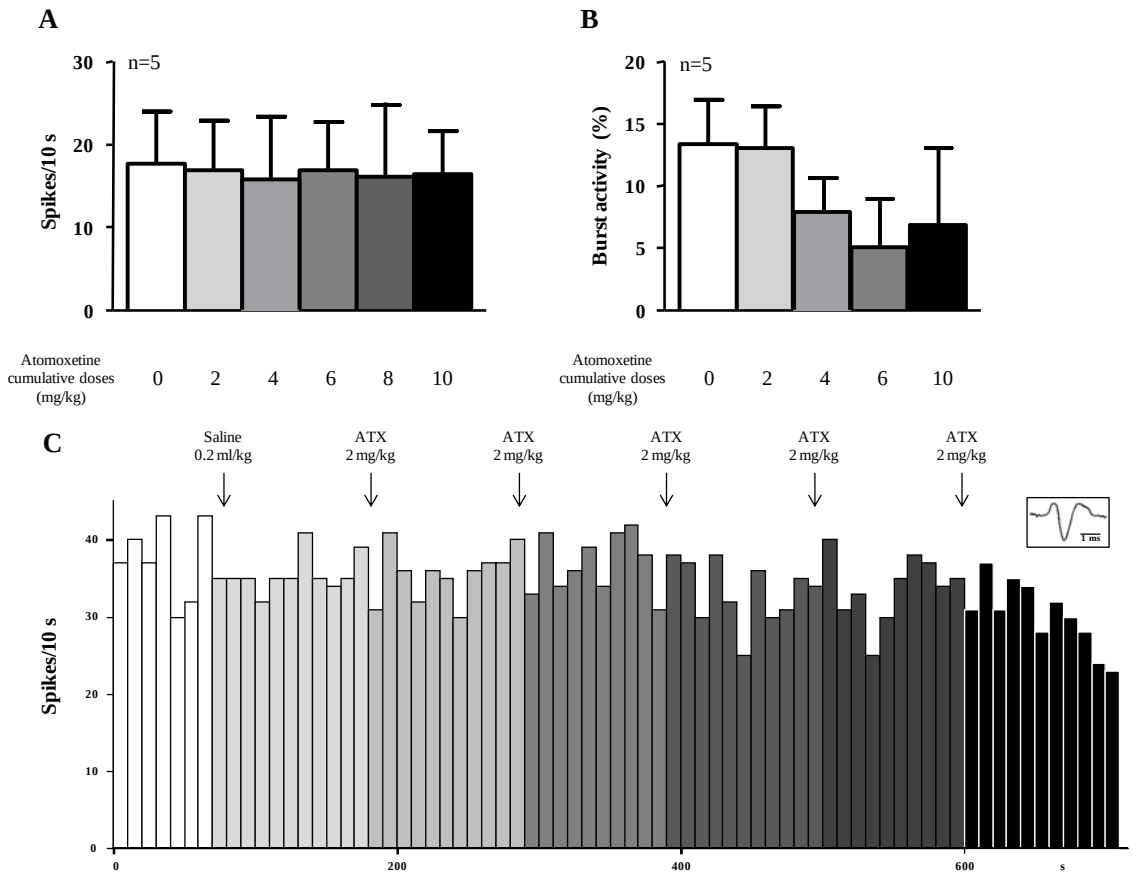


Figure 42: Atomoxetine does not change the electrophysiological activity of midbrain dopamine neurons.
On a small population of VTA dopamine neurons, atomoxetine was administered up to a cumulative dose of 10 mg/kg but did not change the firing (**A**) and burst activities (**B**) of these neurons. (**C**): Representative recording example of a neuron insensitive to cumulative intravenous ATX administration. Boxed is shown a typical action potential waveform of this VTA dopamine neuron.

III-3-B- Methylphenidate exerts hidden excitatory effects on dopamine neurons

As mentioned before, administration of a single 2 mg/kg dose of MPH significantly decreased the firing rate of dopamine neurons (Fig. 43A-C). Subsequent 0.2 mg/kg eticlopride administration, a dopamine receptor D_2 antagonist, rescued baseline firing and burst activities. For most of the neurons tested, the combination of 2 or 4 mg/kg of MPH and 0.2 mg/kg of eticlopride resulted in higher firing rates and burst activities than under baseline conditions (Fig. 43). These results indicate that initial blockade of the dopamine reuptake transporter by MPH increases dopamine concentrations in the synaptic cleft, which in turn activate the G_i-coupled inhibitory dopamine D_2 autoreceptors. Furthermore, we have demonstrated that MPH exerts

hidden excitatory effects on VTA dopamine neurons and that this effect can only be unmasked if dopamine D_2 receptors are inactivated.

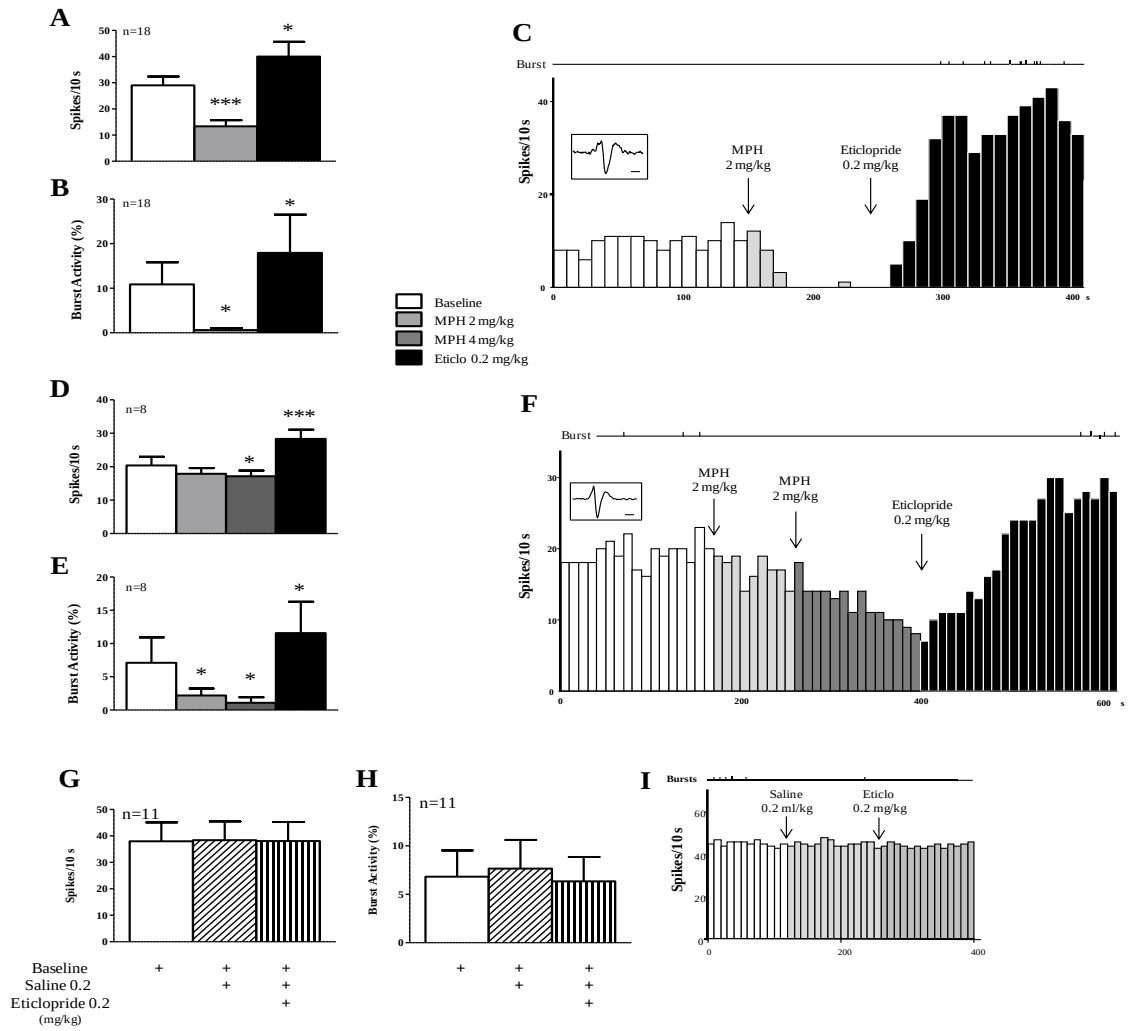


Figure 43: Methylphenidate exerts a hidden excitatory effects on midbrain dopamine neurons.

(**A**, **B**, **C**): Two mg/kg of MPH significantly decreased the firing and burst activities of midbrain dopamine neurons, as observed previously. However, dopamine D_2 receptor antagonism (eticlopride, 0.2 mg/kg) successfully rescued MPH-induced firing rate decreases, and even further increased the firing and burst activities to levels higher than under baseline condition, revealing therefore the excitatory effects of MPH onto VTA dopaminergic neurons. Following eticlopride, burst activities were higher than baseline in 10 out of 20 neurons. Representative firing histogram example of one neuron which was silenced by MPH (**C**), and a less sensitive neuron, still responding to a dose-response of MPH, administered up to 4 mg/kg (**F**). The selective dopamine D_2 receptor antagonist eticlopride not only reversed the MPH-induced effects but produced an additional excitatory effect on the firing rates (**A**, **C**, **D**, **F**) and burst activities (**B**, **C**, **E**, **F**). Boxed are shown the corresponding dopamine neuron action potential waveforms. Note that alone, eticlopride did not alter the electrical activities of midbrain dopamine neurons (**G-I**). *P<0.05, ***P<0.001 *vs.* baseline, Neuman-Keuls test after significant repeated measures ANOVA.

III-3-C- The excitatory component of MPH depends upon both D_1 and alpha-1 receptors

In 23 neurons, the combination of 2 mg/kg of MPH and 0.2 mg/kg of eticlopride (MPH/eti) resulted in a 27% and 74% significantly higher firing and burst activity (respectively Fig. 44A and 44B). Pre-administration of the alpha-1 receptor antagonist prazosin at 1.5 mg/kg, did not prevent (firing: $F_{(1,26)}=0.16$, $p>0.5$, burst: $F_{(1,26)}=0.29$, $p>0.6$) the excitatory effect observed following MPH and eticlopride administration (Fig. 44A-C). Three neurons did not show any increase in firing activity following MPH and eticlopride. Similarly, initial dopamine D_1 receptor antagonism with SCH23390 (0.6 mg/kg) failed to prevent such effects (firing: $F_{(1,26)}=0.08$, $p>0.7$, burst: $F_{(1,26)}=0.53$, $p>0.4$, Fig. 45A-C). However, the excitatory effects of MPH following D_2 blockade were completely lost when the combined administration of both prazosin (1.5 mg/kg) and SCH23390 (0.6 mg/kg) was performed (7/8 neurons, Fig. 46A-C), showing that both D_1 and alpha-1 receptors are required for MPH to exert its excitatory effects. On their own, prazosin and SCH23390 did not exert any significant effects on the firing and burst activities of VTA dopamine neurons.

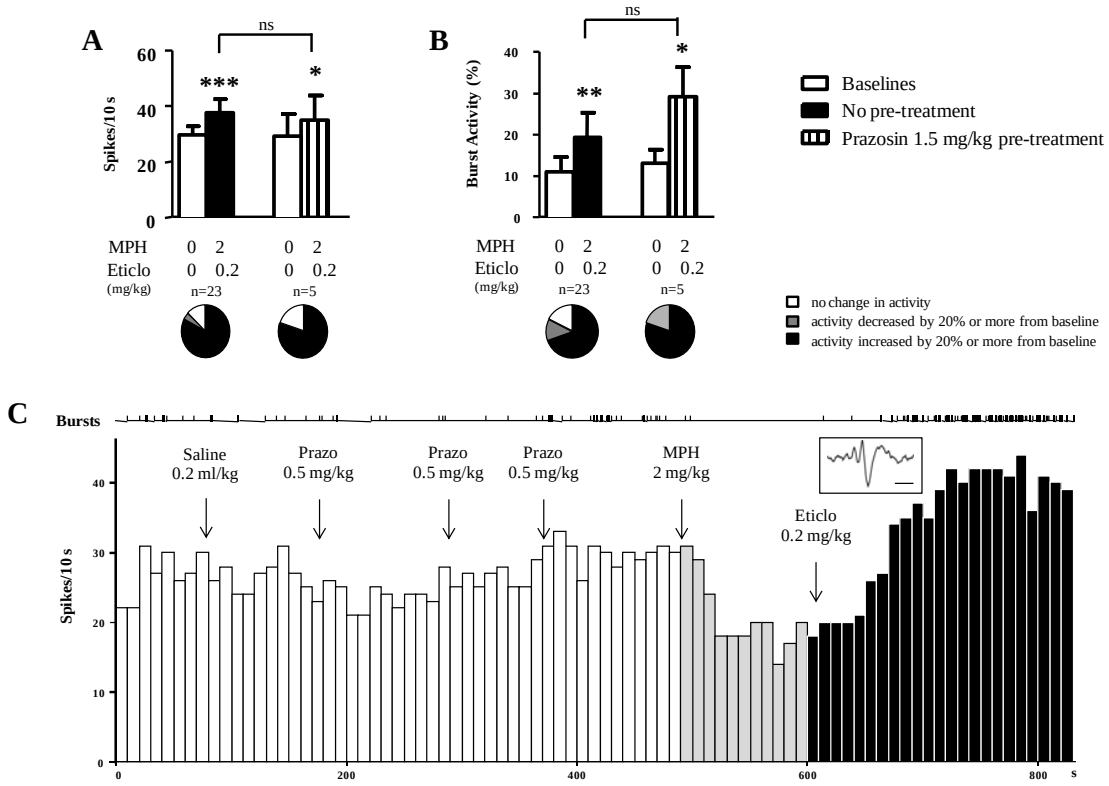


Figure 44: Adrenergic alpha-1 receptors alone are not responsible for the excitatory effects of methylphenidate.

(**A**, **B**): When the alpha-1 receptor antagonist prazosin was pre-administered (1.5 mg/kg), MPH (2 mg/kg) still exerted excitatory effects on VTA dopamine neurons following dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg). (**C**): Representative firing histogram example of a recording where prazosin was cumulatively administered as a pre-treatment and with progressive 0.5 mg/kg increments, up to a dose of 1.5 mg/kg. Boxed is shown the corresponding dopamine neuron action potential waveform. ns: non-significant, *P<0.05, **P<0.01, ***P<0.001 *vs.* baseline, Bonferroni test after significant repeated measures two-way ANOVA.

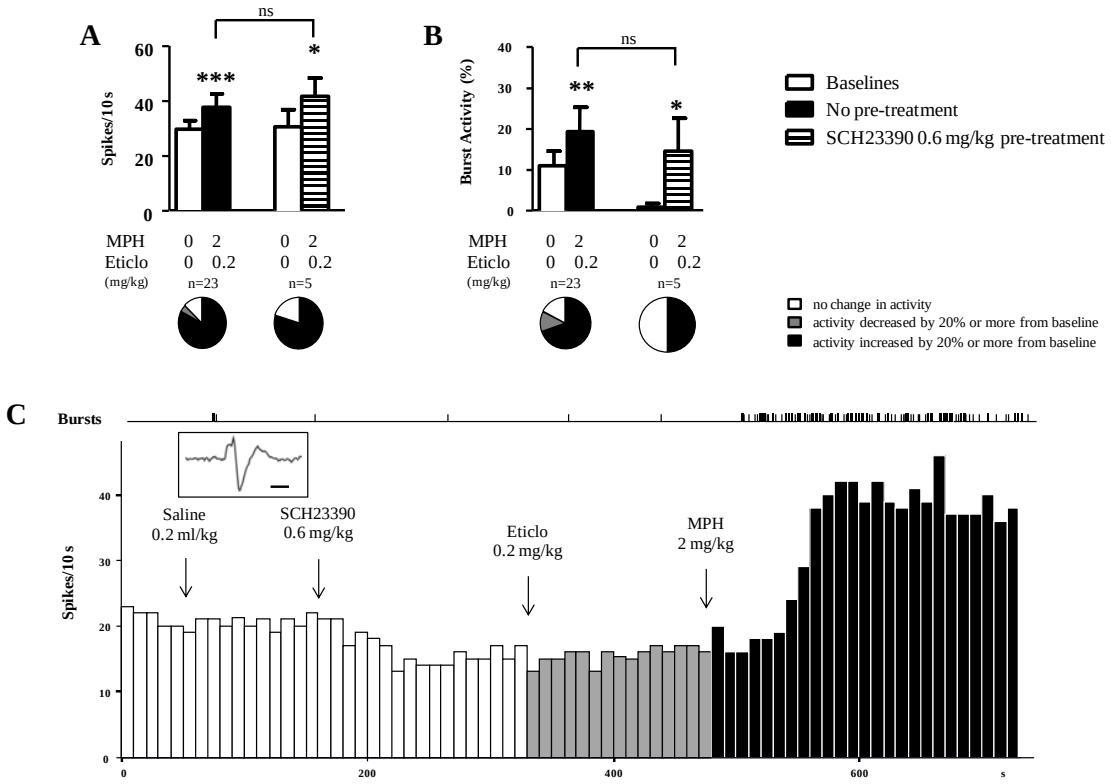


Figure 45: Dopamine D_1 receptors alone are not responsible for the excitatory effects of methylphenidate.

When the dopamine D_1 receptor antagonist SCH23390 was pre-administered (0.6 mg/kg), the combination of MPH (2 mg/kg) and eticlopride (0.2 mg/kg) still induced higher firing (**A**) and burst activities (**B**) of VTA dopamine neurons than under baseline conditions. (**C**): Representative time course example of such recordings. Boxed is shown the corresponding dopamine neuron action potential waveform. In this neuron, eticlopride was administered before MPH. ns: non-significant, *P<0.05, **P<0.01, ***P<0.001 *vs.* baseline, Bonferroni test after significant repeated measures two-way ANOVA.

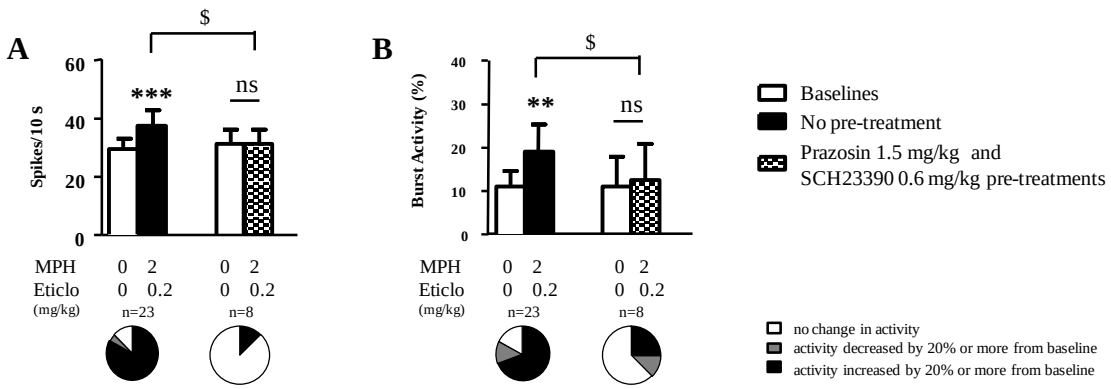


Figure 46: Methylphenidate requires both adrenergic alpha-1 and dopamine D_1 receptors to exert its excitatory effects on midbrain dopamine neurons.
Both alpha-1 receptor antagonism (prazosin 1.5 mg/kg) and dopamine D_1 receptor antagonism (SCH23390 0.6 mg/kg), administered as a pre-treatment, successfully prevented MPH/eticlopride-induced activation of firing (**A**) and burst (**B**) activities in almost all of the neurons recorded this way (7/8). ns: non-significant, **P<0.01, ***P<0.001 *vs.* baseline, $P<0.05 *vs.* specified conditions, Bonferroni test after significant repeated measures two-way ANOVA.

We then examined whether the hidden MPH-induced excitatory effect could be related to glutamate receptor activation. Interestingly, 0.5 mg/kg of intravenous MK 801 administration, an NMDA receptor antagonist, totally prevented the occurrence of higher firing and burst activities observed following MPH and D_2 antagonism in 5 out of 7 neurons tested this way (71%, Fig. 47). MK801 intravenous administration, up to 0.5 mg/kg, did not alter baseline firing nor burst activities of midbrain dopamine neurons (not shown). There was no interaction between intravenous challenges and MK 801 treatment (firing: $F_{(1,28)}=0.37$, $p>0.5$, burst: $F_{(1,28)}=1.02$, $p>0.3$).

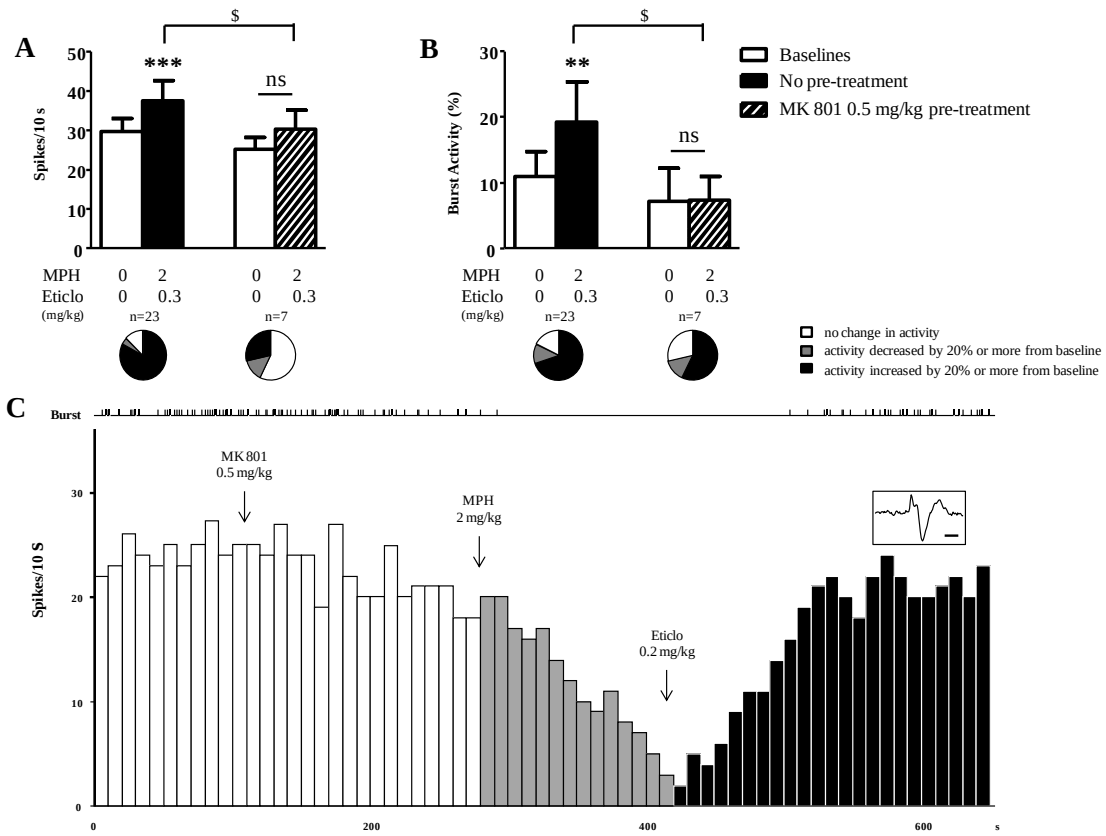


Figure 47: Importance of glutamatergic neurotransmission in methylphenidate-induced excitatory effects.
(**A**, **B**): Pre-treatment with the selective glutamatergic antagonist MK801 (0.5 mg/kg) completely prevented MPH-induced excitatory effects, observed following dopamine D_2 receptor antagonism. (**C**): Representative recording example of such protocol. Boxed is shown the corresponding dopamine neuron action potential waveform. Note a return to baseline firing rate levels following recovery of MPH-induced firing rate reduction by eticlopride. ns: non-significant, **P<0.01, ***P<0.001 *vs.* baseline, $P<0.05 *vs.* specified conditions, Bonferroni test after significant repeated measures two-way ANOVA.

III-3-D- Influences of local catecholamines

Dopamine, norepinephrine and methylphenidate were applied locally onto ventral tegmental area dopamine neurons using iontophoresis. Under baseline conditions, dopamine, norepinephrine and MPH all strongly inhibited the firing activities of midbrain dopamine neurons (Fig. 48A,B). Interestingly, after MPH administration and dopamine D_2 receptor antagonism, dopamine remains an inhibitory neurotransmitter on the firing activity, but its efficacy is significantly lower ($p<0.001$) than under baseline conditions. On the contrary, both MPH and norepinephrine tend to exert small excitatory effects on the firing activity of dopaminergic neurons following D_2 blockade (Fig. 48A,C).

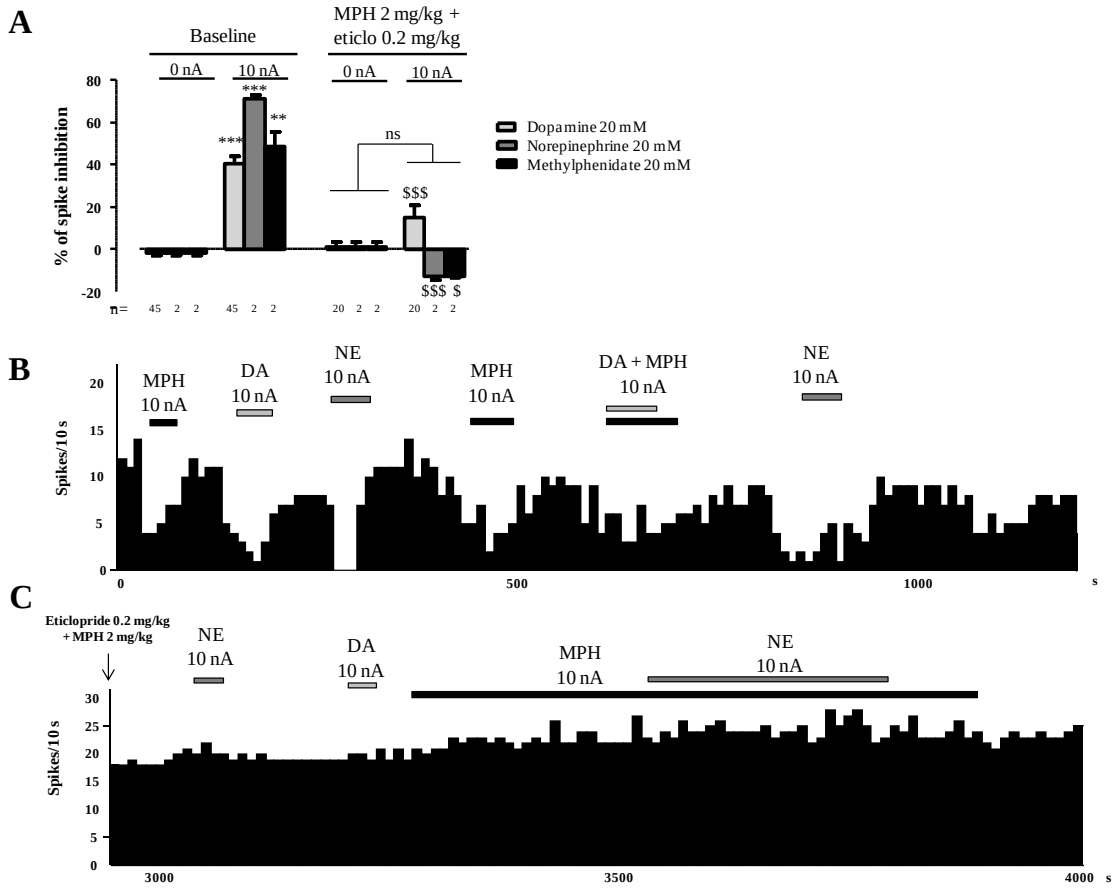


Figure 48: The excitatory effect induced by the combination methylphenidate/eticlopride does not mainly involve local catecholamine receptors. (**A**, **B**): Local iontophoretic applications of dopamine, norepinephrine and methylphenidate on VTA dopamine neurons result in firing activity inhibitions. After MPH and eticlopride administration (**C**), these inhibitions are no longer observed. Under these conditions, norepinephrine and MPH produce only very small excitations, while locally applied dopamine remains devoid of action. Note the strong efficiency of norepinephrine to suppress spike generation under baseline conditions (**A**, **B**), which is a dopamine D_2 receptor dependent mechanism, as these effects were not observed following D_2 receptor blockade (**A**, **C**). Here and in the following figures, horizontal bars indicate the transient ejection periods using microiontophoresis. **P<0.01, ***P<0.001 *vs.* respective 0 nA currents, Neuman-Keuls test after significant ANOVA. $P<0.05, $$$P<0.001 *vs.* respective 10 nA currents, unpaired Student's t-test.

III-3-E- Importance of glutamatergic neurotransmission and NMDA receptors in MPH-induced excitatory effects

The impact of local glutamatergic neurotransmission was investigated on the excitatory effects of MPH. Local application of HA-966, a glycine NMDA receptor antagonist, or kinurenic acid, a ionotropic glutamate receptor antagonist, did not change the overall spontaneous firing (Fig. 49A) or burst activity (Fig. 49B) of dopamine neurons. However, we found that HA-966 had no consequences on VTA midbrain neuron firing rates in 8 out of 11 neurons (73%), increased firing rates in 1 neuron (9%) and decreased firing rates in 2 neurons (18%). On the other hand, kinurenic acid decreased firing rates in 10 out of 13 neurons (77%), increased firing rates in 1 neuron (8%) and had no effect in only 2 neurons (16%). Neither of the two antagonists ever exerted any effect on burst activities in all of the neurons (100%) tested this way. Fig. 49C-E shows that both kinurenic acid and HA-966 tend to exert a stronger inhibition of VTA dopamine neurons following methylphenidate and eticlopride exposure than under baseline conditions. These results indicate that MPH exerts its excitatory effects through mechanisms that may depend upon local ionotropic glutamate receptor activation. The fact that both antagonists did not reduce burst activity was unexpected, as current literature suggests that the burst activity of midbrain dopamine neurons strongly depends upon tonic activation of local NMDA receptors. In the prefrontal cortex, however, HA-966 was very efficient in reducing NMDA-induced firing rate activations of pyramidal neurons (not shown, these results have been recently submitted for publication).

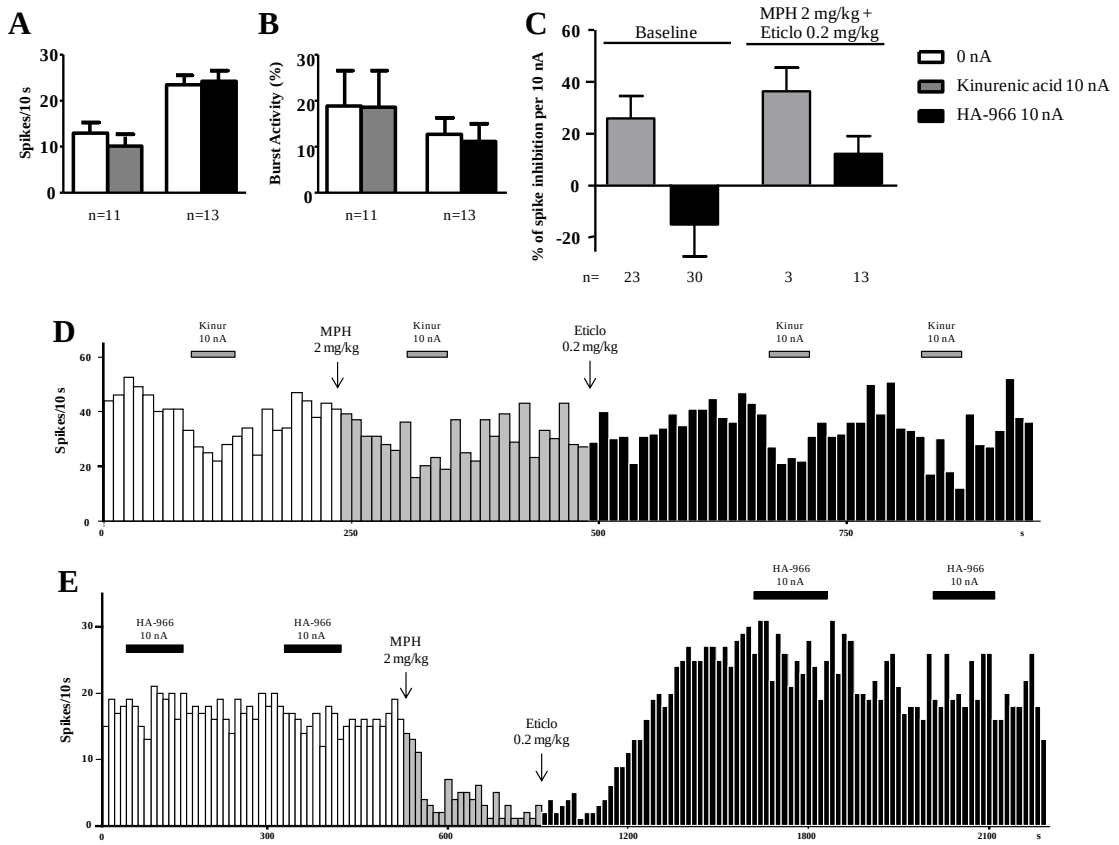


Figure 49: The importance of local glutamatergic neurotransmission in methylphenidate-induced excitatory effects.
Local application of kinurenic acid, a ionotropic glutamate receptor antagonist, or HA-966, a glycine NMDA receptor antagonist, did not change spontaneous firing (**A**) or burst activities (**B**), even following both MPH and eticlopride exposures (**C**). Note that some neurons are more sensitive than others to local glutamatergic receptor blockade (**D**, **E**) and that these firing rate reductions can be potentiated following MPH/eticlopride administration.

As expected, local 10 nA NMDA application induced significant strong increases in firing and burst activity of ventral tegmental area dopamine neurons (respectively increasing activities by 2.4 and 2.2 fold, $p<0.001$ vs 0 nA, Fig. 50). NMDA (10 nA) increased firing rates, which was not reversed by HA-966 (10 nA, Fig. 50A,B), contrary to what was observed in the prefrontal cortex (not shown), whereas kinurenic acid (10 nA) strongly reduced NMDA-induced increase of firing ($p=0.0024$ vs NMDA alone, Fig. 50A) and burst activities ($p=0.012$ vs NMDA alone, Fig. 50B).

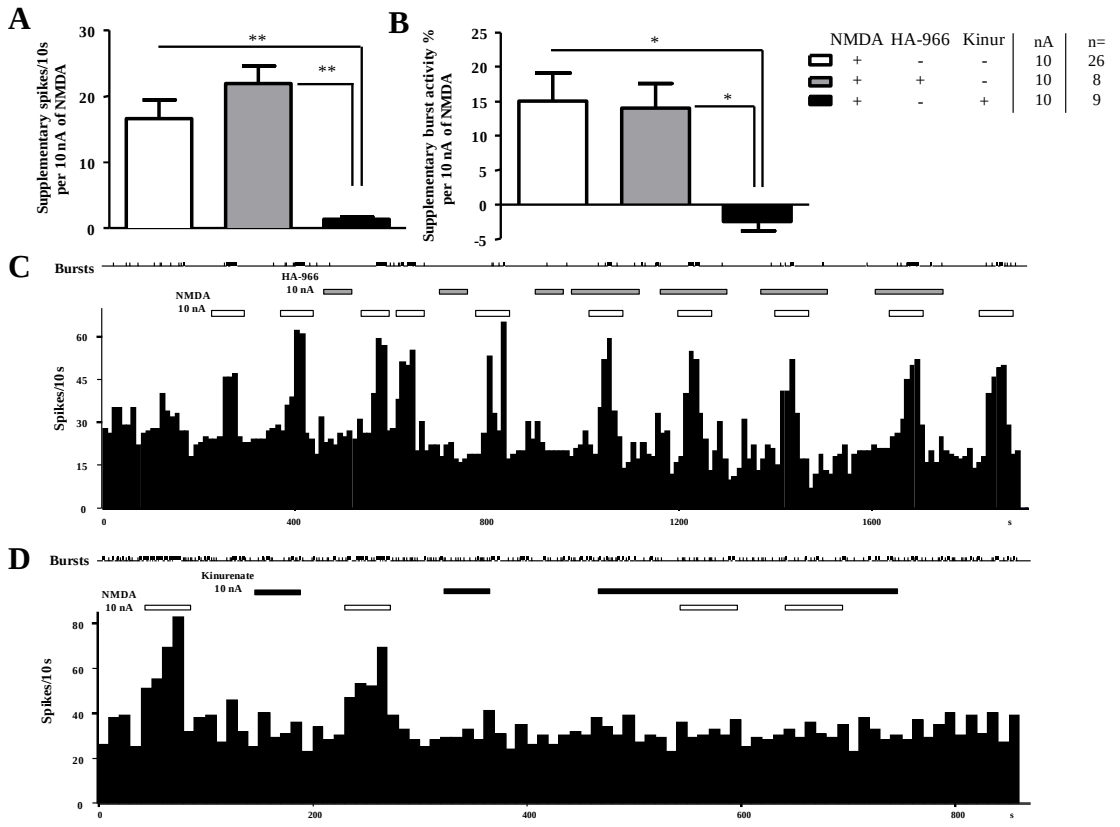


Figure 50: The importance of local NMDA neurotransmission on midbrain dopamine neurons.
(**A**): Local application of NMDA (10 nA) produced strong and transient firing rate increases in VTA dopamine neurons, an effect reversed by kinurenic acid applications (10 nA), but not HA-966 (10 nA). (**B**): Similarly, burst activities were increased following NMDA application, an effect only reversed by kinurenic acid applications. (**C**, **D**): Representative recording examples illustrating such effects. *P<0.05, **P<0.01, *vs.* specified conditions, Neuman-Keuls test after significant ANOVA.

III-3-F- Role of the PFC in MPH-induced excitatory effects

Finally, prefrontal cortex inactivation was achieved by local intra-PFC lidocaine perfusion (Fig. 38B). Under these conditions, no excitatory effects following MPH/eti on cell firing and burst activity were observed in 75% of all recordings (6/8 neurons, Fig. 51A-C). There was no interaction between intravenous challenges and PFC inhibition (firing: $F_{(1,29)}$=0.64, p>0.4, burst: $F_{(1,29)}$=0.11, p>0.7). These results highlight the importance of the neurotransmission connexion between the PFC and the VTA, in the excitatory effects of methylphenidate following D_2 receptor blockade.

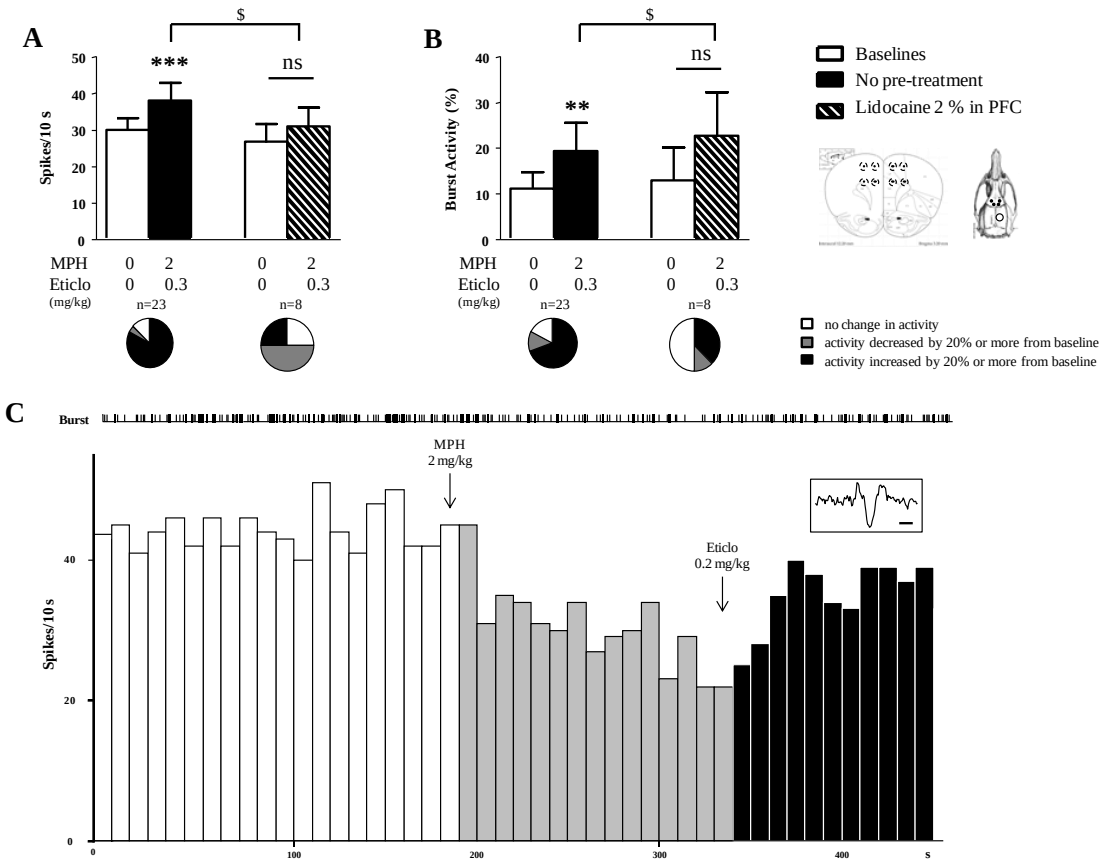


Figure 51: The role of the prefrontal cortex in methylphenidate-induced excitatory effects on midbrain dopamine neurons.
Inactivation of the prefrontal cortex (PFC) using micro-perfusions of lidocaine (2% w/v, 2.5 µl per injection site, 2 µl/min, 8 sites in total) prevented MPH-induced excitatory effects on both the firing (**A**) and burst activities (**B**) of VTA dopamine neurons. Microperfusions of lidocaine were performed immediately prior to the intravenous MPH challenge. ns: non-significant, **P<0.01, ***P<0.001 *vs.* baseline, $P<0.05 *vs.* specified conditions, Bonferroni test after significant repeated measures two-way ANOVA.

III-4- Discussion

Here, we have demonstrated that MPH, following D_2 receptor blockade, exerts strong excitatory effects on ventral tegmental area dopamine neurons. This excitatory input may involve a combination of alpha-1, D_1 and glutamate receptors, as well as functional meso-cortico connexions, which are key centres involved in addiction (Chen *et al.*, 2011).

As expected, methylphenidate induced dose-dependent inhibition of both the firing and burst activities of midbrain dopamine neurons (Fig. 43), as observed before with MPH (Choong *et al.*, 2004; Shi *et al.*, 2004) or other psychostimulants (Einhorn *et*

al., 1988; Panin *et al.*, 2012). MPH blocks the dopamine reuptake transporter, which prevents extracellular dopamine from being reuptaken into the presynaptic element (Markowitz *et al.*, 2008; Volz *et al.*, 2008). This increase in extracellular dopamine concentration (Wilens, 2008) activates the inhibitory dopamine D_2 autoreceptors, therefore producing decreases in both firing rates and burst activities (Centonze *et al.*, 2002), through D_2/D_3-mediated negative feedback (Shi *et al.*, 2000a; Viggiano *et al.*, 2004). Intravenous exposure to ATX did not induce firing or burst activity alterations in midbrain dopamine neurons (Fig. 42). Atomoxetine does not change extracellular levels of dopamine in both the striatum and the nucleus accumbens (Bymaster *et al.*, 2002). Dopamine D_2 receptors are major actors in dopaminergic neurons regulation (Guiard *et al.*, 2008; Mercuri *et al.*, 1997). Dopamine neuron firing activity alterations are believed to play a crucial role in the behavioural abuse responses to psychostimulants (Ellinwood *et al.*, 1983; Wolf *et al.*, 1993). The firing inhibition produced by either MPH or D_2 receptor agonists can be reversed by selective D_2 antagonists (Ackerman *et al.*, 1993; El Mansari *et al.*, 2010; Federici *et al.*, 2005; Tepper *et al.*, 1997). However, we have shown here that firing and burst activity rescuing revealed the excitatory effects of MPH on these neurons (Fig. 43-44). A few studies have brought out the fact that psychostimulant administrations, followed by D_2 receptor antagonism, lead to such effects (Shi *et al.*, 2000b; Shi *et al.*, 2004). However, we found that simultaneous alpha-1 and D_1 receptor antagonisms are required to suppress the MPH-induced excitatory effects (Fig. 46), while either alpha-1 or D_1 antagonism alone failed to reduce such effects (Fig. 44-45). Such results differ from both the aforementioned studies, as the authors found only a partial effect of alpha-1 blockade, but not D_1 blockade (Shi *et al.*, 2000a; Shi *et al.*, 2004). Adrenergic alpha-1 receptor inhibition is known to reduce bursting activity as well as regularising the firing rates of VTA dopamine neurons, while alpha-2 receptor blockade increased burst activities and provoked irregular firing (Grenhoff *et al.*, 1993b). In fact, alpha-1 inhibition sometimes increases, sometimes decreases firing and burst activities, according to some authors and in agreement with what we found, showing no overall effect due to inconsistencies. Another study also demonstrated that increases in extracellular adrenergic levels, induced by reboxetine exposure, will activate bursting discharges of VTA dopamine neurons, an effect that is probably mediated by the activation of excitatory alpha-1 receptors (Linner *et al.*, 2001). In the present study, we did not observe any reduction of burst activity following 1.5 mg/kg of prazosin administration, even after 0.5 mg/kg progressive increments (Fig.

44). Moreover, the selective norepinephrine reuptake inhibitor atomoxetine failed to induce a modification of the firing rates of midbrain dopamine neurons (Fig. 42). Noradrenergic neurotransmission may play a crucial role in the regulation of VTA dopamine neurons, mainly through direct connexion from the locus coeruleus to the VTA, as shown by previous anatomical and electrophysiological studies (Grenhoff *et al.*, 1993a; Jones *et al.*, 1977; Simon *et al.*, 1979). In parallel, VTA projections onto the locus coeruleus have been observed (Beckstead *et al.*, 1979; Deutch *et al.*, 1986; Samuels *et al.*, 2008; Simon *et al.*, 1979; Swanson, 1982), although they remain very sparse (less than 1% of all VTA dopamine neurons project onto the locus coeruleus) (Swanson, 1982). Interactions between the locus coeruleus and the VTA are crucial in mediating the effects of psychostimulants such as amphetamines (Ferrucci *et al.*, 2013). Indeed, following methamphetamine exposure, initial norepinephrine release by noradrenergic neurons from the locus coeruleus, combined with dopamine release from midbrain terminals, will activate PFC functions (Ferrucci *et al.*, 2013). Moreover, norepinephrine-deficient mice present hypersensitivity to cocaine and amphetamine (Schank *et al.*, 2006; Weinshenker *et al.*, 2008). In our study, local noradrenergic pathways may be partially required for MPH-induced excitatory effect. Noradrenergic modulation of midbrain dopamine neurons has not been clearly characterised. Indeed, a few studies have shown that the norepinephrine reuptake inhibitor reboxetine can increase the firing activities of midbrain dopamine neurons (El Mansari *et al.*, 2010; Linner *et al.*, 2001). The alpha-1 receptor antagonist prazosin is known to reduce bursting activity, while the alpha-2 receptor antagonist idazoxan has opposite effects (Grenhoff *et al.*, 1993a; Grenhoff *et al.*, 1993b). Contradictory results emerge within our study, as the norepinephrine reuptake inhibitor atomoxetine did not induce firing rate inhibition of VTA dopamine neurons (Fig. 42), unlike reboxetine. To be noted: alone, eticlopride did not significantly affect the overall electrical discharges of VTA dopamine neurons (Fig. 45C).

Under baseline conditions, local iontophoretic application of dopamine, and norepinephrine exerted strong inhibition of dopamine neuron firing activities (Fig. 48), as emphasised before (Einhorn *et al.*, 1988; Guiard *et al.*, 2008; Kiyatkin *et al.*, 1998; Paladini *et al.*, 2004; Rosenkranz *et al.*, 1999; White *et al.*, 1984; White *et al.*, 1986). According to some studies, norepinephrine strongly binds to the G_i-coupled dopamine D_4 receptor, which belongs to the D_2-like family, hence producing firing inhibition

(Lanau *et al.*, 1997; Newman-Tancredi *et al.*, 1997; Root *et al.*, 2015). In the present study, we witnessed a strong reduction of dopamine and norepinephrine-induced firing rate inhibition following eticlopride administration (Fig. 48), which indicates that dopamine and norepinephrine-induced firing rate inhibitions are also both mediated through local dopamine D_2-like receptor activation. Our study, for the first time, reports that local methylphenidate application onto VTA dopamine neurons produces a similar effect to what is observed using dopamine, a result of dopamine D_2 inhibitory autoreceptors activation following MPH-induced DAT blockade. Surprisingly, when methylphenidate and eticlopride were administered, dopamine, but not methylphenidate or norepinephrine, still produced inhibitory effects (in all of our recordings) on dopaminergic neuron firing rates (Fig. 48A), although such effects are greatly dampened, which could be partially explained by mutual competition on the dopamine D_2 receptor by dopamine (leaking from the pipette) and eticlopride. These results differ from those in the study by Einhorn in 1988, where the author found that local dopamine application does not alter firing parameters following sulpiride administration (Einhorn *et al.*, 1988). In line with our results, one study found that local dopamine application onto VTA dopaminergic neurons leads to firing decreases even during the application of the D_2 receptor antagonist raclopride (Guiard *et al.*, 2008). In this study, the authors also found that norepinephrine application leads to firing inhibition under baseline conditions, an effect strongly attenuated under idazoxan iontophoresis, an alpha-2 receptor antagonist.

In the ventral tegmental area, burst activity is regulated by local neurotransmission and each bursting activity releases greater quantities of dopamine at dopamine terminals versus single or bursting spike activity (Bean *et al.*, 1991; Gonon, 1988; Oster *et al.*, 2015). Alternate firing modes between bursting and single discharges are vital to maintain balanced D_1 and D_2 receptor occupancies (Dreyer *et al.*, 2010). Indeed, in our experiment, local NMDA application induced firing rate increases and burst discharges (Fig. 50), as seen before (Chergui *et al.*, 1993; Johnson *et al.*, 1992; Kitai *et al.*, 1999; Mereu *et al.*, 1997; Overton *et al.*, 1997; Wickham *et al.*, 2015; Zweifel *et al.*, 2009), probably through enhancement of excitatory currents (Wu *et al.*, 1999). However, only the broad ionotropic glutamate receptor antagonist kinurenate, or 7-chloro-kinurenic acid (Mok *et al.*, 2009), was effective in abolishing NMDA-induced activations (Fig. 50A,C). The specific NMDA/glycine antagonist HA-966 failed to

reduce such activations in midbrain neurons (Fig. 50A,B), but strongly dampened NMDA-induced firing rate activations in cortical neurons (not shown). These two antagonists did not significantly modify spontaneous firing and burst activities of midbrain dopamine neurons (Fig. 49A-B), although a small population of neurons were mildly affected by these antagonists under both baseline and after MPH/eti conditions, explaining such variations in the responses (Fig. 49C-E). One study found opposite results, where intraperitoneal administration of kinurenic acid produced firing and burst activity decreases (Linderholm *et al.*, 2007). These differences can be explained by the techniques used. Indeed, the authors administered a rather high intraperitoneal dose of 4-chlorokinurenine, a precursor to 7-chloro-kinurenic acid, that will systemically block all NMDA receptors in the brain. Therefore, glutamatergic neurotransmission pathways, arising from other brain regions, may directly or indirectly play an important role in the regulation of midbrain dopamine neurons, an effect that we did not witness locally, although a previous study from our laboratory showed that 85% of midbrain dopaminergic neurons are activated following 0.5 mg/kg of intravenous MK801, a non-competitive NMDA antagonist (Bennett *et al.*, 2007), probably as a consequence of the inhibition of GABAergic pathways. Here, we suggest that only a minority of dopamine neurons are under direct tonic NMDA receptor activation, while other neurons remain insensitive to local glutamate neurotransmission. Here, we therefore counterbalance previous results showing that tonic activations of NMDA receptors are responsible for bursting discharges of midbrain dopamine neurons. Moreover, some dopamine neurons might be activated by kinurenic acid because of its diffusion onto GABAergic interneurons, located at close proximity to dopaminergic neurons, alleviating the inhibition of firing activities from interneurons on these neighbouring dopamine neurons. Such effects on interneurons have yet to be identified. Moreover, intra-VTA infusion of a GABA antagonist induced strong dose-dependent dopamine release (Giorgetti *et al.*, 2002).

Finally, our results demonstrate the role of NMDA receptors in mediating the hidden MPH-induced excitatory effects. Many studies have found that burst activity is strongly regulated by afferent PFC connexions (Lodge, 2011; Murase *et al.*, 1993; Omelchenko *et al.*, 2007; Overton *et al.*, 1997; Patton *et al.*, 2013; Svensson *et al.*, 1989). Pre-treatment with the non-competitive NMDA antagonist MK 801 at 0.5 mg/kg significantly prevented the MPH-induced excitatory effects (Fig. 47). Local MK 801

application led to dopamine neurons excitation in one study, a result that partially corroborates our findings (French *et al.*, 1993). Another study suggested that local MK 801 application may also target GABA neurons, which are major actors in dopamine neuron modulations within the VTA (Liu *et al.*, 2012a) and the substantia nigra (Nissbrandt *et al.*, 1994). Lidocaine, a blocker of fast voltage-gated sodium channels (Catterall, 2002; Courtney, 1975; Sheets *et al.*, 2003; Vedantham *et al.*, 1999; Yeh *et al.*, 1985), is used in medicine as a local anaesthetic (McDonald *et al.*, 2015; Perniola *et al.*, 2014; Terkawi *et al.*, 2015; Yung *et al.*, 2015) or in intravenous preparations (Hassani *et al.*, 2015; Honarmand *et al.*, 2015; Khan *et al.*, 2015; Kranke *et al.*, 2015; Papapetrou *et al.*, 2015). This powerful anaesthetic is also routinely used in veterinary medicine (DeRossi *et al.*, 2015; Hendrickx, 2015; Hermeto *et al.*, 2015; Morgaz *et al.*, 2014). It has been frequently used to induce cortical inactivation in animal models (Ahn *et al.*, 2002; Martin, 1991; Sara *et al.*, 1995; Tehovnik *et al.*, 1997; Uehara *et al.*, 2007; Valenti *et al.*, 2009). When lidocaine was slowly pre-perfused within the PFC, no hidden excitatory effects of MPH were observed following D_2 receptor antagonism in the majority of the neurons tested (Fig. 51). This highlights the importance of PFC glutamatergic descending connexions onto the VTA in response to MPH/eti administration, in line with another study which clearly demonstrated that PFC lesion prevents MPH behavioural sensitisation (Lee *et al.*, 2008). Moreover, it has been established that these pathways are essential in maintaining normal VTA functions (Almodovar-Fabregas *et al.*, 2002; Carr *et al.*, 2000; Chen *et al.*, 2011; Fujisawa *et al.*, 2011), which can be disrupted under psychostimulant administration (Wolf, 1998), mainly through calcium channel regulations (Rajadhyaksha *et al.*, 2004; Rajadhyaksha *et al.*, 2005).

To conclude, we propose that MPH firstly activates both dopamine D_1 and adrenergic α1 receptors within the PFC, which, in turn, trigger glutamate release in the midbrain originating from cortico-tegmental projections, inducing *in fine* NMDA receptor activation and therefore enhancement of the firing rates of VTA dopaminergic neurons. Such an excitatory input of MPH on midbrain dopamine neurons can only be observed when dopamine D_2 inhibitory autoreceptors are inactivated.

Chapter IV – The impact of chronic methylphenidate administration on adult animals.

IV-1- Introduction

Some studies have reported that chronic stimulant use may lead to some growth deficits in adolescents (Charach *et al.*, 2006; Poulton *et al.*, 2003; Poulton *et al.*, 2013; Rose *et al.*, 2015; Safer *et al.*, 1972; Spencer *et al.*, 2006) and rodents (Komatsu *et al.*, 2012), although this is disputed by a few (Biederman *et al.*, 2003; Harstad *et al.*, 2014; Sirois *et al.*, 2009). Other studies reported changes in fertility (Adriani *et al.*, 2006; Fazelipour *et al.*, 2012; Ramasamy *et al.*, 2014). Concerns arise with acute and chronic psychostimulant uses, as they might induce long-lasting neuronal adaptations (Achat-Mendes *et al.*, 2003; Konova *et al.*, 2015; Lepelletier *et al.*, 2015; Marco *et al.*, 2011; Urban *et al.*, 2013a). As mentioned before, ADHD drugs may sensitise patients to later legal (Bron *et al.*, 2013; Dalsgaard *et al.*, 2014; Rush *et al.*, 2005; Vansickel *et al.*, 2011) and/or illegal drug abuse (Dalsgaard *et al.*, 2014; Dos Santos Pereira *et al.*, 2015; Jordan *et al.*, 2014; Vansickel *et al.*, 2011). Indeed, in adults, acute MPH induces craving for nicotine, increases tobacco consumption and smoking. In humans, ADHD adolescents have higher risks of substance use disorders when compared to the background population. Animal studies performed on hypertensive rats (SHR strain, an animal model of ADHD) have revealed that an adolescent MPH treatment at 1.5 mg/kg for 30 days, orally and followed by 22 days of drug withdrawal, but not an ATX treatment (0.3 mg/kg, i.p., 22 days of withdrawal), can induce higher cocaine sensitivity in adulthood (Jordan *et al.*, 2014; Somkuwar *et al.*, 2013). Interestingly, acute methamphetamine administration leads to a rise in blood sugar (glucose) levels (Graham *et al.*, 2010), probably through elevated levels of circulating corticosterone, as observed in that study. Methamphetamines induce hyperthermia and increase brain extracellular glucose (Pachmerhiwala *et al.*, 2010). Pre-treatment with prazosin (1 mg/kg, intraperitoneally) prevented the MDMA-induced high extracellular glucose

levels, but not hyperthermia. Such effects may arise from independent mechanisms following MDMA administration. Besides, extracellular glucose levels and neuronal activity are closely linked (Fellows *et al.*, 1992; Routh, 2010; Silver *et al.*, 1994; Vazirani *et al.*, 2013), mainly through glia metabolism coupling (Tsacopoulos *et al.*, 1996; Turner *et al.*, 2011a). In this fourth chapter, we will assess how chronic exposure to methylphenidate during adulthood can impact on physiological parameters such as body growth and glycaemia as well as neuronal adaptations of VTA dopamine neurons, PFC pyramidal neurons or striatal medium spiny neurons. With this aim, we will investigate whether chronic MPH can induce long-lasting desensitisation of dopamine D_2 receptors, NMDA neurotransmission adaptations and NMDA receptor 2B expression as well as striatal dopamine release.

IV-2- Material and Method

IV-2-A- Subjects and groups

Seventy-eight male Sprague-Dawley rats were purchased from Charles River, UK. Animals were housed in groups of 2-4 per cage, maintained at 20-22^{o}C with humidity rates above 40% under a 12:12 L/D cycle with lights ON at 07h00. Food and water were provided *ad libitum*. Animals were allowed a 3-day acclimatisation period after delivery. All experiments were performed during the light phase and with permission from the UK Home Office and De Montfort University Ethics Committee under the Project Licence 60/4333 and with the Personal Licence 60/13750.

Post-adolescence animals weighing 150 grams were used at the beginning of each treatment. Animals were randomly assigned to one of the following groups (Fig. 52):

- Animals receiving 4 mg/kg/day of MPH (or vehicle alone) in 1.2 ml/kg of saline, intraperitoneally and for 15 consecutive days.
- Animals voluntarily drinking 5 mg/kg/day of MPH (or vehicle alone) in 2 ml/kg of sucrose 10% (w/v) and for 15 consecutive days.

All experiments were performed the day following the last day of administration, hence allowing a washout period.

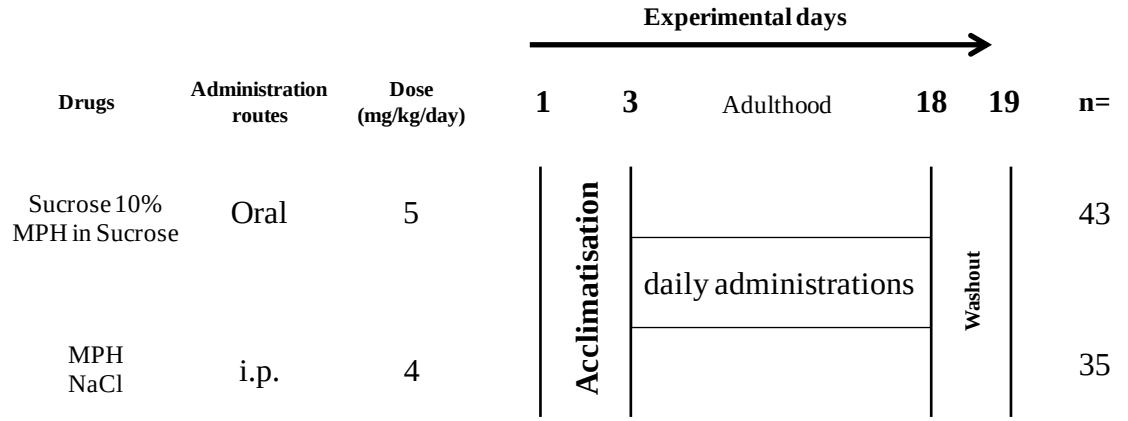


Figure 52: Experimental protocol for chronic methylphenidate treatment during adulthood.
Following 3 days of acclimatisation, rats were given MPH at doses of 4-5 mg/kg/day. Administration routes were either intraperitoneal (using a saline 0.9% w/v as vehicle) or oral (using a sucrose 10% w/v as vehicle). In the oral dosing protocol, each rat voluntarily drank 2 ml/kg of solution. MPH was either dissolved in saline 0.9% or sucrose 10%. Following 15 consecutive days of treatment, animals were allowed a 24-hour washout period. n values represent the number of animals included in each group.

IV-2-B- *In vivo* extracellular single unit electrophysiology

Please refer to paragraph III-2-B. In this chapter, the number of putative ventral tegmental area dopamine neurons per track was recorded by recording the total number of active neurons encountered during one electrode descent within the region of interest.

IV-2-C- Evaluation of glycaemia and growth

Blood sugar level, or glycaemia, was measured immediately following anaesthesia induction. A single blood drop was taken from the lateral tail vein and blood sugar levels were assessed using the Accu-Chek® blood glucose system (Aviva). In the rat, normal blood sugar levels range between 5.5 and 8.1 mM (Akbarzadeh *et al.*, 2007; Florence *et al.*, 2014; Kahn *et al.*, 1991; Wang *et al.*, 2010b). Experimentally-induced

diabetes increases glycaemia to an average of 20 mM (Kahn *et al.*, 1991; Manjunath *et al.*, 2009; Nagai *et al.*, 2013; Rasch, 1979), although some consider the limit to be above 11.1 mM (Lambertucci *et al.*, 2012).

Animals were weighed daily, at 9 AM. Growth parameters were established by comparing the weights of the animals before and at the termination of the treatment. Therefore, the weight ratio between the end and the beginning of the chronic treatment was measured for each animal and was a direct reflexion of body weight gain.

IV-2-D- *In vitro* ^{3}H-dopamine release

At the termination of the treatments, some striata were used to determine the capacity of MPH to induce tritiated dopamine release. For the complete method, please refer back to paragraph I-2-B.

IV-2-E- Western Blots

Please refer to paragraph II-2-D.

IV-2-F- Data analysis

Please refer to previous paragraphs. In our model, glycaemic values above 20 mM (3.6 g/l) were arbitrarily considered diabetic. For cell per track protocols, the total number of spontaneously firing cells per electrode descent was recorded and expressed as a mean ± standard error of the mean (S.E.M.) for each group.

IV-3- Results

IV-3-A- Effects of treatments on growth and blood sugar levels

Oral administrations of a sucrose 10% solution, at a dosing schedule of 4 ml/kg/day and for 15 consecutive days, tended to increase blood sugar levels (p=0.065), compared to animals not receiving oral dosing, whether given MPH or not (Fig. 53A). In parallel, 4-5 mg/kg/day of methylphenidate and for 15 days did not significantly change glycaemia compared to similar vehicle administration (Fig. 53B). Therefore, neither dosing routes nor treatments could significantly alter blood glucose concentrations.

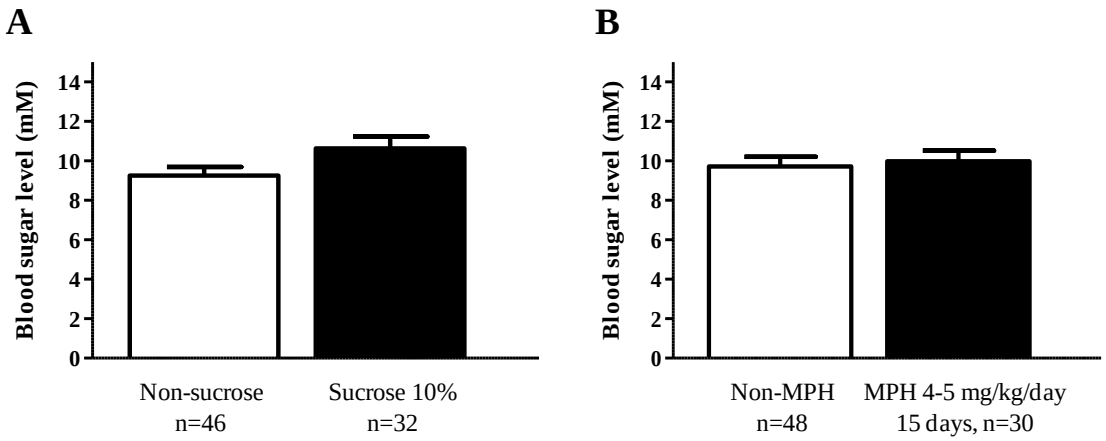


Figure 53: Chronic exposures to methylphenidate or sucrose do not alter blood sugar levels.
(**A**): Chronic oral ingestion of a 10% sucrose solution (2 ml/kg) almost significantly increased blood sugar levels (p=0.065, unpaired Student's t-test), whether MPH was given or not. (**B**): Chronic exposure to MPH (5 mg/kg/day) did not influence blood sugar levels (glycaemia), measured with the Accu-Chek system kit (Aviva).

Animals treated intraperitoneally did not show any signs of slower growth (Fig. 54A). However, it was initially found in our first group of animals that daily intraperitoneal administrations of both vehicle (n=28) and MPH (4 mg/kg/day, n=19) induced a 4% significant reduction in weight gain (p=0.04 *vs* non-i.p., unpaired Student's t-test), compared to animals treated orally (Fig. 54B). Similarly, daily MPH for 15 consecutive days did not affect growth parameters (Fig. 54C), reinforcing the hypothesis that MPH is not positively correlated to slower growth rates.

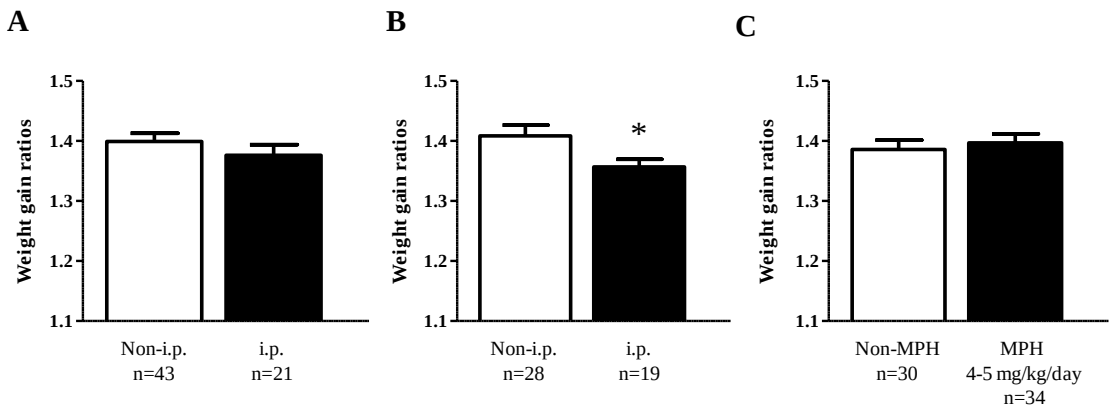


Figure 54: Impact of intraperitoneal dosing and methylphenidate on weight gain. Daily intraperitoneal administrations did not alter total body weight gain (**A**), although one population of animals included in a previous study showed significantly reduced weight gain (**B**). Chronic exposure to 4-5 mg/kg/day of MPH did not delay growth (**C**). *P<0.05, unpaired Student's t-test.

IV-3-B- Chronic methylphenidate induces MPH-insensitivity in VTA dopamine neurons

Different groups were used within our study. Indeed, animals received 4-5 mg/kg of MPH (or corresponding vehicle) either orally or intraperitoneally, for 15 consecutive days. Based upon similar responses to MPH regardless of frequency and administration route, groups were pooled between controls (naive and vehicle) and MPH-treated. Indeed, we did not observe any qualitative or quantitative difference between the two different administration routes (oral and intraperitoneal) and neuronal responses to MPH or eticlopride challenges. On a small naive population (n=10), methylphenidate was administered intravenously at 2 mg/kg. As previously mentioned (Chapter III, Fig. 43A,C), this dose successfully decreased firing and burst activities of ventral tegmental area dopamine neurons (n=9), an effect reversed after dopamine D_2 receptor antagonism using 0.2 mg/kg of eticlopride. Similarly, vehicle-treated animals displayed similar responses to both MPH and eticlopride, whether animals were treated with sucrose only (oral dosing) or saline (intraperitoneal dosing) (Fig. 55). However, in animals chronically treated with 4-5 mg/kg/day of MPH, the intravenous MPH challenge (2 mg/kg) failed to significantly decrease both firing and burst activities of midbrain dopamine neurons (Fig. 55), a result not observed with animals treated with the vehicle only (Fig. 55). Examples of representative firing rate histograms from naive, vehicle and oral MPH animals are represented respectively in Fig. 56A, B and C.

Identical representations are displayed on Fig. 57A and B for intraperitoneal administrations. Note that reversal of the MPH-induced decrease of electrical activities with eticlopride led to significantly higher firing rates compared to baseline activities in the majority of all recordings (Fig. 55A, 57A).

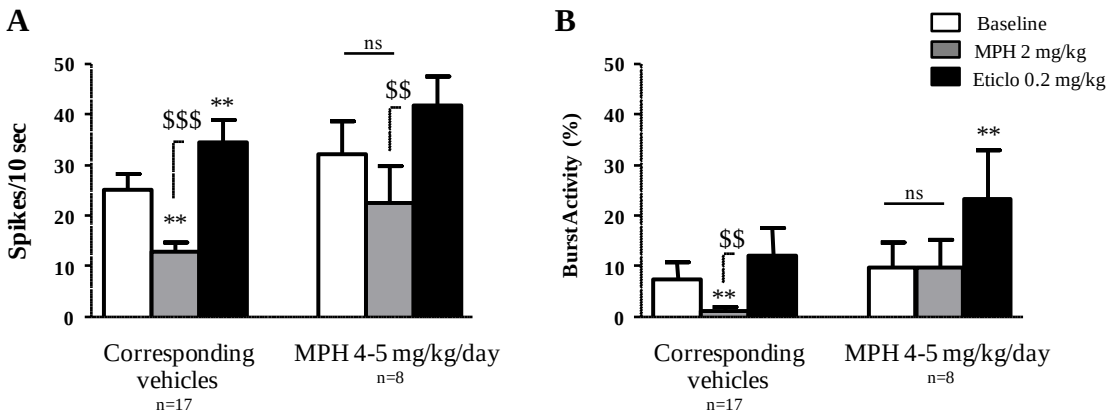


Figure 55: Chronic methylphenidate exposure induces tolerance in midbrain dopamine neurons to subsequent intravenous challenges.
When adult rats were chronically exposed to MPH (4-5 mg/kg/day), tolerance to subsequent intravenous challenges with MPH (2 mg/kg) was observed in VTA dopamine neurons, assessed by no significant change of the firing (**A**) and burst activities (**B**) of such neurons following MPH challenges in MPH-treated animals, contrary to what was seen in control animals (vehicle). Note successful recovery following eticlopride (0.2 mg/kg). Our different dosing routes (oral or intraperitoneal) did not produce any differences in the responses to either MPH or eticlopride. Therefore, here and in some following figures, groups of animals were pooled based upon similar responses. ns: non-significant, **P<0.01 *vs.* respective baselines, $$P<0.01, $$$P<0.001 *vs.* specified conditions, Bonferroni test after significant repeated measures two-way ANOVA.

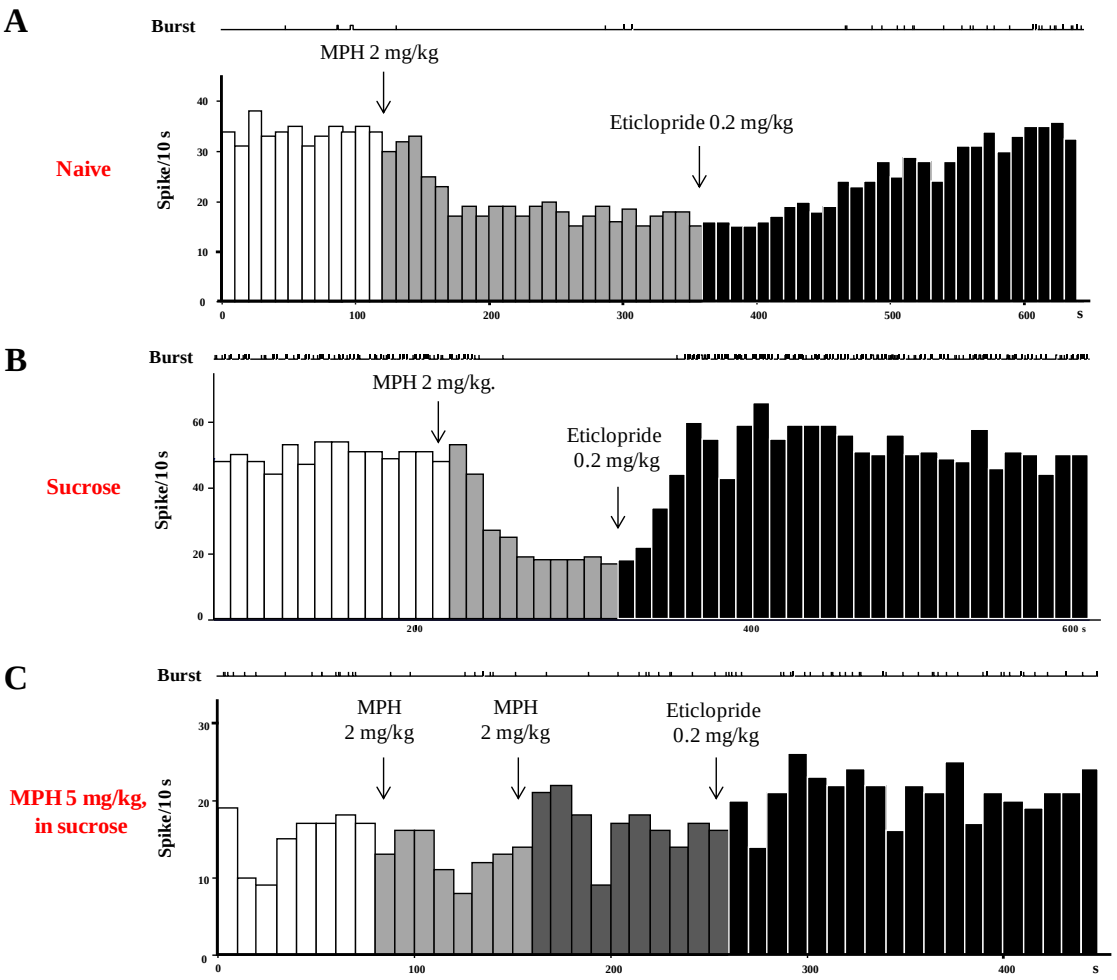


Figure 56: Tolerance to methylphenidate challenges in midbrain dopamine neurons recorded from orally treated animals.
In naive (**A**) and vehicle animals (**B**), intravenous challenge with 2 mg/kg of MPH considerably reduces spike generation of VTA dopamine neurons, an effect lost (tolerance) following chronic MPH treatment (**C**). Note that tolerance was observed even following 4 mg/kg of intravenous MPH challenge.

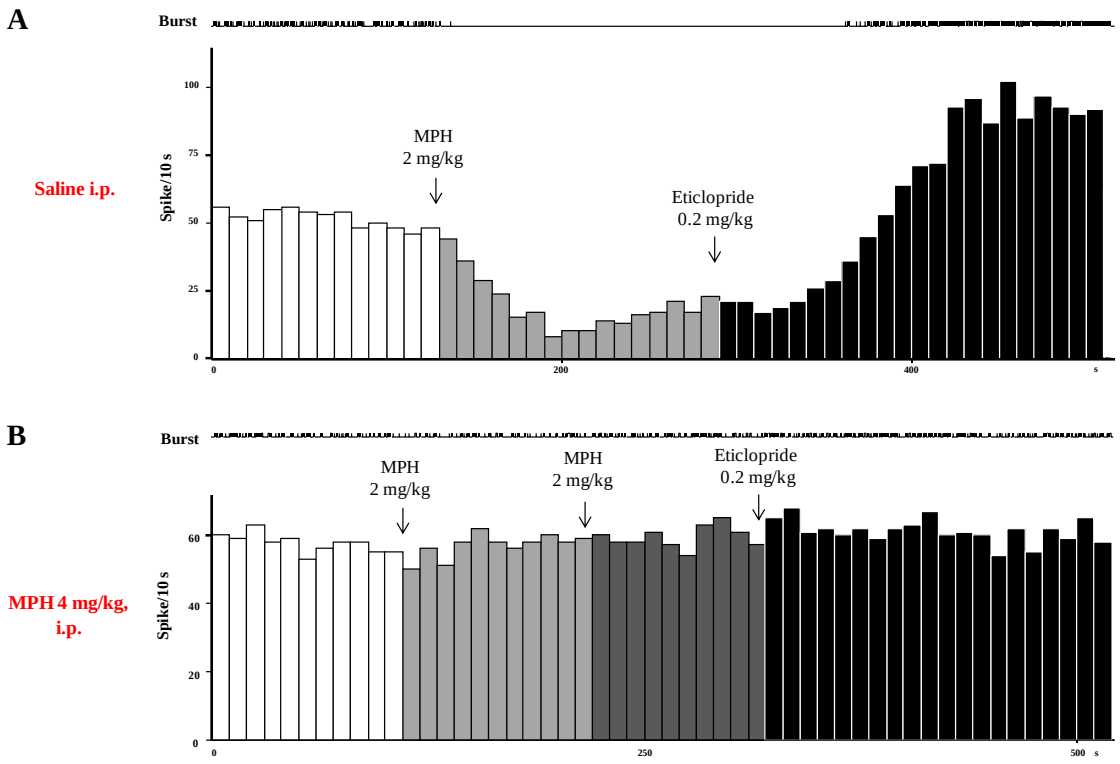


Figure 57: Tolerance to methylphenidate challenges in midbrain dopamine neurons recorded from intraperitoneally-treated animals.
Similarly to what was observed previously, vehicle animals showed strong sensitivity to intravenous MPH challenges (**A**), while tolerance was observed in animals chronically exposed to MPH (**B**). Tolerance was even observed following 4 mg/kg of intravenous MPH challenge.

IV-3-C- Chronic methylphenidate does not modify baseline VTA neuronal population activities but triggers burst activity increases following intravenous MPH and D_2 blockade challenges

We then considered whether chronic MPH could affect the ventral tegmental area neuronal population (Fig. 58A) before and after intravenous MPH and eticlopride challenges. In both control and MPH-treated animals, baseline firing and burst activities as well as the total number of spontaneously discharging dopamine neurons are not significantly altered following 15 days of chronic 4-5 mg/kg/day MPH treatment (respectively Fig. 58B, C and D). After MPH and D_2 blockade challenges (respectively 2 and 0.2 mg/kg), both groups displayed similar neuronal responses (Fig. 58B-D).

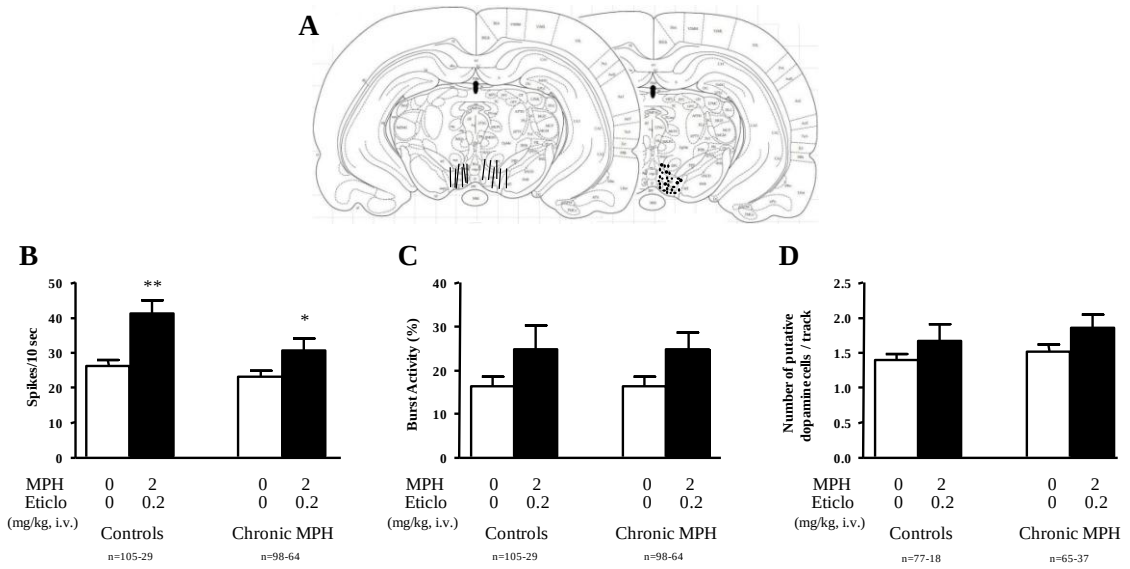


Figure 58: Neuronal populations before/after methylphenidate and eticlopride administrations and their respective responses.
(**A**): In this experiment, a cell per track protocol was performed, where spontaneously discharging VTA dopamine neurons were recorded per electrode descent. Vertical black bars indicate positions of the descents, while black dots indicate locations of some of the spontaneously discharging VTA dopamine neurons. Determined from stereotaxic coordinates. (**B**, **C**): In both control and MPH-treated animals (4-5 mg/kg/day), the global activity of dopamine neurons is not significantly altered after MPH administration and dopamine D_2 receptor blockade. (**D**): The total number of active dopamine neurons found in each track remained unchanged in both groups. n values indicate the number of neurons included. *P<0.05, **P<0.01 *vs.* respective baselines, Neuman-Keuls test after significant ANOVA.

IV-3-D- Chronic methylphenidate leads to dopamine transporter desensitisation rather than D_2 receptor desensitisation

Following our results concerning MPH-insensitive dopaminergic neurons, we tested whether desensitisation arises at the dopamine transporter or the dopamine D_2 receptor level. Intravenous administrations of a single 20 µg/kg dose of quinpirole, a dopamine D_2 receptor agonist, successfully mimicked the 2 mg/kg MPH challenge by significantly decreasing the firing and burst activities of 10 out of 10 VTA dopamine neurons (firing: $F_{(2,16)}=53.99$, $p<0.0001$, burst: $F_{(2,16)}=3.54$, $p<0.05$, Fig. 59). Both groups (MPH-treated animals and vehicle groups) displayed similar sensitivities to D_2 receptor agonism (respectively 77.3% and 76.4%), calculated by the percentage of firing rate decrease before and following quinpirole administration (Fig. 59B). These results indicate that chronic MPH administration does not induce dopamine D_2 receptor desensitisation. It should be noted that the neuronal firing rates returned to the exact

same baseline levels following both quinpirole (D_2 receptor agonism) and eticlopride (D_2 receptor antagonism) challenges (Fig. 59A), unlike what is observed following MPH/eticlopride challenges.

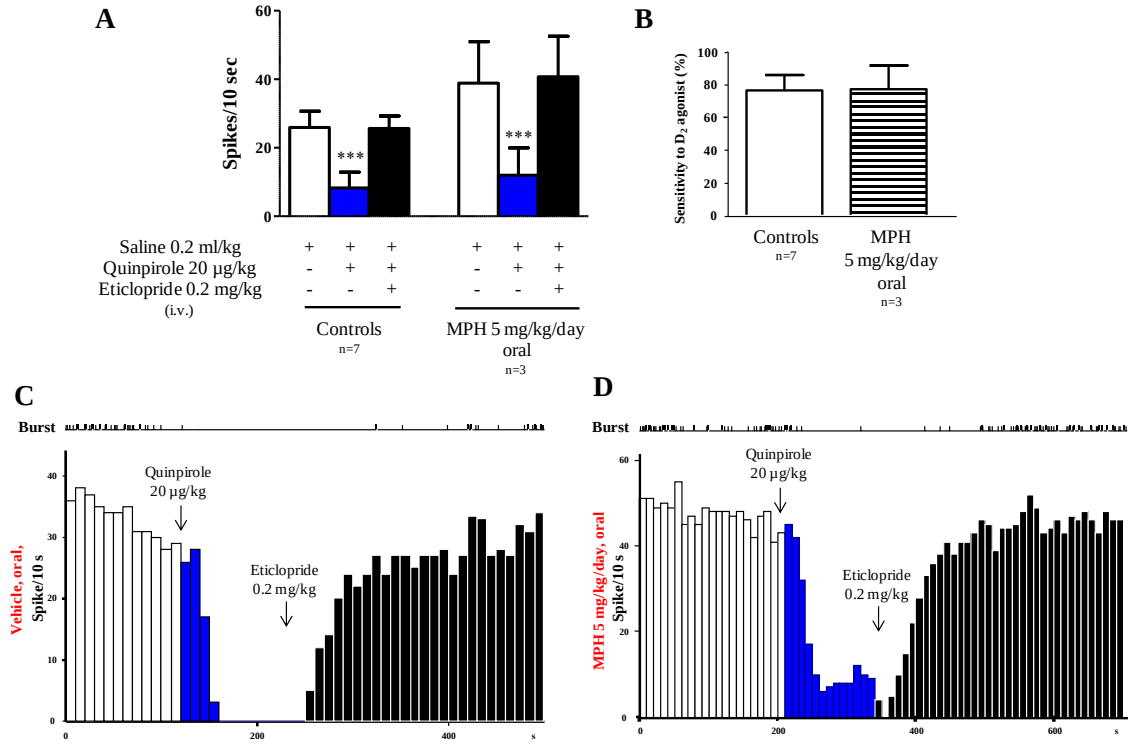


Figure 59: Chronic treatment with methylphenidate does not induce dopamine D_2 autoreceptor desensitisation.
(**A**): In both controls and MPH-treated animals, intravenous challenges with 20 µg/kg of quinpirole, a potent dopamine D_2 receptor agonist, strongly decreased the firing activities of VTA dopamine neurons, an effect completely reversed by dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg). (**B**): Both groups showed similar sensitivities to quinpirole challenges. (**C**, **D**): Representative recording examples in controls and MPH-treated animals. ***P<0.001, Bonferroni after significant repeated measures two-way ANOVA.

Next, we investigated whether the dopamine reuptake transporter could induce such desensitisation, using the *in vitro* neurotransmitter release assay. In control animals, both 10 and 100 µM methylphenidate perfusions successfully induced dose-dependent striatal ^{3}H-dopamine release (Fig. 60), with similar potencies to what was previously seen in naive animal (Chapter I). In contrast, 10 µM of MPH perfusion was not sufficient to induce striatal ^{3}H-dopamine release in animals treated daily with 4-5 mg/kg/day of MPH for 15 days. The subsequent 100 µM MPH perfusion successfully induced tritiated dopamine release, but to a significantly lower degree compared to the control animals ($p<0.0001$, unpaired Student's t-test). Taken together, these results

strongly suggest that chronic MPH leads to dopamine transporter desensitisation or down-regulation, as animals belonging to the MPH-treated groups display milder effects for the same MPH concentration (either 10 or 100 µM) to induce dopamine release, rather than dopamine D_2 receptor desensitisation, as they display similar sensitivity to dopamine D_2 receptor agonism.

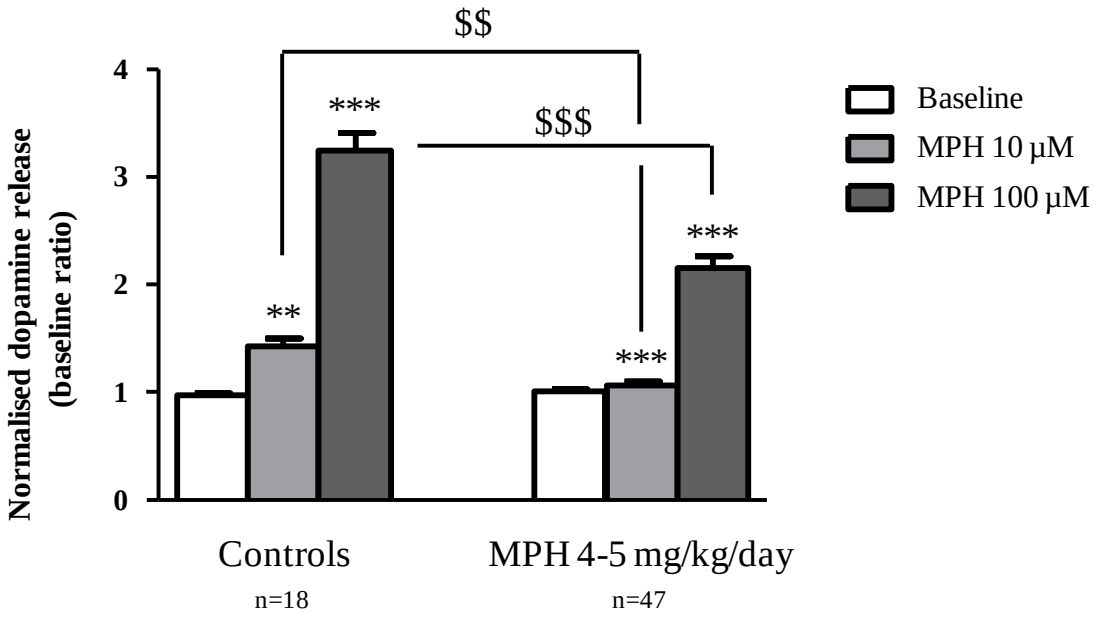


Figure 60: Dopamine release tolerance to methylphenidate perfusion in chronically treated animals.
Perfusion of striatal slices from control animals with 10 and 100 µM of MPH induced *in vitro* dopamine release, as observed previously. However, application of 10 µM of MPH in animals chronically treated with MPH failed to induce dopamine release. In MPH-treated animals, 100 µM superfusions of MPH led to significantly lower dopamine release than striatal slices from control animals. n values represent the number of perfusion chambers used. **P<0.01, ***P<0.001 *vs.* respective baselines, $$P<0.01, $$$P<0.001 *vs.* respective doses, Neuman-Keuls after significant ANOVA. Here, n values represent the number of perfusion chambers used.

IV-3-E- The impact of chronic methylphenidate on the spontaneous and glutamate- induced firing activity of PFC pyramidal neurons, striatal MSN and $NMDA_{2B}$ protein expression

Compared to controls, animals treated during adulthood with a chronic MPH treatment (5 mg/kg/day, orally for 15 days) displayed a significantly higher firing activity of pyramidal neurons in the PFC (p=0.01, unpaired Student's t-test, Fig. 61A). Such treatment did not affect burst discharge (Fig. 61B) or the total number of active pyramidal neurons found in one electrode descent (Fig. 61C), although small but non-significant increased tendencies were observed (p=0.1 and p=0.24, respectively,

unpaired Student's t-test). In a large population of neurons (n>40), NMDA was locally applied (30 mM) onto pyramidal neurons using various iontophoresis currents. In both vehicle (saline) and MPH-treated animals (MPH 4 mg/kg/day, intraperitoneally and for 15 days), different currents of NMDA (5, 10 and 15 nA) all successfully triggered large neuronal firing activation (Fig. 61D). No difference in the potency of NMDA to induce such activation was found between the two groups. When medium spiny neurons (MSN) were studied, we found a significant decrease in the NMDA-induced excitation in animals that were chronically treated with oral MPH during adulthood ($p=0.05$, unpaired Student's t-test, Fig. 61E), suggesting that MPH administration may induce local electrophysiological changes in striatal MSN rather than in PFC pyramidal neurons.

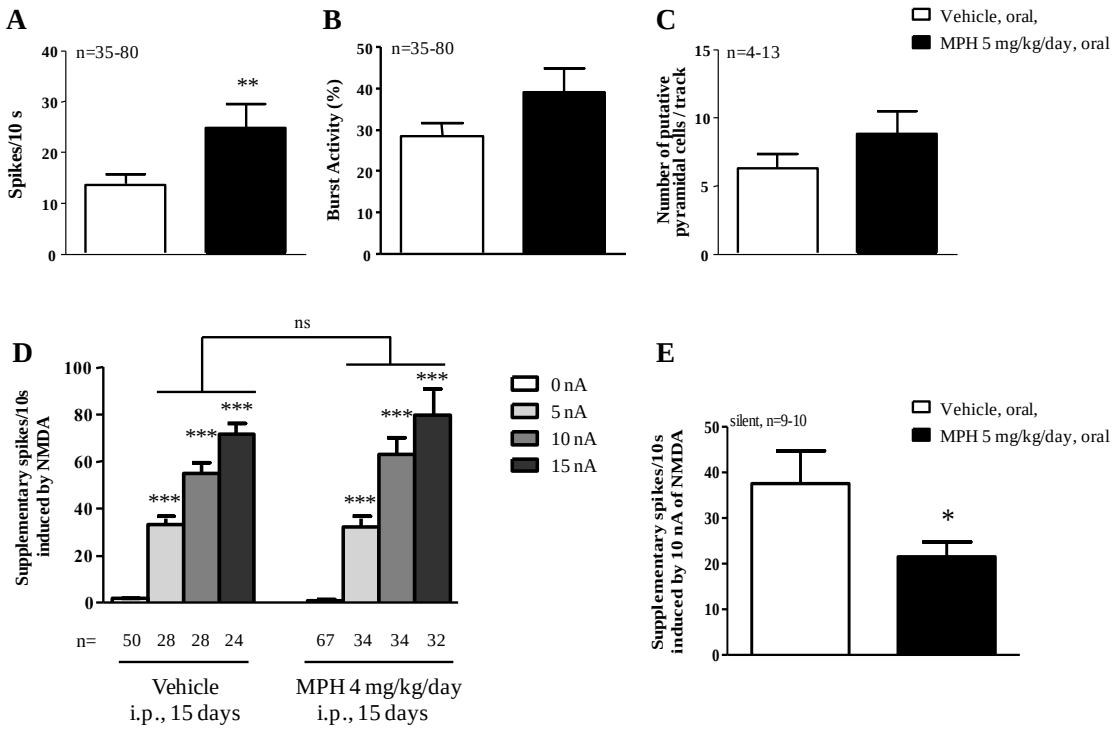


Figure 61: Effect of chronic methylphenidate treatment on spontaneous and NMDA-induced firing activities of prefrontal cortex pyramidal and striatal medium spiny neurons.
Chronic MPH treatment (5 mg/kg/day, oral) significantly increased the firing rates of spontaneously discharging prefrontal cortex pyramidal neurons (**A**), without altering burst discharges (**B**) nor the total number of active neurons (**C**), although tendencies to increase were observed. (**D**): In these neurons, chronic MPH exposure (4 mg/kg/day, i.p.) did not alter NMDA neurotransmission. (**E**): In silent striatal GABAergic medium spiny neurons, chronic MPH (5 mg/kg/day, oral) led to significantly decreased neuronal responses to locally applied NMDA. *P<0.05, **P<0.01 *vs.* vehicle, unpaired Student's t-test (**A**, **E**). ns: non-significant, ***P<0.001 *vs.* respective 0 nA current, Neuman-Keuls after significant ANOVA (**D**).

When dopamine (10 nA) was locally applied on striatal medium spiny neurons, using microiontophoresis, animals exposed to chronic MPH treatment (5 mg/kg/day, orally and for 15 consecutive days) displayed decreased sensitivities of NMDA neurotransmission (Fig. 62). Indeed, as seen in Chapter II (Fig. 34), NMDA application (10 nA) on medium spiny neurons located in the striatum induced either a significant decrease ($p<0.05$) or a non-significant increase ($p>0.05$) in NMDA neurotransmission (Neuman-Keuls test after significant ANOVA, respectively Fig. 34A and B). The fact that some MSN display firing rate inhibition while other MSN display firing rate activation, following iontophoretic application of both dopamine and NMDA, can be explained by two different populations of MSN within the striatum. Indeed,

several studies suggest that dopamine D_1 receptor signalling enhances dendritic excitability and glutamatergic signalling, while D_2 receptor signalling exerts the opposite effect (Nishi *et al.*, 2011; Surmeier *et al.*, 2007). Moreover, such a segregation of MSN according to the expression of either D_1 or D_2 receptors directly parallels the role they play in the direct or indirect pathways to the basal ganglia, respectively (Gerfen *et al.*, 2011; Macpherson *et al.*, 2014; Reinius *et al.*, 2015; Umemiya *et al.*, 1997). To note: some MSN express both D_1 and D_2 dopamine receptors, but are confined within the nucleus accumbens (Bertran-Gonzalez *et al.*, 2008; Nishi *et al.*, 2011; Yager *et al.*, 2015). Consistently with what was previously observed in Chapter II, control animals displayed identical responses (potentiation or dampening of NMDA) to iontophoretically applied dopamine, in conjunction with NMDA (Fig. 62A and B). However, in striatal medium spiny neurons, adult animals chronically exposed to MPH presented decreased sensitivities of the NMDA neurotransmission to locally applied dopamine, an effect not observed following vehicle exposure (controls).

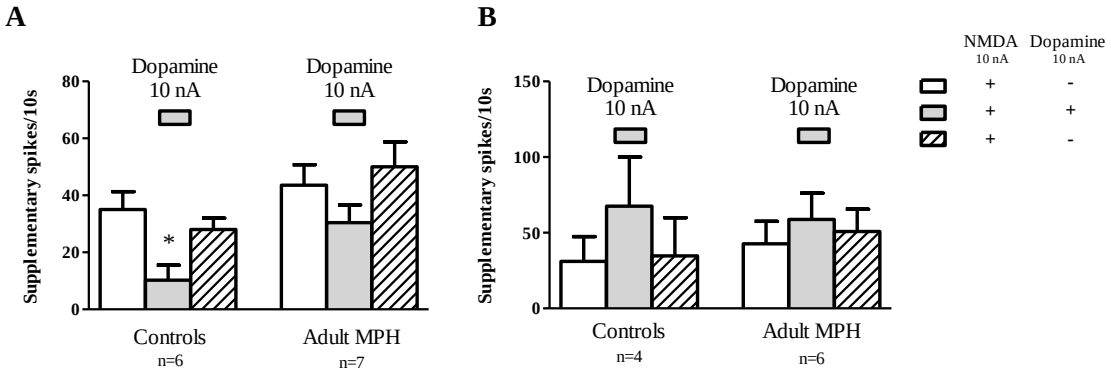


Figure 62: Chronic exposure to methylphenidate induces dampening of striatal medium spiny neurons to locally applied dopamine.
In control animals, locally applied dopamine (10 nA) induces either dampening (**A**) or potentiation (**B**) of the responses of striatal GABAergic medium spiny neurons (MSN) to local NMDA applications (10 nA), as seen previously in Fig. 34. In comparison, animals treated chronically with MPH during adulthood (5 mg/kg/day, for 15 consecutive days) displayed decreased sensitivities (**A**, **B**) of the NMDA neurotransmission to locally applied dopamine. *P<0.05 *vs.* NMDA 10 nA, Neuman-Keuls after significant ANOVA.

Based upon such results and in order to further examine NMDA neurotransmission in the striatum, we then focused on NR_{2B} protein expression. In the prefrontal cortex and the hippocampus, adult MPH treatment induced small but non-significant $NMDA_{2B}$ protein expression decreases, whilst it significantly decreased

NR_{2B} protein expression in the striatum (Fig. 63). These combined results indicate that chronic MPH exposure during adulthood leads to decreased NMDA neurotransmission in striatal medium spiny neurons, in correlation with a decreased striatal NR_{2B} expression.

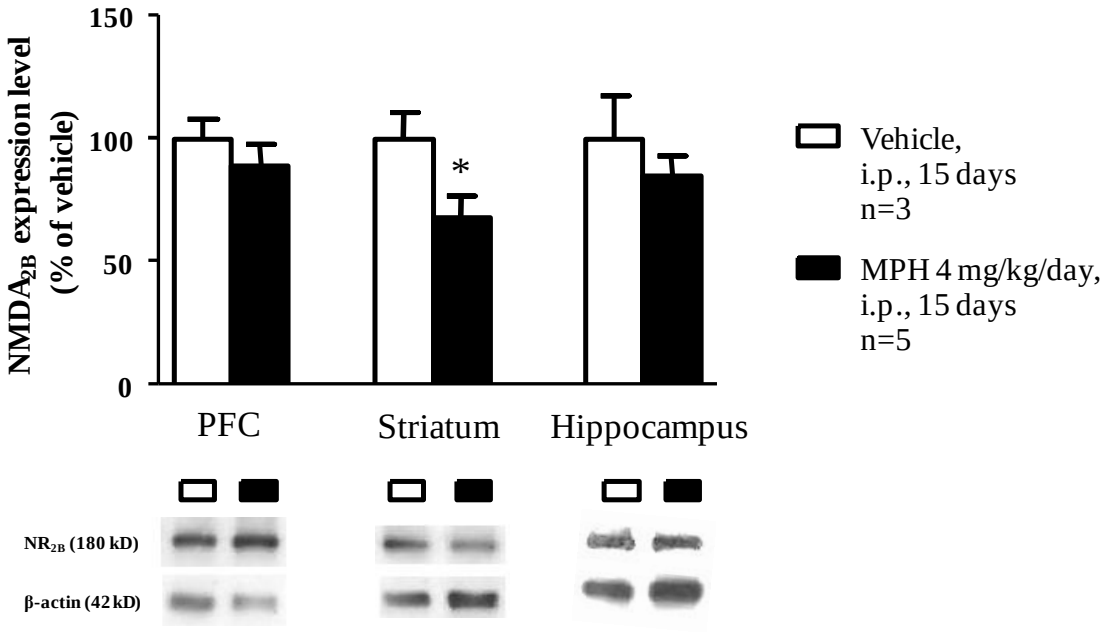


Figure 63: Chronic methylphenidate exposure leads to decreased striatal NMDA 2B receptor expression.
Chronic exposure to MPH (4 mg/kg/day, intraperitoneally) significantly decreased NR_{2B} protein expression in the striatum, but not in the prefrontal cortex, nor in the hippocampus. Bottom are displayed representative immunoblots with protein sizes in kilodaltons (kD). *P<0.05 *vs.* vehicle, unpaired Student's t-test.

IV-4- Discussion

In this chapter, we have first demonstrated that dissolving up to 5 mg/kg of methylphenidate in a 10% sucrose solution (w/v) can be considered an efficient and stress-free method for oral drug delivery to rats. Such a protocol also closely reproduces the pharmacokinetic profiles in humans following methylphenidate intake (Calipari *et al.*, 2013). Indeed, we did not find any long-lasting increase in the blood sugar levels (glycaemia) of animals following chronic 2 ml/kg sucrose administrations (Fig. 53A). These observations confirm previous findings where efficient drug delivery was performed using the exact same protocol in Lister hooded rats (Atcha *et al.*, 2010). Here, we avoid the stress that can result from using gastric gavage techniques (Hoggatt

et al., 2010; Turner *et al.*, 2011b; Turner *et al.*, 2012). It also reduces the risk of the potentially life-threatening consequences of gavage (Germann *et al.*, 1994). Nowadays, countless alternative techniques are used for voluntary oral drug dosing in rodents, many using sweet or palatable substances such as chocolate (Huang-Brown *et al.*, 2002), peanut butter (Diogo *et al.*, 2015; Taylor *et al.*, 2016), cookie dough (Corbett *et al.*, 2012), jelly (Flecknell *et al.*, 1999), juice (Wheeler *et al.*, 2007), milk (Matsumoto *et al.*, 2014) or even honey (Kuster *et al.*, 2012).

Intraperitoneal or oral methylphenidate, administered at 4-5 mg/kg/day during 15 consecutive days, did not influence glycaemic parameters after the last administration (Fig. 53B). Amphetamines are known to induce hypoglycaemia in mice (Moore *et al.*, 1965) whilst inducing hyperglycaemia in humans (Asser *et al.*, 2015). Local fluctuations in cerebral glucose metabolism within the prefrontal cortex, the limbic cortex and some subcortical structures such as the caudate or the thalamus, have been observed following either acute MPH (0.35 mg/kg) or D-amphetamine (0.15-0.25 mg/kg) injections (Ernst *et al.*, 1997; Matochik *et al.*, 1993), a result that might be explained by higher neuronal glucose demands upon activation (Gobel *et al.*, 2013). In humans, consumption of another psychostimulant, caffeine, has been linked to systemic increases of glucose concentration (Olateju *et al.*, 2015), although this was not observed in rats (Jarrar *et al.*, 2014). It remains difficult to fully establish a linear relationship between local brain glucose metabolism and systemic blood glucose levels, although they may vary proportionally (Magnoni *et al.*, 2012; Zetterling *et al.*, 2011). Our results did not show any significant change in blood sugar levels following 15 days of chronic 5 mg/kg/day MPH administration. The long delay between the last MPH administration and the blood sugar level measurement could explain such results, although it is more than likely that MPH has no effect on glycaemia, regardless of the presence or absence of drug withdrawal.

Neither the chronic MPH treatment nor the intraperitoneal administration route were shown to alter the growth parameters of adult rats (Fig. 54A,C). However, we earlier found in another group of animals that the intraperitoneal route (administration of both vehicle and MPH) led to a slightly but significantly lower body weight gain (Fig. 54B). Here, we put forward the hypothesis that stress, caused by the injection, may induce such an effect. This partly corroborates previous findings, where a 13 week long

MPH treatment in young rats induced a delayed body weight gain (Komatsu *et al.*, 2012), as observed before on neonatal and juvenile rats (Pizzi *et al.*, 1986). However, these results were observed using very high doses of subcutaneous MPH, between 60 to 200 mg/kg/day. As a matter of fact, Pizzi *et al* observed a high mortality rate within the 200 mg/kg/day group, justifying the need to discontinue such a protocol (Pizzi *et al.*, 1987). Young developing rats may be more sensitive to the high doses of psychostimulant than adults and this may explain such differences. In our present study, MPH dosing remained low (4-5 mg/kg/day) compared to these studies (60-200 mg/kg/day). Studies in monkeys have revealed that chronic MPH (20-60 mg daily, for 1 year) does not affect growth or weight (Gill *et al.*, 2012). Gill *et al* used MPH doses of 6 +/- 1 mg/kg, which were chosen to mimic human serum levels following chronic MPH in children, resulting in MPH serum concentration of 11-13 ng/ml (Gill *et al.*, 2012; Swanson *et al.*, 2003). Studies on humans have shown that chronic MPH does not affect growth (Biederman *et al.*, 2010; Rapoport *et al.*, 2002), although this has been contested by others (Safer *et al.*, 1972; Swanson *et al.*, 2007; Zhang *et al.*, 2010a). Nevertheless, our study observed possible body weight gain velocity decreases following chronic MPH treatments using slightly supratherapeutic doses, in one group of animals.

Next, we observed that animals receiving chronic MPH do not respond to subsequent intravenous MPH challenges, which only induce firing rate and bursting activity decreases in VTA dopaminergic cells in naive and vehicle groups (Fig. 55-57). This can be interpreted as a possible drug tolerance, where the same dose of a drug has less effect following repeated administration (Sellers, 1978; Wang *et al.*, 2007b). In pharmacology, drug tolerance is also named desensitisation (Wolf *et al.*, 2010; Yamamoto *et al.*, 2013). We note with interest that MPH tolerance following chronic MPH exposure (using widely ranging doses, between 2 to 20 mg/kg) has been described before in rodents (Alam *et al.*, 2015; Emmett-Oglesby *et al.*, 1981; Jones *et al.*, 2014; Pearl *et al.*, 1976) and humans (Lakhan *et al.*, 2012; Patrick *et al.*, 2005; Ross *et al.*, 2002; Swanson *et al.*, 1999; Swanson, 2005). Indeed, these behavioural studies show that long-term MPH administration induces: 1/ tolerance on cognition, assessed by the water maze test, where animals swam for a longer period following chronic MPH exposure (2 mg/kg/day), while acute MPH improved cognition (2 mg/kg); 2/ locomotion tolerance, where acute MPH exposure (0.6-10 mg/kg) induces increases in horizontal locomotion, while repeated exposure (10 consecutive days) failed to do so;

3/ tolerance to sweetened milk consumption, assessed by a return to initial baseline levels of milk consumption following chronic MPH exposure (15 mg/kg daily or every fourth day), while acute exposure resulted in a significant decrease in milk intake. In humans, tolerance to MPH can develop following repeated intake and is characterised by the inability of MPH to reduce ADHD symptoms (Ross *et al.*, 2002; Swanson *et al.*, 1999; Winsberg *et al.*, 1987), if using the same daily dose, hence requiring a higher dosage regimen for the alleviation of the same symptoms. Tolerance to psychostimulants in ADHD children is seen in "most patients", requiring dose adjustments (Pliszka *et al.*, 2007; Yanofski, 2011). In non-human primates, chronic MPH exposure leads to decreased dopamine D_2/D_3 receptor availability, assessed by positron tomography (Gill *et al.*, 2012). Moreover, in an animal model of ADHD (using young spontaneously hypertensive rats), chronic MPH led to decreased dopamine transporter density (Simchon *et al.*, 2010). Methylphenidate tolerance could be the consequence of two mechanisms. First, the dopamine reuptake transporter may not be sensitive enough to MPH in order to induce enough extracellular dopamine concentration increases in the synaptic cleft to produce enough dopamine D_2 receptor activation, due to downregulation of dopamine transporters. The second hypothesis is that MPH still efficiently blocks the dopamine transporter to the same degree as in control animals, but the dopamine D_2 autoreceptor has become insensitive to rises in extracellular dopamine.

Here, we have shown that dopamine D_2 receptors are not significantly affected by chronic MPH, as both vehicle and MPH-treated animals displayed similar sensitivity to the D_2 receptor agonist quinpirole (20 µg/kg, Fig. 59). Interestingly, another study also found that neurons decreased their firing rates by 80% following quinpirole intravenous administration in both controls and MPH-treated rats (Shen *et al.*, 2006), as observed in our present study (78%). These results corroborate the study of Volkow in 2012 where the authors found no change in dopamine D_2 receptor binding following chronic MPH in ADHD adults (Volkow *et al.*, 2012). Therefore, one hypothesis arises from these results. The pre-synaptic dopamine reuptake transporter (DAT) may be down-regulated, resulting in the inefficiency of MPH in inducing DAT blockade. Interestingly, some studies have witnessed DAT internalisation in mice following chronic (20 mg/kg, twice a day for 3 days) cocaine exposure (Peraile *et al.*, 2010) as well as a DAT downregulation in patients following 3 months of 0.25-0.6 mg/kg/day of

MPH, assessed by positron emission tomography (Vles *et al.*, 2003). Cocaine and MPH both block the DAT with similar *in vivo* affinities in humans (Volkow *et al.*, 1999a). Vles in 2003, however, also found a post-synaptic downregulation of the dopamine receptor (Vles *et al.*, 2003), which has not been observed in our study. Paradoxically, one imaging study found a DAT up-regulation in adult patients that were never previously medicated (Wang *et al.*, 2013), whilst another study found a higher DAT availability following sub-chronic cocaine exposure in mice (Koff *et al.*, 1994). DAT membrane levels are rapidly regulated by intracellular mechanisms to maintain adequate dopamine homeostasis in the synapse (Gulley *et al.*, 2003). After acute psychostimulant use, such regulations are believed to arise more rapidly and transiently than after chronic administration, which leads to more persistent changes in the transporter expression (Zahniser *et al.*, 2001). Our study also demonstrated that perfusion of striatal slices with 10 µM of MPH fails to significantly induce radio-labelled dopamine release in animals chronically treated with 4-5 mg/kg/day of MPH for 15 days, while 100 µM of MPH superfusion induces lower dopamine release than animals treated with vehicle (Fig. 60), as observed in another study using rats sub-chronically treated (4 consecutive days followed by a 2-week washout period) with 4 mg/kg of MPH (Sproson *et al.*, 2001). Therefore, in MPH-treated animals, methylphenidate challenges fail to sufficiently block dopamine transporters in order to induce significant synaptic dopamine concentration rises to levels that are high enough to activate inhibitory dopamine D_2 autoreceptors. This might be a consequence of a possible DAT down-regulation after chronic MPH exposure. Further studies are needed to examine whether chronic MPH exposure induce long-lasting consequences on DAT local expression, an effect that may require careful monitoring in patients.

When a large neuronal population was examined, chronic MPH did not lead to modification in the neuronal activity of dopamine neurons (Fig. 58). In the study by Choong *et al*, the authors found no significant changes in the neuronal excitability of VTA dopaminergic neurons after an acute 1 mg/kg MPH intraperitoneal administration (Choong *et al.*, 2004). However, the same laboratory found increases in the excitability of midbrain dopamine neuron after 1 mg/kg/day of MPH for 3 weeks, whilst decreases in neuronal excitability were found following 30-60 days of washout (Shen *et al.*, 2006). Chronic adolescent exposure to MPH (2 mg/kg/day for 7 days) led to increased neuronal firing of the midbrain dopamine neurons in adolescent rats, an effect lost after

2-3 weeks of washout (Brandon *et al.*, 2003). However, chronic exposure to D-amphetamine at 1.5 mg/kg/day for 20 days in adolescent rats, followed by 20 days of washout, induced firing enhancement of VTA dopamine neurons, an effect that was not observed using a higher dose of 5 mg/kg/day (Labonte *et al.*, 2012). The consequences of adolescent psychostimulant exposures will be further discussed in the next chapter (Chapter V).

In the prefrontal cortex, chronic MPH treatment did not significantly alter NMDA-induced firing rate activation in a large population of neurons (Fig. 61D). These results that are different to what we have found following acute MPH and D-amph exposures (3 mg/kg, Chapter II), may indicate that chronic MPH exposure is not likely to induce persistent changes in the glutamatergic neurotransmission of cortical pyramidal neurons. The PFC is necessary to induce behavioural sensitisation following chronic MPH (Lee *et al.*, 2008). However, we have found that chronic methylphenidate increases the firing rate of PFC pyramidal neurons (Fig. 61A). One study on freely behaving animals, using microarray electrodes, revealed that both acute and chronic methylphenidate (2.5 mg/kg, for 5 consecutive days, followed by a 3-day washout period) induce increases of the PFC pyramidal neuron firing rate in the majority of all neurons tested, but the authors did not characterise the underlying mechanisms of such increases (Salek *et al.*, 2012). We put forward the hypothesis that chronic MPH treatment increases tonic input of PFC glutamate neurons onto midbrain dopamine neurons. In 2015, Schmitz *et al* observed higher glutamate levels as well as decreased glutamate uptake capacity in the PFC of juvenile rats after 30 days of daily 2 mg/kg MPH (Schmitz *et al.*, 2015). It will be vital to assess the possible impacts of such glutamate neurotransmission, as excessive glutamate in the synaptic cleft leads to excitotoxicity (Ha *et al.*, 2006; Han *et al.*, 1997; Kritis *et al.*, 2015; Tirosh *et al.*, 2000), which can also be a result of excessive NMDA receptor stimulation (Schubert *et al.*, 2001). In our model, we did not observe any alteration of the NMDA-induced firing activity of pyramidal cells after chronic MPH. One can assume that the initial glutamatergic plasticity remains temporary, because of prolonged activation of NMDA receptors, but is crucial for therapeutical effects. In juvenile rats, chronic MPH administration (1 mg/kg/day for 3 weeks, 5 days per week) increases hyperpolarising currents in cortical pyramidal neurons and decreases EPSC (excitatory post synaptic current) frequencies (Urban *et al.*, 2012). Such a depression of cortical activity within

the PFC of juvenile rats is not observed in adult animals in the present study (Fig. 61). Next, we observed a significant reduction in $NMDA_{2B}$ protein receptor expression in the striatum (Fig. 63) and a parallel decreased responsiveness of striatal medium spiny neurons to local NMDA delivery (Fig. 61E), which strengthens our observations. These results on chronic MPH intake are in total opposition to what we found using acute MPH exposure (Fig. 35, 37). A previous observation, using acute MPH (1 mg/kg), has shown decreased NR_{2B} expression in the PFC (Urban *et al.*, 2013b).

Our results which show that chronic adult exposure to MPH tends to induce a dampening of the sensitivity of striatal medium spiny neurons (MSN) to locally applied dopamine (Fig. 62) are in line with previous findings. Indeed, one study in mice observed increased MSN dendritic spine formation following chronic psychostimulant exposure (cocaine, 30 mg/kg, intraperitoneally, over 4 weeks) in neurons located in the nucleus accumbens and which specifically express dopamine D_1 and D_2 receptors (Kim *et al.*, 2009). These increases in spine densities are believed to trigger long-term potentiation (Matsuzaki *et al.*, 2004). Another study witnessed behavioural sensitisation or tolerance (in a 1:1 ratio) to chronic MPH (0.6-10 mg/kg for 5 days), closely associated with electrophysiological sensitisation or tolerance in MSN of the nucleus accumbens (Claussen *et al.*, 2014a). Therefore, chronic methylphenidate exposure may reduce striatal plasticity and might not be without long-term consequences. As a matter of fact, chronic MPH exposure (6 mg/kg, twice a day for 28 days, orally) significantly decreased dopamine D_2 receptor availability in the striatum (Caprioli *et al.*, 2015). Similarly, chronic oral methylphenidate intake (2 mg/kg/day) for 2 months significantly decreased striatal dopamine D_2 receptor availability (Thanos *et al.*, 2007). Our results need to be studied further in order to determine the role of D_1- or D_2-like receptors after chronic exposure to methylphenidate.

To conclude, we have shown here that chronic intraperitoneal treatments during adulthood can alter body growth parameters in some animals. Voluntary oral MPH administration can be easily performed using sucrose solutions, as suggested in previous studies. Chronic MPH treatment probably induces dopamine reuptake transporter down-regulation, possibly by internalisation, assessed by dopamine neurons insensitivity to intravenous MPH challenges, but may not affect post-synaptic dopamine D_2 inhibitory autoreceptors. Besides, striatal tissues from MPH-treated animals induce significantly

lower dopamine release using the same concentration of MPH compared to control tissues. The global VTA dopamine neuron population is not significantly affected by chronic treatment, although some neurons have higher burst activities following MPH and D_2 receptor antagonism in MPH-treated animals than the respective control animals (Fig. 58), suggesting MPH-induced neuronal adaptations. Finally, *in vivo* NMDA-induced neuronal activation of striatal MSN is reduced following chronic MPH, in parallel with significant decreases of striatal $NMDA_{2B}$ protein expression, which can be the visible result of the on-going side effects on neuronal plasticity.

Chapter V – The long-term consequences of methylphenidate treatment during adolescence on adult brain functions.

V-1- Introduction

ADHD occurs mainly during childhood and adolescence (Adesman, 2001; Hurtig *et al.*, 2007; Wilens *et al.*, 2010) but can persist throughout the entire adult lifespan in a widely ranged proportion of patients (ten to sixty percent), depending on the different cohorts, if previously diagnosed with childhood ADHD (Gentile *et al.*, 2006; Kessler *et al.*, 2005; Pehlivanidis, 2012). Evidence for long-term side effects is limited. While some studies reported risks and/or side effects such as insomnia, tics, erythema, dyskinesia, sleep disturbance, abdominal pain, headache and appetite loss for ADHD patients under chronic psychostimulant therapy (Ahmann *et al.*, 1993; Efron *et al.*, 1997; Greenhill *et al.*, 2002; Lerner *et al.*, 2008; Martinez-Raga *et al.*, 2013; Rappley, 1997; Rodrigues *et al.*, 2008; Senecky *et al.*, 2002), other studies reported no serious side effects to such treatments (Gadow *et al.*, 1999). However, ADHD drugs, at clinically relevant doses, are known to improve academic performance in children and adolescents (Chacko *et al.*, 2005; Evans *et al.*, 1991; Greenhill *et al.*, 2002; Hechtman *et al.*, 2004; Najib, 2009; Pelham *et al.*, 1993; Powers *et al.*, 2008; Swanson *et al.*, 2004), therefore outweighing the side effects, if correctly dosed (Manos, 2008; Martinez-Raga *et al.*, 2013). In rodents, adolescent methylphenidate treatment is associated with altered behavioural responses in adulthood (Bolanos *et al.*, 2003), impaired reproductive axis (Chatterjee-Chakrabarty *et al.*, 2005; Fazelipour *et al.*, 2012), impaired learning (Rowan *et al.*, 2015) and increased hippocampal BDNF (brain-derived neurotrophic factor) mRNA levels (Simchon-Tenenbaum *et al.*, 2015), a crucial protein for plasticity, memory, synapse establishment/maintenance and neuronal regeneration (Bekinschtein *et al.*, 2008; Benraiss *et al.*, 2001; Huang *et al.*, 2001; Pencea *et al.*, 2001; Yamada *et al.*, 2003; Zigova *et al.*, 1998). Another study found that chronic treatment with oral MPH (2 mg/kg, twice a day) leads to increased impulsiveness in adulthood (Pardey *et al.*, 2012). In parallel, chronic adolescent amphetamine exposure at high doses (0.5-5

mg/kg/day) induced increases in the risk-taking behaviour of adult rats and increased firing activities of dopamine and serotonin neurons (Labonte *et al.*, 2012). Interestingly, serotonin neurotransmission, alone or with dopamine neurotransmission, has been linked to impulsivity in humans (Dalley *et al.*, 2012; Pattij *et al.*, 2008; Seo *et al.*, 2008; Stein *et al.*, 1993; Walderhaug *et al.*, 2010; Worbe *et al.*, 2014). In rodents, adolescent exposure to stimulants may lead to drug-seeking behaviour in adulthood (Brandon *et al.*, 2001; Carlezon *et al.*, 2004; Crawford *et al.*, 2011; Jordan *et al.*, 2014), although this was not seen in rhesus monkeys (Gill *et al.*, 2012; Martelle *et al.*, 2013) or humans (Barkley *et al.*, 2003; Wilens *et al.*, 2003). However, it may be too early to draw conclusions on such matters. Adolescent exposure to drugs may not be without risk, knowing that the brain still develops up to 24 years of age (Andersen, 2003).

In this fifth chapter, we will examine the impact of chronic adolescent methylphenidate treatment on growth, neuronal activities and glutamate neurotransmission, in an attempt to characterise any possible long-term consequences of such treatments. A focus will also be put on the link between depression-like behaviours and the electrical activities of dorsal raphe nucleus serotonin neurons. Finally, assessments of dopaminergic neurotransmission, combined with dopamine autoreceptor sensitivity as well as behavioural sensitivity to D-amphetamine, will be assessed in rats that received adolescent exposure to methylphenidate.

V-2- Material and Methods

V-2-A- Subjects and groups

Fifty-two male Sprague-Dawley rats were purchased from Charles River, UK. Animals were housed in groups of 2-4 per cage, maintained at 20-22^{o}C with humidity rates above 40% under a 12:12 L/D cycle with lights ON at 07h00. Food and water were provided *ad libitum*, except in the sucrose preference test. Animals were allowed a 3-day acclimatisation period after delivery. All experiments were performed during the light phase and with permission from the UK Home Office and De Montfort University Ethics Committee under the Project Licence 60/4333 and with the Personal Licence 60/13750.

Adolescent animals weighing 70-90 grams (Levin *et al.*, 2007; McCutcheon *et al.*, 2009; Milstein *et al.*, 2013; Morris *et al.*, 2010; Sengupta, 2013) were used at the beginning of each treatment. Animals were randomly assigned to one of the following groups (Fig. 64):

- Animals voluntarily drinking 5 mg/kg/day of MPH (or vehicle alone) in 2 ml/kg of a sucrose 10% (w/v) solution, orally and for 15 consecutive days, followed by a 28-day washout period. n=42.

- Animals receiving 4 mg/kg/day of MPH (or vehicle alone) in 1.2 ml/kg of saline, intraperitoneally and for 15 consecutive days, followed by a 28-day washout period. n=10.

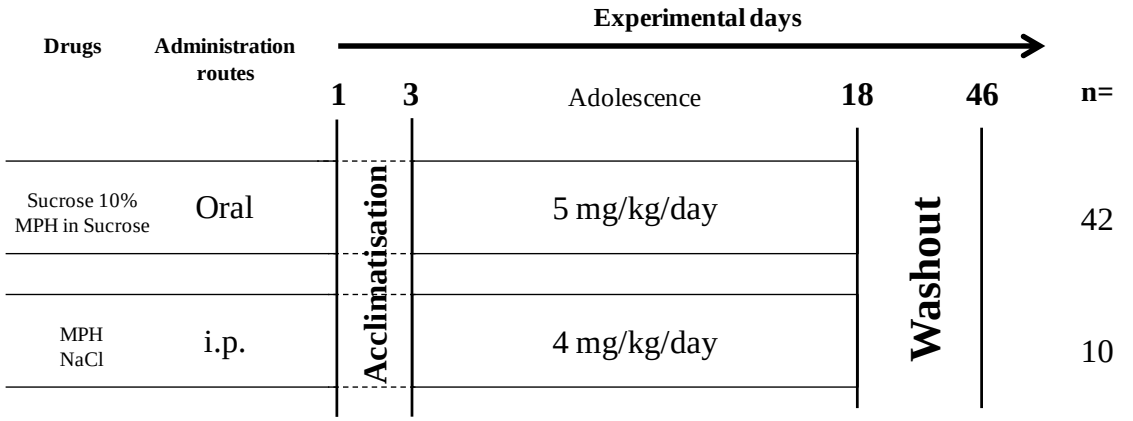


Figure 64: Experimental protocol for chronic methylphenidate treatment during adolescence.
Following 3 days of acclimatisation, young rats weighing 70-90 grams were given MPH for 15 consecutive days, followed by a 28-day washout period. Administration routes were either intraperitoneal (4 mg/kg/day) or oral (5 mg/kg/day). n values represent the number of animals included in each group.

V-2-B- Sucrose preference test

After at least 20 days following the last MPH administration, some animals were subjected to a sucrose preference test (Fig. 65), adapted from previous published protocols (Li *et al.*, 2015a; Mateus-Pinheiro *et al.*, 2014; Mileva *et al.*, 2015; Overstreet, 2012; Tang *et al.*, 2015). Briefly, rats were tested for sucrose preference over a five day period using a two-bottle choice test. Animals had *ad libitum* access to food and water throughout the experiment. On the first day, rats were housed singly and accustomed to drinking from two water bottles. On the following three days, rats were trained on the sucrose preference test, in which one out of the two water bottles was

replaced by a bottle containing a 2% sucrose solution (w/v). Rats were allowed to drink freely from both bottles during the 12-hour nocturnal phase (7.30 PM until 7.30 AM). During the light phase, both bottles were replaced by bottles containing water only. The bottles were weighed and refilled each day at the same time in the morning. The positions of the bottles were switched daily to avoid position preferences, which has been observed in mice (Bachmanov *et al.*, 2002). After removing the bottles at the end of the last habituation day, rats were subjected to a preference test during 1 hour. Sucrose preference was determined after the last trial, on the fifth day, by the quotient of sucrose consumption over 12 hours to the total liquid intake, consisting of both water and sucrose intakes. A sucrose preference score lower than 65% was considered a depressive-like phenotype (Briones *et al.*, 2012; Couch *et al.*, 2013; Strekalova *et al.*, 2004; Willner, 1997).

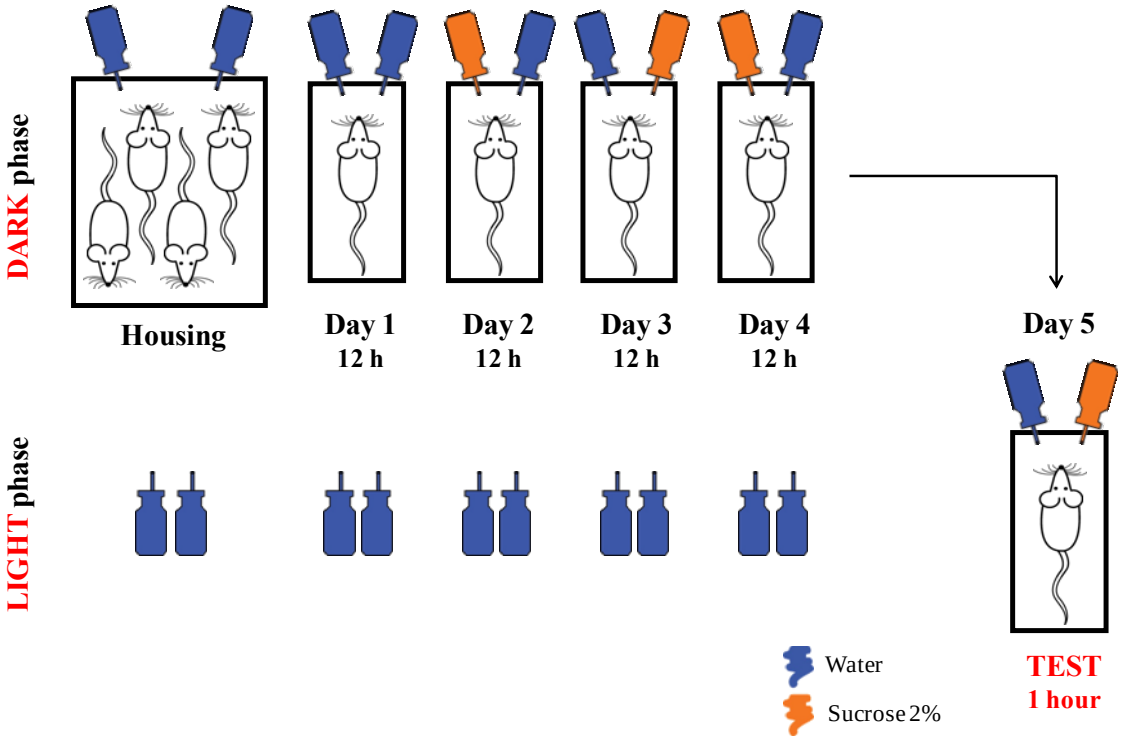


Figure 65: Sucrose preference test protocol.
Animals were group-housed before being singly housed on the first day of the sucrose preference test. Animals had *ad libitum* access to food and water throughout the experiment. During the dark phase and starting on the second day (Day 2), animals could choose between two drinking bottles, containing either tap water (blue) or a 2% sucrose solution (orange). Sucrose preference was calculated from results obtained during a 1-hour test period, performed on the fifth day and during the light phase. Animals were kept in polypropylene cages (black rectangles) measuring 56x38x17 cm, whether singly- or group-housed.

V-2-C- *In vivo* extracellular single unit electrophysiology

Please refer to paragraph III-2-B. In this chapter, the number of putative dorsal raphe nucleus (DRN) serotonin neurons per track was recorded by recording the total number of active serotonin neurons encountered during one electrode descent within the DRN, at the following coordinates (Fig. 66): anteroposterior -7.5 to -8 mm to Bregma, lateral 0 mm, dorsoventral 5-7 mm below cortical surface. Here, only putative serotonin neurons presenting a triphasic extracellular waveform and a regular firing activity (1-40 spikes per 10 seconds) were recorded (Oosterhof *et al.*, 2015; Vandermaelen *et al.*, 1983). Bursting of DRN serotonin neurons was calculated with the following criteria: at least two bursts occurring within 20 ms or less and followed by a silence period of at least 40 ms. These parameters were adapted from previous electrophysiological investigations (Hajos *et al.*, 1995; Labonte *et al.*, 2012; Manta *et al.*, 2009; Rouchet *et al.*, 2008). To further confirm the specific recording of serotonergic neurons only, systemic exposure to 8-OH-DPAT (5-Hydroxy-*N*,*N*-dipropyl-2-aminotetralin) was performed at the end of some recordings, which induced neuronal silencing that could be reversed by WAY-100135 administration.

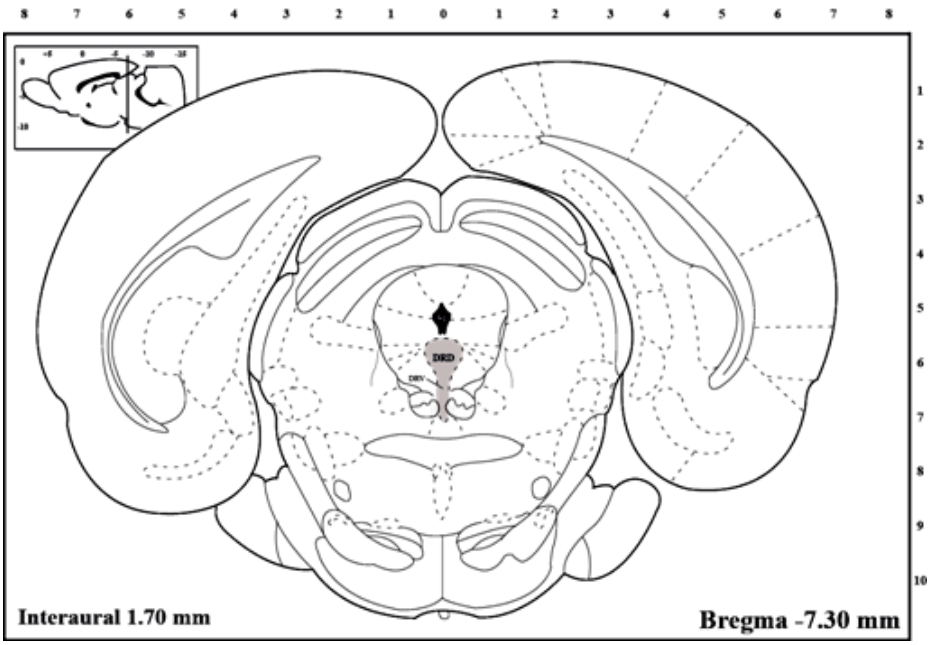


Figure 66: Dorsal raphe nucleus location.
The shaded area delimits the location of the dorsal raphe nucleus (DRN), where serotonin neurons are found. Scales represent distances (in mm) from the midline and the surface of the brain. DRD dorsal raphe nucleus, dorsal part. DRV dorsal raphe nucleus, ventral part. Coronal slices adapted from Paxinos and Watson (1997).

V-3- Results

Within this chapter, 115 putative serotonergic neurons were recorded in the DRN and 179 putative dopaminergic neurons were recorded within the midbrain, from 52 animals.

V-3-A- Adolescent treatment with methylphenidate does not induce growth deficits

The impact of a chronic methylphenidate treatment during adolescence on growth parameters was investigated. We noted with interest that MPH, administered orally at 2.5 mg/kg twice a day and for 15 days, did not induce any body weight gain deficiency during the treatment period (D18, Fig. 67A). After the 28-day washout period, no differences were observed between MPH-treated animals and vehicle-treated animals (D46, Fig. 67A). Similarly, a dosing schedule of 5 mg/kg under the same protocol failed to produce differences in body weight gain. MPH intraperitoneal administration, at 2 mg/kg for 15 days and twice a day, did not induce any significant change in body weight gain (Fig. 67B). Here, we show that adolescent MPH treatments in the range of 4-5 mg/kg/day do not induce weight gain deficiency.

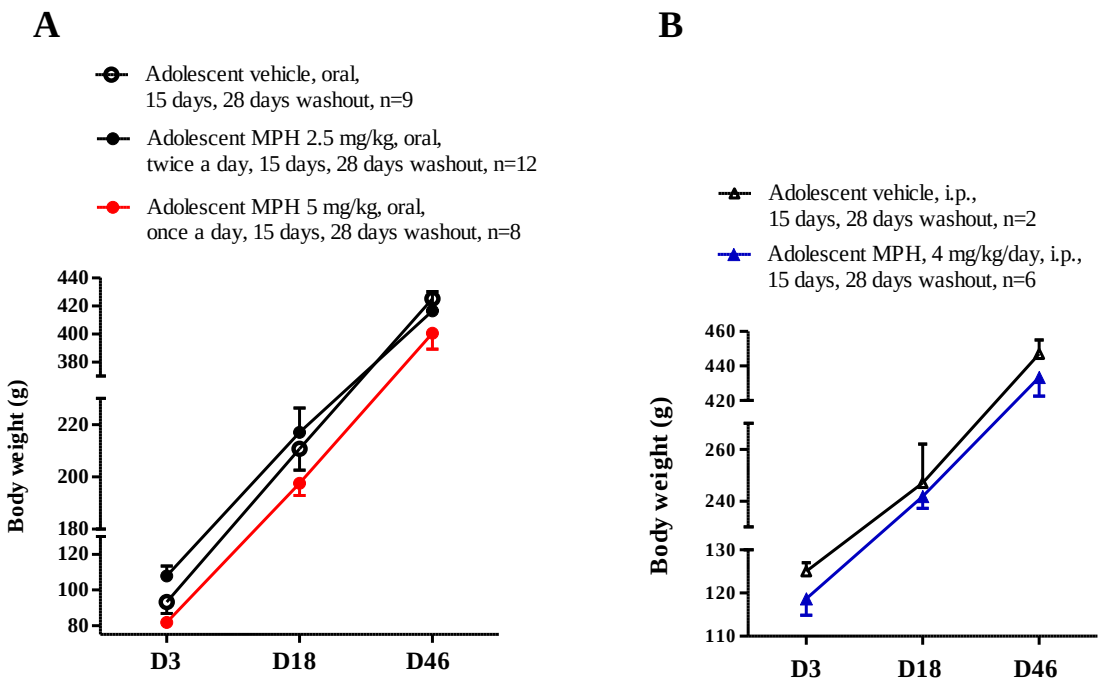


Figure 67: Adolescent exposure to chronic methylphenidate does not induce body growth delay.
(**A**, **B**): Body weight gain was not significantly affected by treatment with MPH. D values indicate experimental day numbers.

V-3-B- Adolescent MPH treatment and depressive-like phenotypes during adulthood

Five days before the end of the washout period (D41), animals were assessed for a depression-like behaviour using the sucrose preference test. Using this protocol, we found that animals successfully learnt to discriminate water from sucrose. Indeed, the transition from the first day to the second day is marked by an increase in liquid consumption in 11 out of 12 animals (92%, Fig. 68A). Interestingly, after this initial increase in sucrose consumption, animals tend to decrease their sucrose consumption on the third day to finally maintain this level of consumption (Fig. 68A). However, for the sucrose preference test, we found that 25% of all animals display a sucrose preference below 65% in both vehicle and MPH-treated animals (respectively 1/4 and 2/8 animals, Fig. 68B). MPH-treated animals and vehicle-treated animals did not differ in their sucrose preferences (Fig. 68C). The low scores, observed in 25% of all animals tested, were not due to individual decreases in total volume intake, as both groups displayed similar liquid intake patterns (Fig. 68D). Although the 65% threshold appears arbitrary, it has been proven accurate in discriminating depression-like

phenotypes (Briones *et al.*, 2012; Couch *et al.*, 2013; Strekalova *et al.*, 2004; Willner, 1997). Therefore, MPH treatment did not induce any long-term depressive traits in rats. This trait was found in 25% of all animals studied (1/4 in the vehicle group, 2/8 in MPH-treated animals for a global 3/12 animals). This puzzling result was further examined. To our surprise, in naive animals (n=10), the same frequency of depression-like phenotypes were observed in 3 of these animals (30%, Fig. 68E). Therefore, such behavioural characteristics, as assessed in our study by the sucrose preference test, might appear "spontaneously" in naive animals maintained under standard laboratory conditions. This result could be partly due to the fact that animals were switched from being group-housed to single-housed or that some animals naturally present such traits when maintained under artificial conditions. Sucrose preference tests could be routinely performed in laboratories to reveal naturally-occurring depression-like phenotypes in rodents.

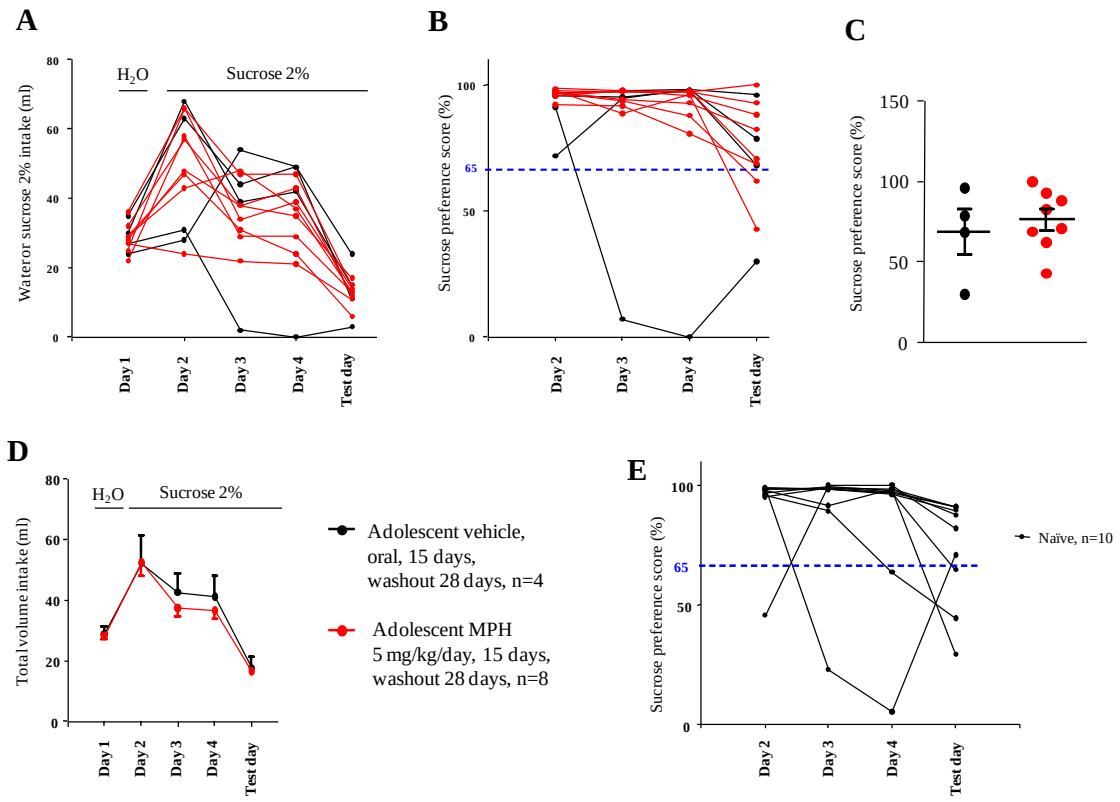


Figure 68: Chronic adolescent exposure to methylphenidate does not induce depression-like behaviour at adulthood.

(**A**): Control animals and MPH-treated animals successfully learned to discriminate water from sucrose, assessed by a drastic increase of volume intake from day 1 to day 2, corresponding to the transition between water and sucrose. (**B**): On the test day, animals from both groups showed similar sucrose preference scores. Both groups (1 animal out of 4 in controls, 2 animals out of 8 in MPH-treated) displayed similar levels of depression-like behaviour, assessed by a sucrose preference score below 65% (dashed blue line, see Briones *et al.*, 2012). Note that one control animal displayed an anhedonic-like behaviour after the third experimental day, by showing practically no interest in sucrose. (**C**): Adolescent exposure to MPH (5 mg/kg/day, for 15 days, followed by a 28-day washout period) does not induce anhedonic-like behaviour. (**D**): Total volume intakes (water or sucrose) were identical between the two groups during the entire experimental protocol. (**E**): To confirm if any anhedonic-like behaviour was naturally occurring in our batch of animals, 10 naive animals were also scored for sucrose preference. Surprisingly, on the test day, we observed that 30% of all animals (3/10) could be considered as displaying depression-like behaviour (score below 65%), confirming our results in **B**.

V-3-C- Adolescent MPH leads to long-term adaptations in serotonin neurons in adulthood

After the recovery period, serotonin neurons from the dorsal raphe nucleus (DRN) were examined *in vivo*. MPH, administered orally at 2.5 mg/kg twice a day for 15 consecutive days during adolescence, did not change the global firing activity of DRN serotonin neurons (Fig. 69A) or the total number of spontaneously active neurons within the DRN (Fig. 69C) found during adulthood, but significantly increased ($p<0.05$, Student's t-test) burst discharges in these neurons by 5 fold (Fig. 69B). Indeed, in the MPH-treated group, 26% of all neurons (13/50) displayed bursting activities whereas only 12% of all neurons in the control group (8/65) did so. These results suggest that VTA dopamine neurons shift their firing rates from regular to bursting activities, without altering the global firing rate frequency. No correlation between sucrose preference results and the electrical activities of these neurons could be observed (Fig. 69D-F). In control animals (n=3, vehicles), we did not observe any correlation either. To be noted: average firing rates, burst activities and cells per track were assessed in at least 7 neurons per animal. Here, we show that low sucrose preference may be linked to high burst activities of serotonergic neurons.

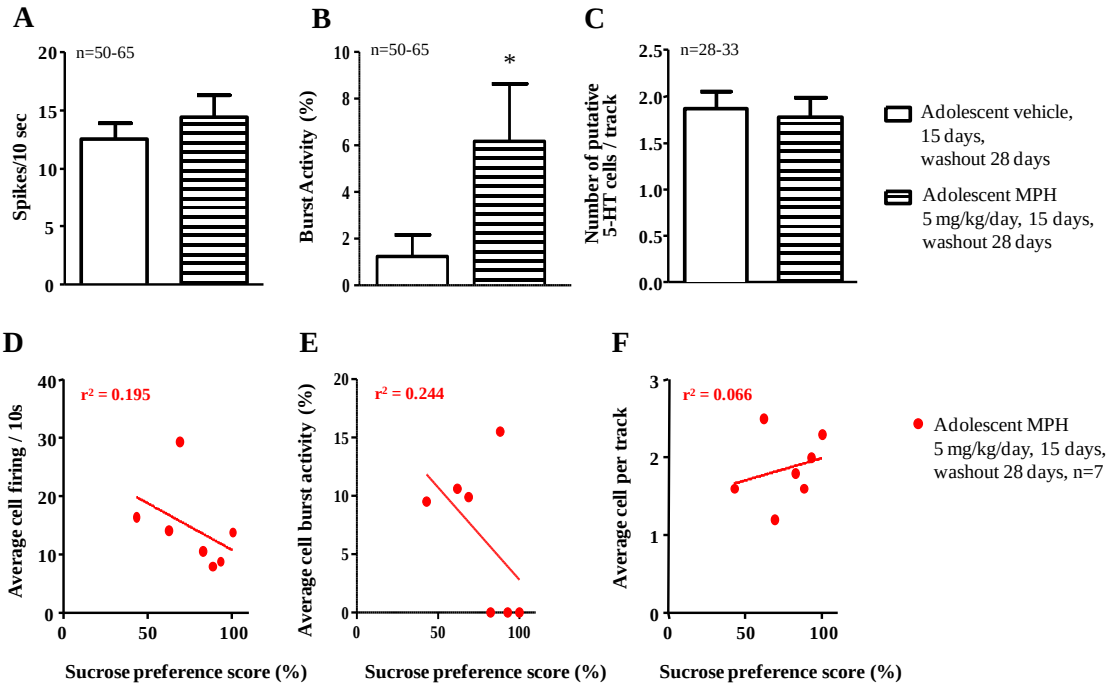


Figure 69: Responses of dorsal raphe nucleus serotonin neurons to chronic methylphenidate in correlation to anhedonia.
(**A**, **B**, **C**): Chronic MPH treatment during adolescence increased the burst activities of dorsal raphe nucleus (DRN) serotonin neurons at adulthood, without significantly changing the firing rates nor the total number of active neurons per electrode descent, suggesting a shift of firing rate activity from regular to bursting activities. (**D**, **E**, **F**): No correlation between the electrical activities of DRN neurons and sucrose preference scores could be found. In sections **D**, **E** and **F**, vehicle animals (n=3) were excluded due to a very low bursting activity in these animals and lack of samples. *P<0.05 *vs.* vehicle, unpaired Student's t-test. r^2 values indicate the correlation coefficient.

V-3-D- Adolescent MPH leads to long-term adaptations in midbrain dopamine neurons in adulthood

Compared to controls, animals that received adolescent MPH exposure (5 mg/kg/day for 15 days) displayed significantly higher ($p<0.04$) firing activities of ventral tegmental area (VTA) dopaminergic neurons (Fig. 70A). Burst activities were not significantly affected by chronic MPH treatment (Fig. 70B). The total number of spontaneously discharging midbrain dopamine neurons (firing rate > 4 spikes per 10 seconds) was significantly increased ($p<0.05$) following chronic adolescence exposure to MPH (Fig. 70C).

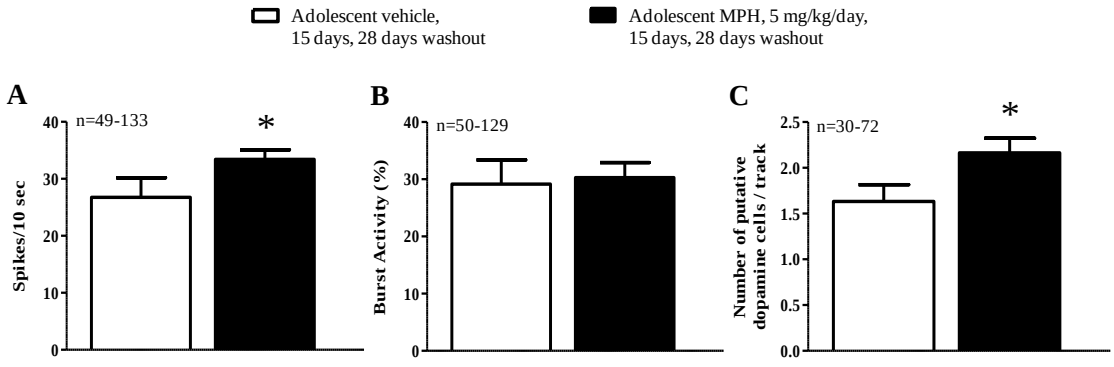


Figure 70: Long-term neuronal adaptations of midbrain dopamine neurons following adolescent exposure to methylphenidate.
(**A**): Chronic administrations of MPH (5 mg/kg/day) during adolescence significantly increased ($p<0.04$) firing activities of VTA dopaminergic neurons in adulthood. (**B**): No change in burst activity was observed following such chronic treatment. (**C**): Chronic exposure to MPH led to significantly greater number of active dopamine neurons found per electrode descent. *$P<0.05$ *vs.* vehicle, unpaired Student's t-test.

V-3-E- Adolescent MPH induces partial dopamine D_2 receptor desensitisation in adulthood

In both controls and MPH-treated animals in adolescence, quinpirole intravenous administration (20 µg/kg) led to strong and reversible firing rate reductions of VTA dopamine neurons ($F_{(2,28)}=37.18$, $p<0.0001$, Fig. 71A). Moreover, animals treated with MPH during adolescence displayed significantly lower sensitivities (% of firing rate decrease) to the high dose (20 µg/kg) of the dopamine D_2 receptor agonist quinpirole ($p<0.05$, unpaired Student's t-test, , Fig. 71B). Indeed, this dose of quinpirole completely stopped the firing activity of 5 out of 9 neurons from the control group, but in none out of 7 neurons in the MPH group. This prompted us to carry out a more thorough study on dopamine D_2 autoreceptor sensitivity, within a large range of doses with another D_2 receptor agonist: apomorphine. When apomorphine was intravenously injected, animals previously exposed to MPH during adolescence (5 mg/kg/day for 15 days followed by 28 days of washout) displayed a significant shift in their sensitivity to the D_2 agonist ($F_{(1,11)}=5.38$ $p<0.05$), as seen in Fig. 71C. Moreover, in such animals, the sensitivity of dopamine D_2 autoreceptor to each apomorphine dose (assessed as the percentage of the decrease in the firing rate), administered with progressive 10 µg/kg increments and up to a cumulative dose of 50 µg/kg, was significantly reduced ($F_{(1,8)}=12.57$, $p<0.01$) when compared to control animals (Fig. 71D).

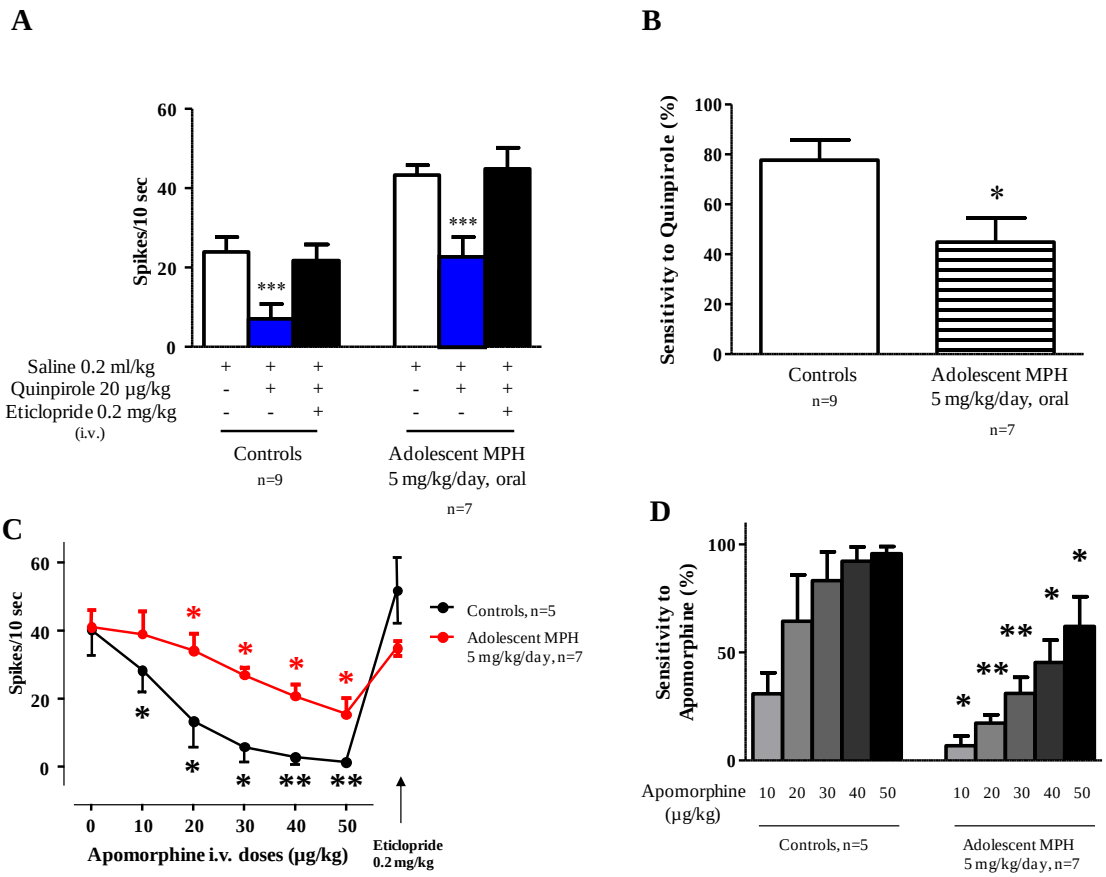


Figure 71: Adolescent exposure to methylphenidate partially induces dopamine D_2 autoreceptor desensitisation in adulthood.
(**A**): Intravenous challenges with quinpirole (20 µg/kg) significantly reduced the firing activities of VTA dopamine neurons in both control and MPH-treated animals ($F_{(2,28)}$=37.18, p<0.0001). Eticlopride (0.2 mg/kg) successfully recovered such MPH-induced firing rate reduction in 15 out of 16 neurons. ***P<0.01 *vs.* saline, Bonferroni after significant repeated measures two-way ANOVA (**B**): Methylphenidate-treated animals during adolescence presented a significantly lower (p<0.05, unpaired Student's t-test) sensitivity to quinpirole challenges in adulthood. (**C**): Similarly, compared to control animals, MPH-treated animals during adolescence displayed significantly lower firing rate reductions following progressive apomorphine (another D_2 receptor agonist) challenges $F_{(1,11)}$=5.38 p<0.05, administered in a dose-response manner and with progressive 10 µg/kg increments. *P<0.05, **P<0.01 *vs.* respective 0 µg/kg apomorphine, Bonferroni after significant repeated measures two-way ANOVA. (**D**): Compared to controls, animals previously exposed to chronic MPH in adolescence had significantly lower sensitivities to dopamine D_2 receptor agonism ($F_{(1,8)}$=12.57, p<0.01). *P<0.05, **P<0.01 *vs.* controls, Bonferroni after significant repeated measures two-way ANOVA.

Intravenous methylphenidate challenge (2 mg/kg) significantly decreased midbrain dopamine neuron firing rates in all groups by 50-65% (Fig. 72A,C,D), an effect reversed by injection of the dopamine D_2 receptor antagonist eticlopride (0.2 mg/kg). In the vast majority of neurons (78%, 29 neurons out of 37) from all groups, the combination

of both dopamine transporter blockade (induced by MPH) and D_2 receptor antagonism (eticlopride) led to significantly higher firing rates, compared to baseline levels (Fig. 72A,C,D), as observed in Chapter III on naive animals (Fig. 43). Similar results were observed for burst activities in 18/37 neurons (Fig. 72B,D).

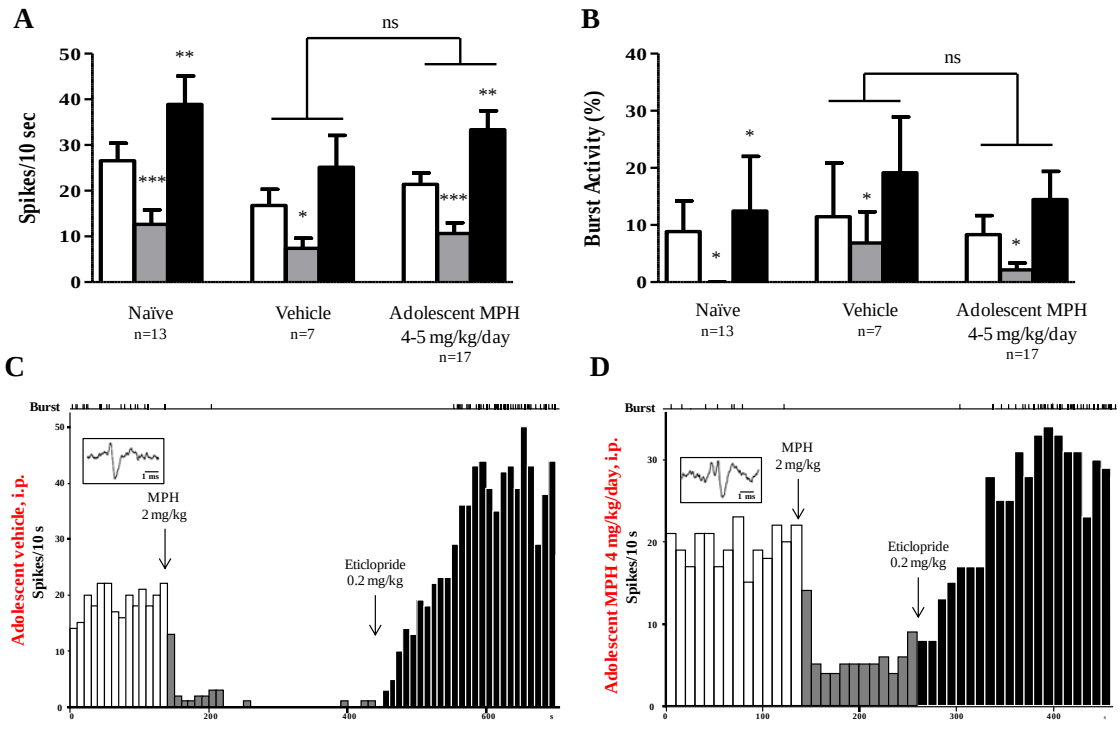


Figure 72: Adolescent exposure to methylphenidate does not induce tolerance to methylphenidate challenges in adulthood.
Intravenous challenges with MPH (2 mg/kg) significantly decreased the firing rates (**A**) and burst activities (**B**) of VTA dopamine neurons in MPH-treated animals. In the control group (vehicle), MPH induced significant firing rate reductions, but did not induce burst activity alterations. (**C**, **D**): Representative firing histogram examples of recordings performed in control and MPH-treated animals (intraperitoneal), respectively. Boxed are shown typical action potential waveforms. Please note that in 29 animals out of 37 (78%), reversal of MPH-induced firing rate reduction by eticlopride (0.2 mg/kg) led to significantly higher firing rates than under baseline conditions (**A**, **C**, **D**). ns: non-significant, *P<0.05, **P<0.01, ***P<0.001 *vs.* respective baselines, Newman–Keuls after significant ANOVA.

V-3-F- Adolescent MPH does not modify $NMDAR_{2B}$ protein expression in the PFC at adulthood but induces behavioural sensitisation to D-amphetamine

Neither 2.5-5 mg/kg of oral MPH, nor 2 mg/kg of i.p. MPH during adolescent (once or twice a day, leading to 4-5 mg/kg/day) had a long-term impact on NMDA receptor 2B protein expression in adulthood in the prefrontal cortex (Fig. 73), confirming our previous results with chronic adult MPH treatments (Fig. 63).

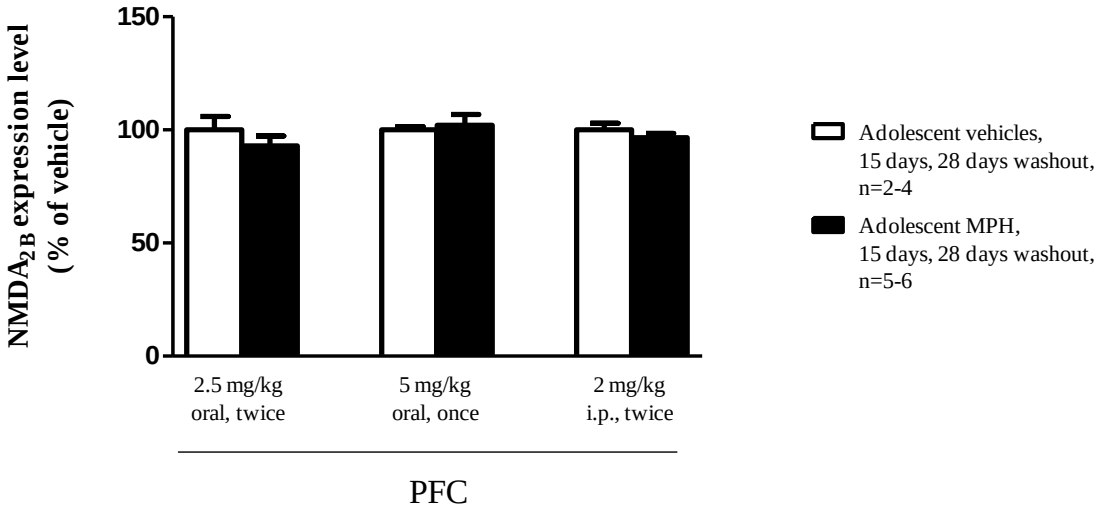


Figure 73: Adolescent exposure to methylphenidate does not increase prefrontal cortex NR_{2B} protein expression.
Chronic adolescent exposure with different regimens of MPH did not significantly alter NMDA receptor 2B protein expression in the prefrontal cortex.

When 3 mg/kg of D-amphetamine was administered intraperitoneally to naive adult rats, we observed a significant increase of rearing events (Fig. 36C2). Next, using a lower dose of D-amph (1 mg/kg), we observed that animals previously treated with MPH during adolescence (2.5 mg/kg/day, orally) displayed significantly more frequent rearing events during the first 30 minutes of observation ($p<0.05$, unpaired Student's t-test) compared to animals that had received vehicle only during their adolescence (Fig. 74). Peak rearing events activity was observed during the first 30 minutes of observation, which was followed by progressive time-dependent decreases of such events, in both groups. Such results indicate a possible immediate behavioural cross-sensitisation between MPH and D-amphetamine, which does not appear to persist in time. Besides, such a result can also indicate a possible risk of multiple drug abuse for previously-treated ADHD patients.

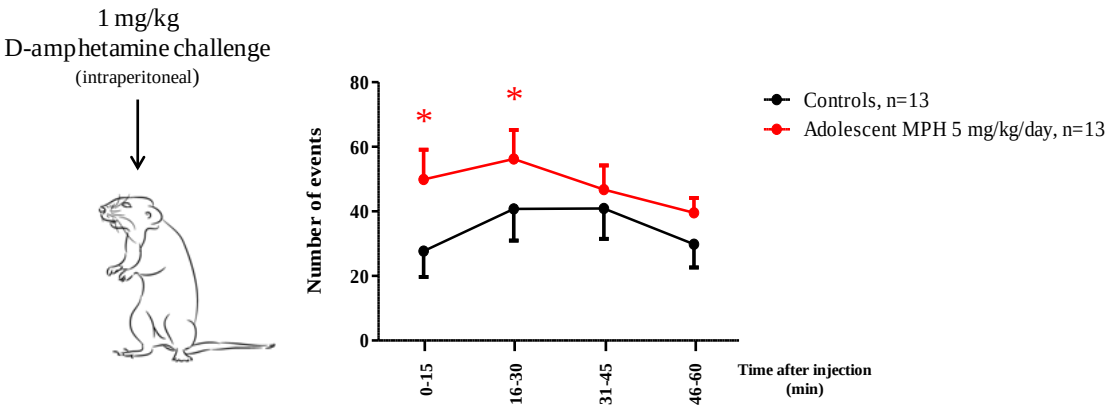


Figure 74: Adolescent exposure to methylphenidate induces behavioural sensitisation to later D-amphetamine challenge.
During the first 30 minutes of observation following D-amphetamine (1 mg/kg, intraperitoneally) challenges, MPH-treated animals displayed significantly greater rearing events than control animals. *P<0.05 *vs.* vehicles, Bonferroni after significant repeated measures two-way ANOVA.

V-4- Discussion

Our results, showing that adolescent exposure to MPH does not induce growth velocity deficits (Fig. 67), corroborates previous observations (Achat-Mendes *et al.*, 2003; Pizzi *et al.*, 1987; Spencer *et al.*, 2006). Contradictory results were also reported. Indeed, while some studies have reported growth alterations throughout the treatment period (Germinario *et al.*, 2013; Poulton *et al.*, 2003; Poulton *et al.*, 2013; Powell *et al.*, 2015; Safer *et al.*, 1972), others confirmed growth or height rebounds after MPH withdrawal in rodents and humans (Klein *et al.*, 1988; Pizzi *et al.*, 1986; Renes *et al.*, 2012; Safer *et al.*, 1975). However, in one group of sub-adult rats investigated in Chapter IV (Fig. 54B), a slight weight reduction was observed following intraperitoneal administrations, but this effect could not be replicated in another batch of animals investigated later on. These findings suggest that stimulant medications may induce growth deficits in ADHD patients under stimulant pharmacotherapy, but such effects may only occur occasionally rather than systematically. Whether these consequences have any significant clinical implications, such as final stature and posture, is a question that requires further research (Poulton, 2005).

In adult rodents, high methylphenidate dosing (20 mg/kg, i.p., once a day for 14 days) induces depression and anxiety, assessed by increased immobility and a decreased latency to become immobile in the forced swim test (Brookshire *et al.*, 2012;

Motaghinejad *et al.*, 2015). Paradoxically, in humans, high MPH dosing has off-label use in the treatment of depression. Indeed, some depressive patients improve following MPH administration (Ayache *et al.*, 2001; Hardy, 2009; Kerr *et al.*, 2012; Lavretsky *et al.*, 2015; Patkar *et al.*, 2006; Rozans *et al.*, 2002). Patients found in palliative care or in geriatrics appear to respond well to MPH therapy (with dosages varying from 2.5 to 40 mg/day), which efficiently reduces symptoms of depression. Consistent with these findings, our study supports the assumption that adolescent MPH administration does not induce more depression-like behaviour in adulthood (Fig. 68B,C) than what is generally found in the background population (Fig. 68E). In our sucrose preference test, the proportion of depression-like behaviour was high in both cohorts (25-30%, Fig. 68). One can wonder if this could be attributed to the transition from social to individual housing (inducing social isolation), which was necessary for assessing individual criteria for depression. Parallel observations in non-human monkeys and mice found isolation to be an important factor in inducing this type of behaviour (Fischer *et al.*, 2012; Hennessy *et al.*, 2014; Li *et al.*, 2013; O'Keefe *et al.*, 2014). In rats, similar conclusions were drawn (Djordjevic *et al.*, 2012; Jahng *et al.*, 2012; Zanier-Gomes *et al.*, 2015). However, whether low sucrose preference scores directly reflect depression-like behaviours remains an open question and one could assume that such a test rather measures vulnerability to depression. It is important to note that in humans, social isolation, following heart failure, stroke or coronary disease, leads to poorer recoveries (Friedmann *et al.*, 2006; O'Keefe *et al.*, 2014; Wang *et al.*, 2006).

Our results, showing that some control animals display sucrose preferences below 65%, stand in contrast to recent investigations using the same protocol and strain of animals. Indeed, Boyko in 2015 and Wang in 2014 found that all control rats (naive, n=30) showed sucrose preference above 70% (Boyko *et al.*, 2015; Wang *et al.*, 2014). However, such rates are easily explained in both studies by the housing conditions, where rats were group-housed until the 1 hour test. In our model, rats were individually-housed at the start of the experiment, on day 1, for acclimatisation to the test conditions. Therefore, initial social isolation may have contributed to the induction of depressive-like traits, although adolescent MPH treatment, given under the exact same conditions, did not affect depression-like rates in rats. However, adolescent exposure to MPH (2 mg/kg, i.p., twice a day for 16 days) was found to significantly decrease the sucrose preference of adult rats for solutions ranging from 0.1 to 0.5% but not with a 1%

sucrose concentration (Bolanos *et al.*, 2003), while we used a 2% concentration (Fig. 68B,C). The study by Bolaños also highlights the fact that there is no difference in total liquid intake between control and MPH-treated rats, as seen in our results (Fig. 68D). One very interesting study, using slightly different experimental protocols, has reported similar results to ours. Indeed, adolescent chronic MPH administration (2-5 mg/kg, once daily, i.p., 10 days) led to unaltered sucrose preference (using 0.125% sucrose solution) in adulthood in singly-housed rats. However, the authors also witnessed sucrose preferences, in controls and MPH-treated animals, ranging between 59 and 72% (Crawford *et al.*, 2013), consistent with our own observations. In naive wild type mice, several sucrose contents in the test solutions (0.5, 1 and 2% w/v) resulted in proportionate (45, 60 and 75%, respectively) sucrose preference scores (Couceyro *et al.*, 2005). Taken together, these observations suggest that adolescent MPH exposure does not induce subsequent depression-like behaviour in adulthood in rodents. Moreover, the 65% sucrose preference depression threshold needs to be further investigated, or even reassessed, as it may not accurately reflect the phenotypes of these traits in rodents kept under standard laboratory conditions.

When dorsal raphe nucleus (DRN) serotonin neurons were recorded during adulthood, we did not see any change in the firing activity or in the total number of active neurons per track following chronic adolescent MPH treatment (Fig. 69A,C), although a small but non-significant ($p<0.1$) firing rate increase was observed. However, an adolescent exposure to MPH leads to increased burst activities of DRN serotonin neurons ($p<0.01$ vs vehicle, unpaired Student's t-test, Fig. 69B). Each bursting activity results in a greater efficiency of neurotransmitter release at terminals (Overton *et al.*, 1997; Paladini *et al.*, 2003; Zhang *et al.*, 2011). This indicates that some serotonin neurons in the DRN may have adapted their firing mode following chronic MPH, by switching from single firing activities to bursting activities, without increasing their firing rates, an effect that persists in time. Baseline burst levels in the control group (around 3%), was similar to previous findings (Manta *et al.*, 2009). A recent study found that adolescent exposure to 1.5 mg/kg of daily D-amphetamine for 21 days, followed by 20 days of washout, significantly increases the firing rate of both DRN serotonin and VTA dopamine neurons, without affecting burst activities (Labonte *et al.*, 2012). This study also shows that higher doses of D-amphetamine (5 mg/kg) do not lead to any long-lasting change in the firing activities of both types of neurons. Although

DRN serotonin neurons have long been considered as incapable of producing burst discharges, mainly due to their slow firing mode (5-25 spikes per 10 seconds), evidence for a large bursting neuronal population now exists (Allers *et al.*, 2003; Gartside *et al.*, 2000; Hajos *et al.*, 1995; Jennings, 2013; Schweimer *et al.*, 2010). One of the physiological significances of burst activity from DRN serotonin neurons may be to mediate the anticipation of reward, but not punishment. Indeed, during the anticipation period of a sucrose reward, mice displayed strong burst activity of DRN serotonin neurons, an effect not seen during the rewarding event itself (Li *et al.*, 2016). Odour-related reward has also been found to trigger bursting discharges of these neurons (Cohen *et al.*, 2015). Besides, bursting DRN serotonin neurons induce high serotonin release in the forebrain, including the prefrontal cortex (Gartside *et al.*, 2000; Hajos *et al.*, 2007; Hajos *et al.*, 1996).

In our experiments, there was no correlation between sucrose preference and the electrical parameters of DRN serotonin neurons (Fig. 69D-F). A recent study on head-restrained and water-deprived mice observed bursting activities of such neurons after rewarding (water) but not punishing (puff of air) events (Cohen *et al.*, 2015). Indeed, burst discharges were observed in the anticipation of rewarding events, whilst punishing events did not induce such effects. Moreover, clues (in the form of odours) to subsequent rewarding or punishing events strongly induce bursting activities of DRN serotonin neurons. Finally, all the neurons recorded in this study displayed firing rate modifications during these behavioural tasks (Cohen *et al.*, 2015). Our results may indicate that "anhedonic" rats displayed higher burst activities of DRN serotonin neurons compared to non-anhedonic rats (Fig. 69E). Anhedonic-like behaviour, measured in our methods through the sucrose preference test, are the typical symptoms of endogenous and non-endogenous depression (Li *et al.*, 2015b; Liu *et al.*, 2012b; Ng *et al.*, 2014; Yee *et al.*, 2015). Anhedonia can reflect a state of stress, occurring at the same frequency in both MPH-treated and control animals (Fig. 68). Mild stress has been linked to decreased sucrose preferences in rodents, an effect alleviated by chronic antidepressant therapies (Gupta *et al.*, 2014; Liang *et al.*, 2015; Tianzhu *et al.*, 2014; Willner *et al.*, 1987). More studies will be needed to characterise the exact relationship between depression-like traits in rodents and the electrical activities of serotonergic neurons. Nevertheless, adolescent MPH treatment does not seem to induce long-lasting depressive phenotypes in rodents in adulthood.

In adulthood, in the ventral tegmental area (VTA), dopaminergic neurons increased their firing rates following chronic adolescent MPH exposure (Fig. 70A). The total number of spontaneously active dopamine neurons in the VTA was greater in MPH-treated animals (Fig. 70C). These results are in line with previous findings, where adolescent amphetamine exposure, followed by a washout period, led to increased firing rates of VTA dopamine neurons, without affecting burst activity (Labonte *et al.*, 2012).

Compared to controls, MPH-treated animals during adolescence showed a non significant ($p=0.14$) decrease of dopamine D_2 receptor sensitivities to the 20 µg/kg dose of quinpirole (Fig. 71A,B), associated with a significantly lower proportion of neurons that were completely inactivated by the agonist. When apomorphine, another potent dopamine D_2 receptor agonist routinely used in electrophysiology, was given intravenously to adult rats up to a total dose of 50 µg/kg, animals previously exposed to chronic MPH during adolescence displayed significantly lower firing rate reductions, proving a partial desensitisation of dopamine D_2 autoreceptors following adolescent MPH treatment (Fig. 71C,D). It is possible that this partial desensitisation is responsible for the moderate increase in firing rate and population activity observed in the present study in MPH-treated animals. Indeed, these dopamine inhibitory autoreceptors are known to be particularly important to control and modulate the firing activity of VTA dopamine neurons (Eddine *et al.*, 2015; Ford, 2014; Pothos *et al.*, 1998). Both groups (controls and MPH-treated animals) were equally sensitive to intravenous MPH re-challenges (2 mg/kg), inducing a reversible (eticlopride 0.2 mg/kg) strong reduction (50-65%) of firing rates, although the MPH-treated group tends to show a slightly reduced response to methylphenidate (Fig. 72). These results indicate that, contrary to what was previously observed following a chronic adult MPH treatment, adolescent MPH treatment, followed by a washout period, does not induce long-lasting dopamine transporter desensitisation (Chapter IV, Fig. 55-56) but may alter the binding properties of dopamine D_2 receptor agonists onto D_2 autoreceptors (Fig. 71). However, one could have expected a larger decrease in MPH-induced inhibition of firing activity in MPH-treated animals, as we demonstrated that the dopamine D_2 autoreceptor is partially desensitised in these animals. The reason for this discrepancy probably lies in the fact that dopamine D_2 agonists target more potently a larger population of dopamine autoreceptors, with which they interact directly, unlike methylphenidate. Whether it is the total density of midbrain dopamine D_2/D_3 autoreceptors, or their intracellular

signalling capacity, that has been altered, needs to be addressed. In midbrain dopamine D_2 receptor deficient mice (using conditional dopamine D_2 receptor knockout), cocaine induced stronger locomotor activity than in wild-type animals (Bello *et al.*, 2011). Very interestingly, another recent electrophysiological study has shown that obese mice (fed with a high-fat diet) present milder quinpirole-induced firing rate reduction than control mice (Koyama *et al.*, 2014). Therefore, one could assume that chronic psychostimulant use can induce long-term dopamine D_2 receptor desensitisation, which, in turn, could affect food and drug seeking behaviour. It would be particularly important to examine whether this partial desensitisation observed in our study is only observable on the dopamine autoreceptors found in the VTA, or if such a desensitisation can also affect other populations of dopamine D_2 receptors, such as the post synaptic D_2 autoreceptors found in striatal medium spiny neurons.

Here, we also observed higher firing rates of VTA dopamine neurons in the majority of the recordings (79%) after the combination of both MPH and eticlopride administrations, regardless of previous adolescent exposure to MPH or not, suggesting that adolescent exposure to MPH does not prevent the later excitatory component of MPH (Fig. 72). Another study (Shen *et al.*, 2006), using a similar protocol, did not observe any change in VTA dopamine neuron electrical activities following adolescent MPH administration (1 mg/kg for 21 days followed by extensive washout periods of either 30 or 60 days). Interestingly, this study also focused on intravenous dopamine D_2 receptor agonism challenges (apomorphine 20 µg/kg and quinpirole 0.1-70 µg/kg) and found no differences between controls and adolescent MPH-treated animals (Shen *et al.*, 2006). Another study showed that adolescent exposure to MPH (10 mg/kg for 7 days) increases significantly locomotor sensitivity to cocaine challenges following 14 days of washout (Brandon *et al.*, 2001).

We did not observe any changes in $NMDA_{2B}$ protein expression levels in the prefrontal cortex (PFC) of adult rats following adolescent exposure to 4-5 mg/kg/day of chronic MPH, regardless of the types of administration routes (Fig. 73). The study by Mao in 2009 using repetitive amphetamine exposure (4 mg/kg for 7 days, i.p., 14-60 days washout) found a persisting downregulation of striatal NMDA receptor 2B protein levels (Mao *et al.*, 2009). However, such differences can be explained by the use of higher doses, adult animals (175-200 g) and different administration routes, as well as

different molecular targets (MPH exerts blockade of extracellular dopamine reuptake while D-amphetamine blocks vesicular dopamine reuptake). It must be noted that NMDA receptor 2B protein levels in the prefrontal cortex are high at adulthood, compared to levels found in the striatum and are required for normal persistent activity (Monaco *et al.*, 2015; Urban *et al.*, 2014; Wang *et al.*, 2008). Moreover, these studies suggested that the ratio between $NMDA_{2B}$ and $NMDA_{2A}$ is responsible for the establishment of both long-term potentiation and adaptation. The authors also reported decreased NMDA receptor 2B protein levels in the prefrontal cortex following a single (1 mg/kg, intraperitoneal) juvenile MPH administration (Urban *et al.*, 2013b), a result opposite to what we observed in the present study (Chapter II, Fig. 37). Previously, we have found that chronic MPH treatment does not induce alterations of $NMDA_{2B}$ protein expression in the PFC of adult rats (Chapter IV, Fig. 63). Here, we also demonstrate that chronic adolescent exposure to MPH, followed by an extensive washout period, does not change cortical $NMDA_{2B}$ protein expression, which is consistent with our previous observations (Fig. 37).

Finally, adolescent exposure to MPH induced behavioural sensitisation to D-amphetamine, assessed by an immediate but relatively short-lasting increased number of rearing events induced by D-amphetamine (1 mg/kg, intraperitoneally) in adult animals previously exposed to MPH during adolescence (Fig. 74). Such cross-sensitisation between these two psychostimulants can underlie plastic mechanisms than occur following stimulant exposure during adolescence. Moreover, early MPH exposure may lay grounds for later substance abuse. This results stand in contrast to a previous study, where the authors found no relationship between adolescent exposure to MPH and sensitisation to methamphetamine in later life (Kuczenski *et al.*, 2002). Nevertheless, our behavioural findings stand in complete concordance with our electrophysiological data showing partial desensitisations of dopamine D_2 autoreceptors. As mentioned earlier, specific VTA inactivation of presynaptic dopamine D_2 receptor has been found to be associated with increased sensitivities to cocaine (Bello *et al.*, 2011). We therefore hypothesised that chronic MPH treatment during adolescence can induce persistent changes in dopamine D_2 receptor sensitivity, which can then translate into increased sensitivities to addictive drugs.

In this fifth chapter, we have demonstrated that adolescent exposure to MPH, followed by 28 days of drug withdrawal, does not induce delayed growth, depression, or cortical $NMDA_{2B}$ protein expression variation. We have also reported that such treatment can induce long-term consequences on the electrical functions of DRN serotonin and VTA dopamine neurons. After drug withdrawal, midbrain dopaminergic neurons may display partial dopamine D_2 autoreceptor desensitisation. It would also appear that adolescent exposure to MPH can induce sensitisation to later D-amphetamine exposure in adulthood, which we believe to be associated with a partial desensitisation of dopamine D_2 autoreceptors, within the midbrain. Further research will be necessary to understand other possible long-term modifications of brain functions that can arise following chronic adolescent psychostimulant exposure.

Chapter VI – The conjunction of caffeine or nicotine, two other freely available psychostimulants, with methylphenidate.

VI-1- Introduction

Caffeine and nicotine, two licit drugs, are widely consumed in the population, among other drugs, like alcohol (Crocq, 2003; Martin *et al.*, 2008). Both drugs are easily available to the public.

Caffeine, produced by the *coffea* plant genus and by tea plants, belongs to the Xanthine family and possesses psychoactive effects (Nehlig *et al.*, 1992). Caffeine exerts non-specific blockade of A_1, A_2 and A_3 adenosine receptors with widely ranging IC_{50} and K_i values (Table 1) (Daly, 2007; Hunter *et al.*, 1990; Schwabe *et al.*, 1985; Snyder *et al.*, 1981). One cup of coffee, containing on average 80 mg of caffeine and therefore resulting in 0.4 to 2.5 mg/kg of caffeine intake, already significantly blocks adenosine receptors (Fredholm *et al.*, 1999). In rats, caffeine binds to adenosine A_1, A_{2A} and A_{2B} receptors with respective K_D values of 20, 8.1 and 17 µM but also possesses low-affinity binding (190 µM) for A_3 receptors (Fredholm *et al.*, 1999). Because of its structural resemblance to strychnine, caffeine also mildly blocks ionotropic glycine receptors, with IC_{50} values below the millimolar range (Duan *et al.*, 2009). In humans, caffeine is ingested to increase locomotor activity and combat fatigue (Glade, 2010; Nehlig *et al.*, 1992), although some do not experience such effects. According to one study, adjunct methylphenidate therapy (10 mg) with a low dose of caffeine (10 mg) produced a stronger attenuation of behavioural symptoms in ADHD children, compared to methylphenidate alone (Garfinkel *et al.*, 1981). Therefore, caffeine supplementation may be an effective tool to decrease total MPH intake. In young adults, caffeine use can modulate ADHD symptoms (Broderick *et al.*, 2004), but a recent study has observed that caffeine can only be linked to ADHD if taken in combination with nicotine (Dosh *et al.*, 2010). In young adolescents, one study reported a clear association between high caffeine consumption (4 or more beverages per day) and ADHD (Martin *et al.*, 2008).

Early caffeine consumption in young rats leads to increased locomotor activities following adult methylphenidate challenge, proving a cross-sensitisation between the two drugs (Boeck *et al.*, 2009). However, some animal studies have reported the beneficial effects of caffeine on ADHD symptoms (Ioannidis *et al.*, 2014). In an animal model of ADHD (juvenile administration of the dopamine and norepinephrine neurotoxin 6-hydroxy-dopamine), a 14-day chronic caffeine treatment improved spatial attention (Caballero *et al.*, 2011), providing potential evidence for caffeine to be used as an adjuvant to psychostimulants, as shown previously (Garfinkel *et al.*, 1981). Another study on adolescent spontaneous hypertensive rats (an animal model of ADHD) revealed that chronic caffeine administration (2 mg/kg, twice a day) for 21 days can normalize ADHD traits (Pandolfo *et al.*, 2013).

Nicotine is a potent agonist of nicotine acetylcholine receptors. To date, 12 subunits ($\alpha_{2\text{-}10}$, $\beta_{2\text{-}4}$) can enter into the composition of neuronal nicotine acetylcholine receptors (Itier *et al.*, 2001; McGehee *et al.*, 1995; Sargent, 1993). Extracted from tobacco plants, nicotine also belongs to an alkaloid family. EC_{50} values for nicotine onto α_7, $\alpha_4\beta_2$ and $\alpha_3\beta_4$ were found to be of 13.2, 0.1-2.5 and 87 µM (Table 1) (Papke *et al.*, 2007; Xiu *et al.*, 2009). Although the legal age for nicotine consumption in the UK is 18 years old, many abuses have been recorded in adolescents. In fact, a recent European study found that 17.3% of adolescents smoke every week in the UK (Pfortner *et al.*, 2015). Current literature on nicotine and ADHD remains contradictory. Some clinical studies reported increased craving for nicotine after methylphenidate intake in ADHD adults (Bron *et al.*, 2013), while another study reported higher nicotine use among young adults with ADHD, compared to the background population (Symmes *et al.*, 2015). Contrary to what is observed in humans, one study did not find any correlation between adolescent MPH exposure (10 mg/kg *per os* once a day for 7 consecutive days) and adult nicotine sensitisation (0.4 mg/kg, subcutaneous) in rats (Justo *et al.*, 2010). It is necessary to assess the questions regarding the fact that ADHD medication may lead to smoking. A Dutch study reported that smoking amongst ADHD patients is twice the national average and that methylphenidate use increases tobacco consumption (Bron *et al.*, 2013). Acute methylphenidate is also responsible for increases in nicotine consumption in ADHD patients (Vansickel *et al.*, 2011) and non-ADHD patients (Rush *et al.*, 2005). However, some studies suggest that methylphenidate could be an efficient medication for reducing nicotine intake in ADHD patients (Covey *et al.*, 2010;

Gehricke *et al.*, 2011; Hammerness *et al.*, 2013), but another study has proved its inefficiency (Hurt *et al.*, 2011).

Findings concerning nicotine, ADHD and methylphenidate remain very contradictory and therefore require further investigation. In this final chapter, we will investigate how nicotine or caffeine could interfere with methylphenidate on dopaminergic neurons, using the same technique as described previously. A special focus will be placed upon neuronal sensitisation to MPH following pre or post-exposure to either nicotine of caffeine.

VI-2- Material and Methods

VI-2-A- Subjects and groups

Eighty-three male Sprague-Dawley rats were purchased from Charles River, UK. Animals were housed in groups of 2-4 per cage, maintained at 20-22^{o}C with humidity rates above 40% under a 12:12 L/D cycle with lights ON at 07h00. Food and water were provided *ad libitum*. Animals were allowed a 3-day acclimatisation period after delivery. All experiments were performed during the light phase and with permission from the UK Home Office and De Montfort University Ethics Committee under the Project Licence 60/4333 and with the Personal Licence 60/13750.

Animals belonged to one of the three groups:

- Naive animals
- Animals voluntarily drinking 5 mg/kg/day of MPH (or vehicle alone) in 2 ml/kg of a sucrose 10% (w/v) solution, orally and for 15 consecutive days, followed by a 28-day washout period.
- Animals receiving 4 mg/kg/day of MPH (or vehicle alone) in 1.2 ml/kg of saline, intraperitoneally and for 15 consecutive days, followed by a 28-day washout period.

VI-2-B- *In vivo* extracellular single unit electrophysiology

Please refer to paragraph II-2-B. Here, only single barrel borosilicate electrodes were used. A total of 67 neurons were recorded within this chapter.

VI-2-C- Behaviour

Sixteen naïve rats weighing 250 grams were housed individually at the beginning of the behavioural experiments. All drugs were dissolved into saline. Animals received a single intraperitoneal injection of either: 0.8 ml/kg of saline (NaCl 147 mM), 0.1 mg/kg of nicotine, 3 mg/kg of D-amphetamine or a combination of both 3 mg/kg of D-amphetamine and 0.1 mg/kg of nicotine. Four animals were used within each group. Animals were then scored for behavioural parameters during 15 minute time periods and up to a total of 60 minutes. The counting of well-defined behavioural traits such as rearing, scratching, grooming, jumping, running, climbing, catalepsy and stereotypical movements was done manually. At the end of the 60 minute observation period, animals were sacrificed by cervical dislocation and the brains were quickly removed, dissected out and immersed into liquid nitrogen. Brain regions were kept at -80^{o}C for further protein analysis.

VI-2-D Data analysis

Please refer to the data analysis explained in paragraph II-2-E.

VI-3- Results

VI-3-A- Caffeine does not change baseline firing or burst activity of VTA dopamine neurons, or the efficiency of the combination of both MPH and eticlopride

When the adenosine antagonist caffeine was administered at 2 mg/kg increments and up to a cumulative dose of 10 mg/kg, no change in the firing or burst activity of midbrain dopamine neurons was observed in 6 out of 6 recordings (Fig. 75), although a small tendency to decreased firing rates were observed from 2 to 8 mg/kg.

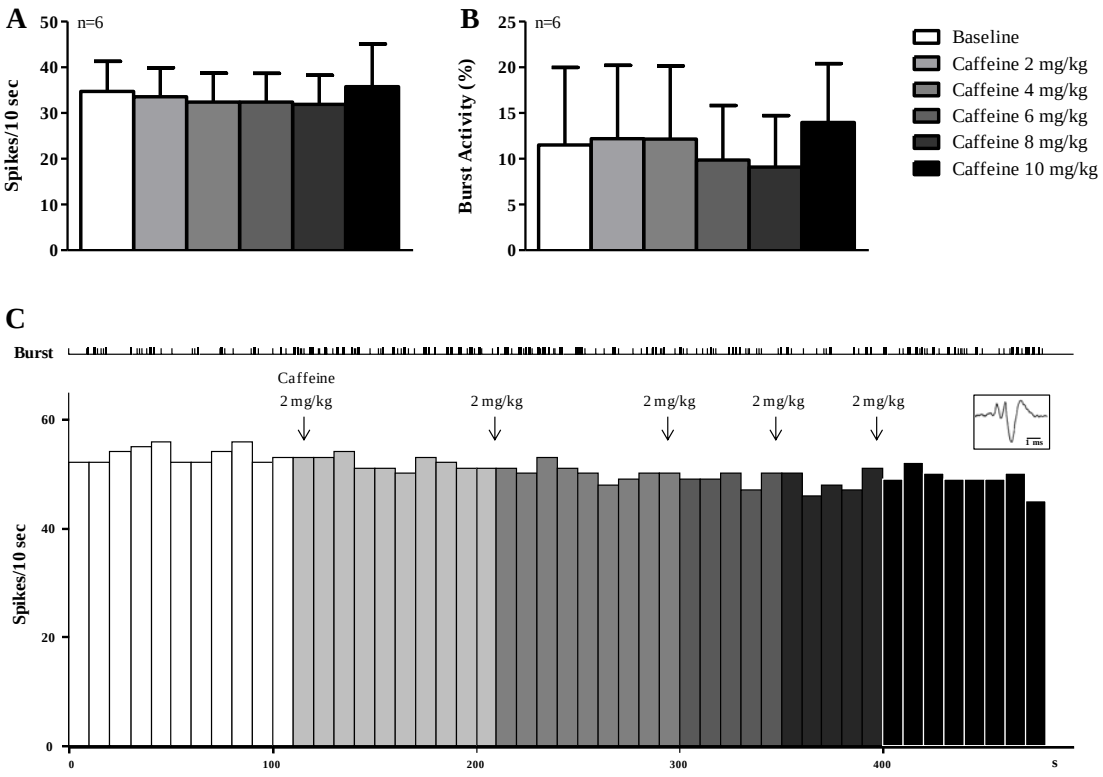


Figure 75: Caffeine administration does not induce significant electrophysiological changes to midbrain dopamine neurons.
(**A**): Caffeine intravenous administration, using progressive 2 mg/kg increments and up to a cumulative dose of 8 mg/kg, induces small but non-significant reductions of the firing rate of VTA dopamine neurons. (**B**): Similar results were observed on burst discharges. (**C**): Representative time course neuronal recording with progressive caffeine administration. Boxed is shown the typical action potential waveform of this neuron.

Pre-administration of caffeine (10 mg/kg) did not induce a significant change (firing: $F_{(1,27)}=0.05$, $p>0.8$, bursts: $F_{(1,27)}=1.16$, $p>0.3$) in the neuronal responses to subsequent MPH (2 mg/kg) and eticlopride (0.2 mg/kg) intravenous challenges (Fig. 76). However, the excitatory effect induced by both drugs (MPH and eticlopride) was observed in all of the recordings after caffeine pre-treatment (100%, 6/6, Fig. 76), a result that does not differ from recordings performed without such pre-treatment (83% of all neurons, 19/23, $p>0.54$, Fisher exact test, Fig. 77).

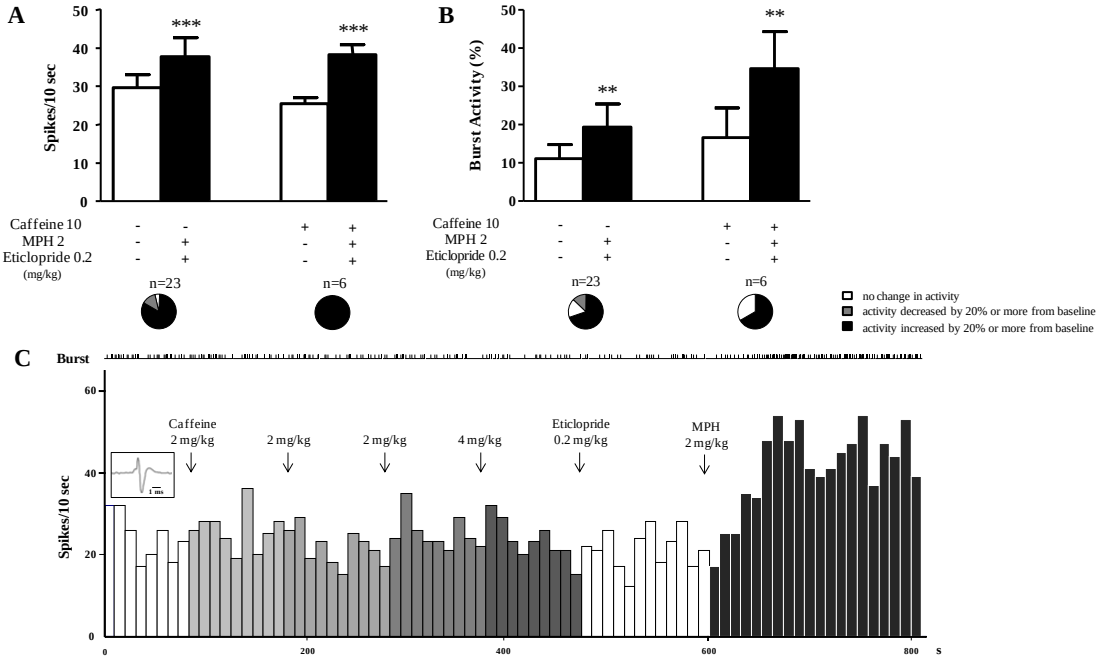


Figure 76: Pre-treatment with caffeine does not prevent the excitatory component of methylphenidate following dopamine D_2 receptor antagonism.
Pre-treatment with 10 mg/kg of caffeine did not alter the excitatory component of MPH (2 mg/kg) on the firing rates (**A**) and burst activities (**B**) of VTA dopamine neurons following dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg). (**C**): Representative time course recording of such protocol. Boxed is shown the typical action potential waveform of this neuron. **P<0.01, ***P<0.001 *vs.* respective baselines, Bonferroni after significant repeated measures two-way ANOVA.

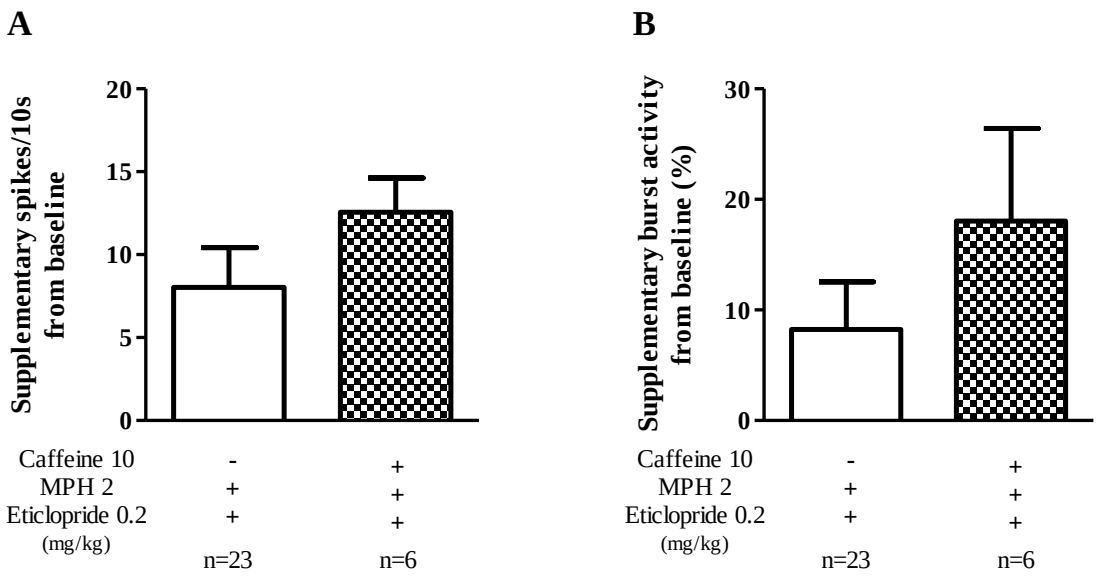


Figure 77: Pre-treatment with caffeine tends to increase the excitatory component of methylphenidate following dopamine D_2 receptor antagonism.
Caffeine pre-exposure (10 mg/kg) tends to non-significantly increase the responses of VTA dopamine neurons to MPH/eticlopride administrations, assessed by the number of supplementary spikes (**A**) or burst activities (**B**) induced by the combination of MPH and eticlopride (respectively with p=0.36 and p=0.3, unpaired Student's t-test).

VI-3-B- The distinction between three neuronal populations following nicotine administration

The acetylcholine receptor agonist nicotine, administered at 50 µg/kg increments and up to a cumulative dose of 100 µg/kg, failed to induce significant changes in the firing and burst activities of midbrain dopamine neurons when a large population of neurons was recorded (n=20, Fig. 78A,B). However, based upon individual responses, three populations of neurons could be differentiated. Indeed, some neurons (6/20, 30%) significantly increased (by more than 20% from baseline levels) their firing rates and burst activities following 50 µg/kg of intravenous nicotine (Fig. 78C,D). Other neurons (5/20, 25%) significantly decreased (by more than 20% from baseline levels) their firing rates and burst activities following the same dose of nicotine. Some other neurons (9/20, 45%) did not respond to the 50 µg/kg dose of nicotine. In the neurons that were originally insensitive to nicotine, a further 50 µg/kg dose of nicotine was administered (cumulative dose of 100 µg/kg). Following such administration, 1 neuron had its firing rate significantly increased while 4 neurons had their firing rates significantly decreased. Four neurons (20%) remained rate-insensitive to any of the nicotine doses. Both doses of nicotine (50 and 100 µg/kg) significantly

increased firing rates in 6 and 7 neurons out of 20 ($p<0.01$ and $p<0.001$, respectively, compared to saline response, Fisher's exact test) and significantly decreased firing rates in 5 and 9 neurons out of 20 ($p<0.01$, compared to saline response, Fisher's exact test). Burst activities followed the same trend, except for the 100 µg/kg where it induced burst increases in 8/20 neurons while decreasing burst activities in 5/20 neurons. Compared to the 50 µg/kg dose, the 100 µg/kg dose did not induce significant changes in population responses (Fig. 78C,D, Fisher's exact test). Typical recordings showing the three different responses of such neurons to nicotine administration (insensitivity, decreases and increases of firing rates) are respectively displayed in Fig. 78E. To be noted: following saline administration, both the burst and the firing activities increased in 1 out of 20 neurons, decreased in 1 out of 20 neurons and no significant changes in the electrical discharges were observed in the remaining 18 neurons.

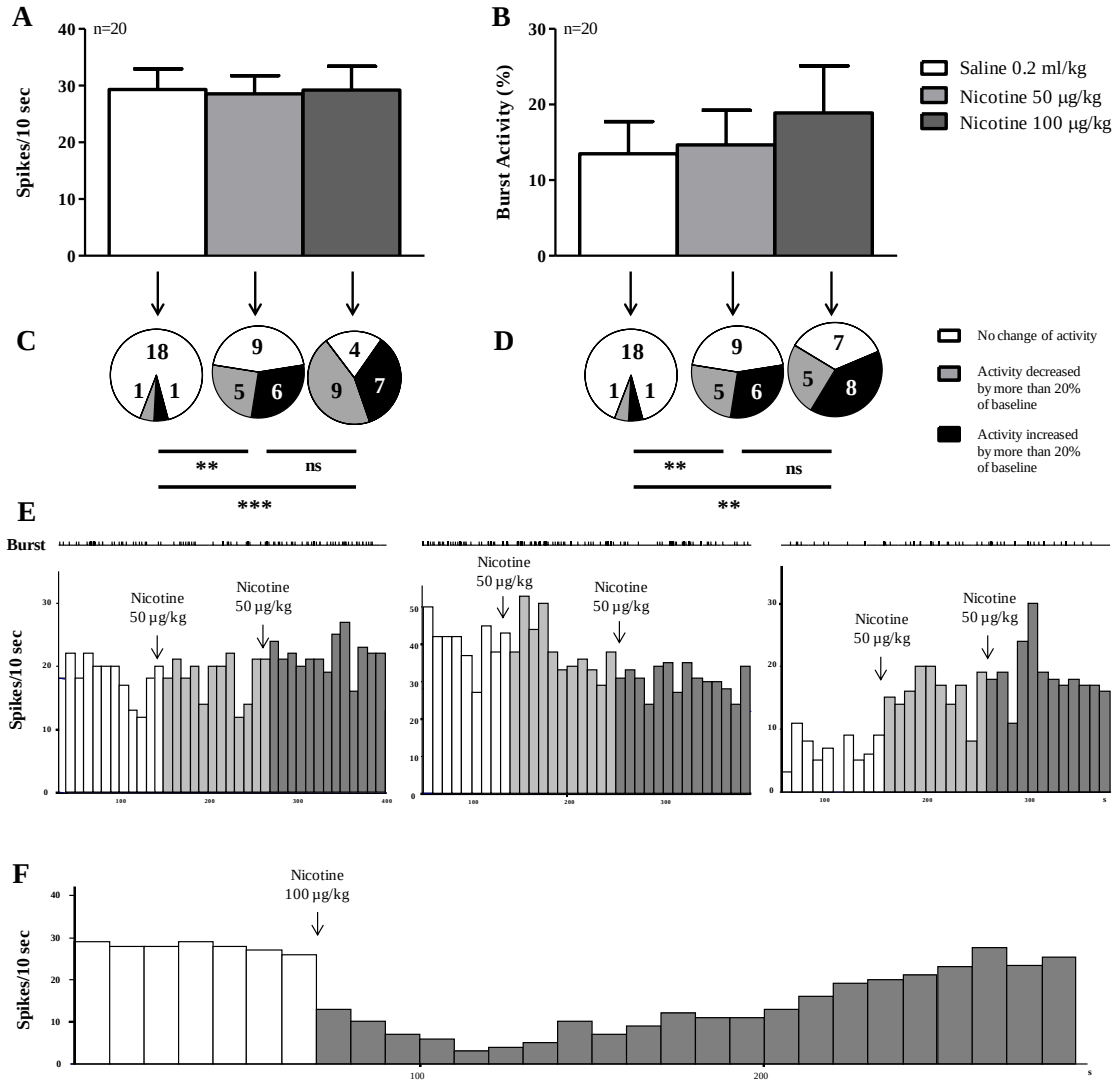


Figure 78: Responses of midbrain dopamine neurons to intravenous nicotine challenges.

In a neuronal population consisting of 20 neurons, cumulative intravenous doses of nicotine (50-100 µg/kg) did not significantly alter the spontaneous firing rates (**A**) or burst discharges (**B**) of such a neuronal population. (**C**, **D**): Following 50 and 100 µg/kg of nicotine, some neurons remained insensitive to progressive nicotine exposure, while others displayed firing rate/burst activations (increase of baseline activity by more than 20%) or inhibitions (decrease of baseline activity by more than 20%). Numerical values indicate the number of neurons in each group. (**E**): Three neuronal populations could therefore be distinguished, based upon their responses to nicotine: neurons insensitive to nicotine, neurons responding by a decrease in firing rate and those responding by an increase in firing rate. (**F**): In one neuron (5%, 1/20), a transient decrease of firing rate was observed following nicotine exposure. **P<0.01, ***P<0.001, Fisher exact test when compared with the proportion of respective responses in the saline group. ns: non-significant.

VI-3-C- Nicotine does not change the efficiency of the combination of both MPH and eticlopride

Nicotine pre-administration (100 µg/kg) did not reduce the efficiency of MPH (2 mg/kg) to induce firing rate decreases (Fig. 79A). Eticlopride (0.2 mg/kg) successfully recovered baseline firing rates in 6 out of 6 neurons (Fig. 79A,C). In this population of neurons, no significant change in burst activities was observed following either nicotine, MPH or eticlopride (Fig. 79B). Here, we conclude that the combination of nicotine with MPH, compared to MPH alone, neither enhances nor reduces the efficiency of MPH in inducing midbrain dopamine neuron electrical activity alterations. Moreover, the excitatory component of MPH/eticlopride still persists in animals pre-treated with nicotine.

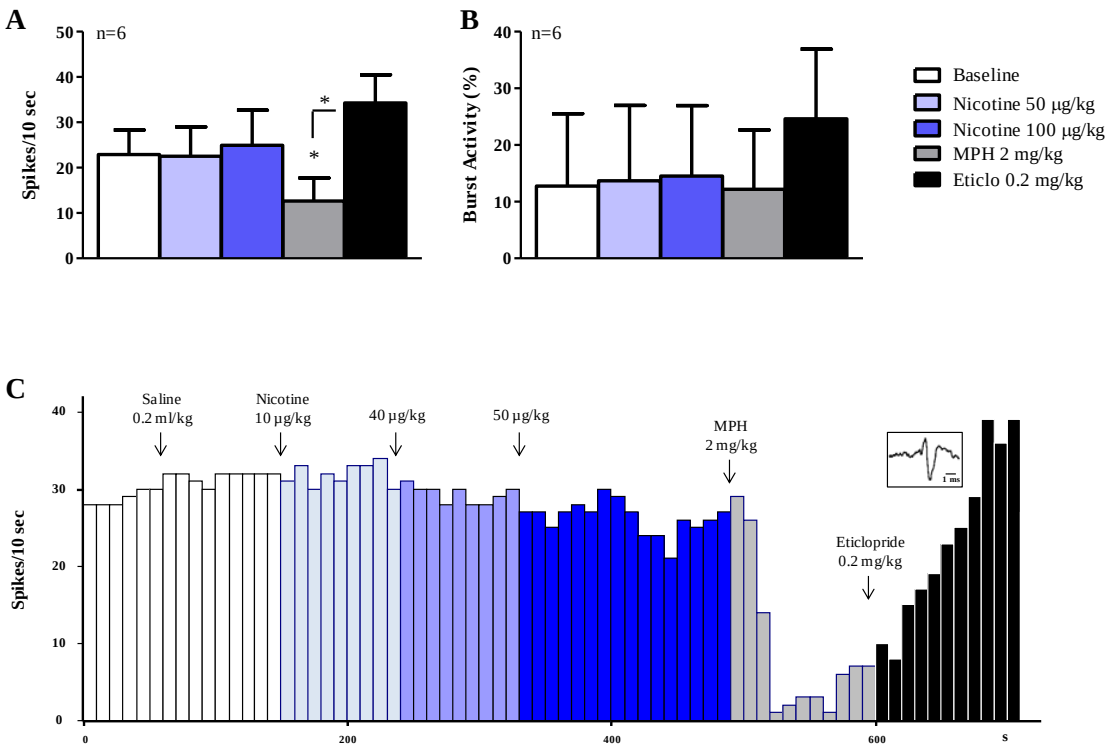


Figure 79: Pre-treatment with nicotine does not alter the excitatory component of methylphenidate following dopamine D_2 receptor antagonism.
Nicotine pre-exposure (100 µg/kg) did not significantly modify the excitatory component of MPH (2 mg/kg) on the firing rates (**A**) and burst activities (**B**) of VTA dopamine neurons following dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg). (**C**): Representative time course recording of such protocol. Boxed is shown the typical action potential waveform of this neuron. *P<0.05 *vs.* baseline or specified conditions, paired Student's t-test.

In these 6 neurons pre-treated with 100 µg/kg of nicotine, 4 neurons (67%) still presented higher firing rates following MPH/eticlopride (Fig. 80), providing evidence for the previously observed excitatory effect of both MPH and eticlopride on dopamine neurons, exactly as noted before in Chapter III. Nicotine pre-treatment did alter neuronal responses to MPH/eticlopride (Bonferroni after significant repeated measures two-way ANOVA).

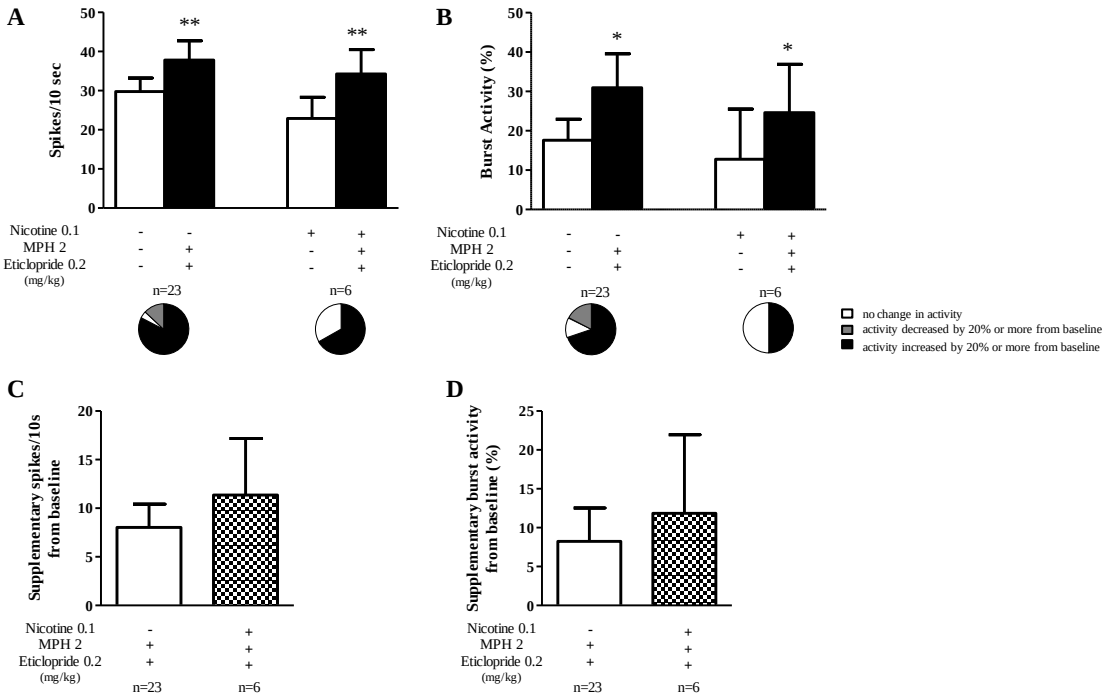


Figure 80: Pre-treatment with nicotine does not alter the methylphenidate-induced excitatory effects on midbrain dopamine neurons.
(**A**, **B**, **C**, **D**): Nicotine pre-exposure (100 µg/kg) did not significantly modify the excitatory component of MPH (2 mg/kg) on the firing rates ($F_{(1,27)}$=0.38, p>0.5) and burst activities ($F_{(1,27)}$=0.21, p>0.6) of VTA dopamine neurons following dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg). *P<0.05, **P<0.01 *vs.* baseline, Bonferroni after significant repeated measures two-way ANOVA.

VI-3-D- Adolescent exposure to MPH tends to reduce neuronal sensitivity to the combination of nicotine, MPH and eticlopride

In 9 adolescent animals that received 5 mg/kg/day of oral MPH for 15 days followed by a 28-day washout period, nicotine pre-administration did not change neuronal responses to subsequent intravenous challenges with MPH and eticlopride (Fig. 81-82). Adolescent MPH exposure does not alter neuronal responses to nicotine, MPH and eticlopride (firing: $F_{(1,21)}$=0.47, p>0.5, burst: $F_{(1,21)}$=1.61, p>0.2, Bonferroni

after significant repeated measures two-way ANOVA). Administration of nicotine (100 µg/kg) as a post-treatment (following MPH/eticlopride challenges) also failed to induce significant alterations in burst or firing activities (Fig. 83) in animals that received chronic adolescent MPH treatments (4 mg/kg/day, i.p. and for 15 days, followed by a 28-day washout period). However, we observed a small reduction in neuronal sensitivity to nicotine, MPH and eticlopride when animals were previously exposed to chronic MPH treatment during adolescence. We pooled all data and observed lower excitatory effects of MPH following eticlopride and/or nicotine pre/post-treatments (Fig. 84). Therefore, nicotine does not alter neuronal responses to intravenous challenges of either MPH or eticlopride, in either naive or MPH-treated animals during adolescence, but adolescent exposure to MPH may lead to decreased sensitivity of VTA midbrain neurons in adulthood to intravenous challenges of the combination of nicotine, MPH and D_2 receptor antagonism.

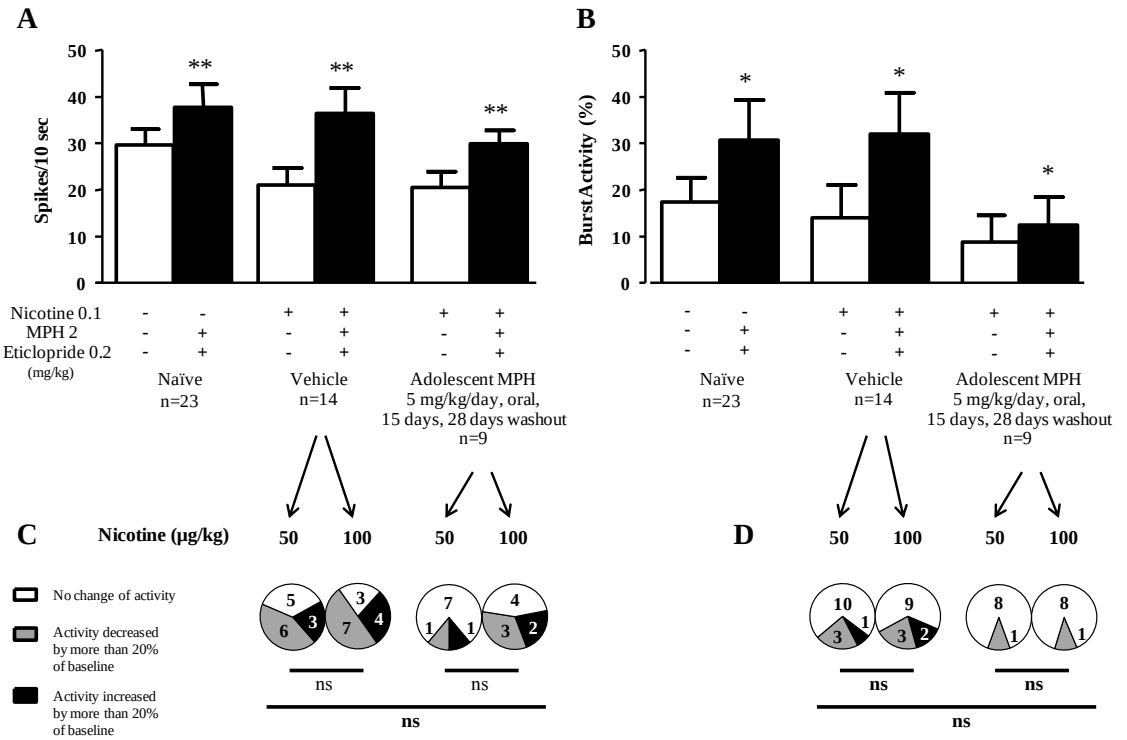


Figure 81: Absence of cross-sensitisation between adolescent exposure to methylphenidate and adult exposure to nicotine.

(**A**, **B**): Adolescent exposure to chronic MPH did not alter the responses of VTA dopaminergic neurons to nicotine administrations in adulthood. (**C**, **D**): Firing rates and burst discharges alterations following cumulative nicotine administration (50 and 100 µg/kg) in control and MPH-treated animals. As observed previously, some neurons remained insensitive to progressive nicotine exposure, while others displayed firing rate/burst activations or inhibitions. Based upon neuronal responses to nicotine, control and MPH-treated animals did not display significantly different responses. n values indicate the number of neurons in each group. Note that adolescent exposure to MPH tends to decrease the efficiency of nicotine to induce firing rate inhibitions (**C**). *P<0.05, **P<0.01 *vs.* baselines, Bonferroni after significant repeated measures two-way ANOVA. ns indicates a non-significant difference using the Fisher exact test.

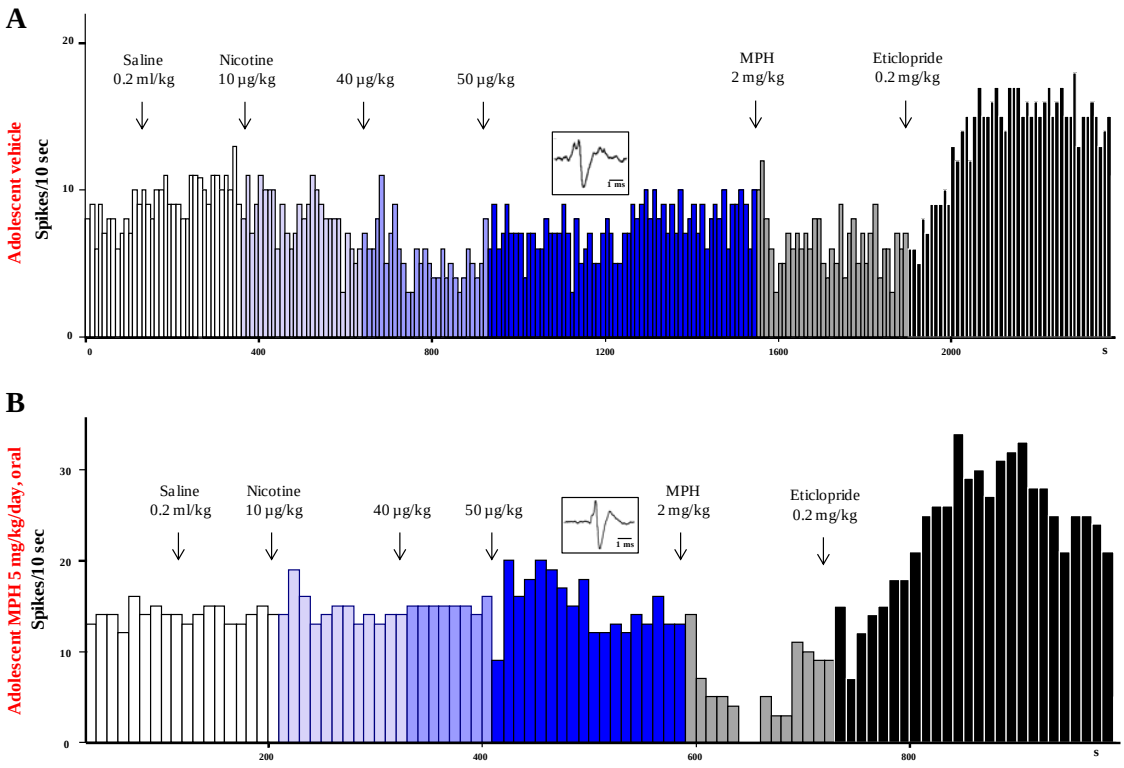


Figure 82: *In vivo* extracellular single cell recordings illustrating the absence of cross-sensitisation between adolescent exposure to methylphenidate and later exposure to nicotine.
Representative time course recording of such a protocol in control animals (**A**) and MPH-treated animals (**B**). No differences between the two groups were observed. Boxed are shown the typical action potential waveforms of these two midbrain dopamine neurons.

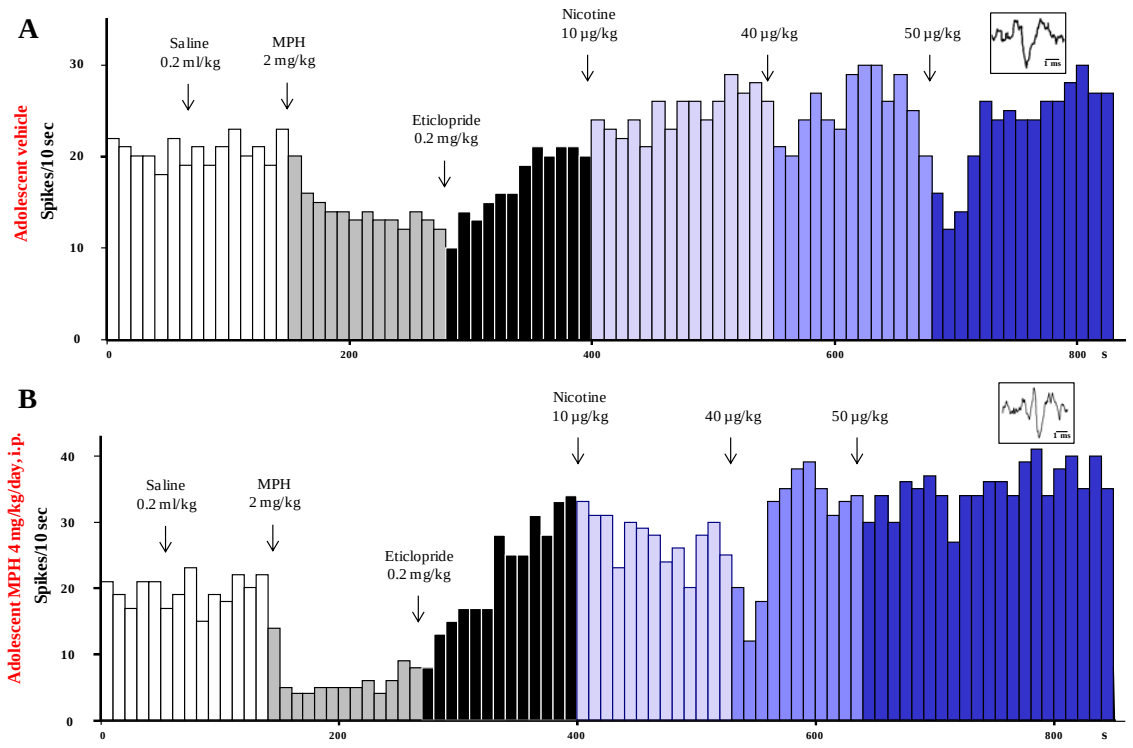


Figure 83: *In vivo* extracellular single cell recordings illustrating the absence of cross-sensitisation between adolescent exposure to methylphenidate and post-exposure to nicotine.

Representative time course recording of such a protocol in control (n=2, **A**) and MPH-treated animals (n=5, **B**). No significant differences between the two groups were observed. Boxed are shown the typical action potential waveforms of these two midbrain dopamine neurons.

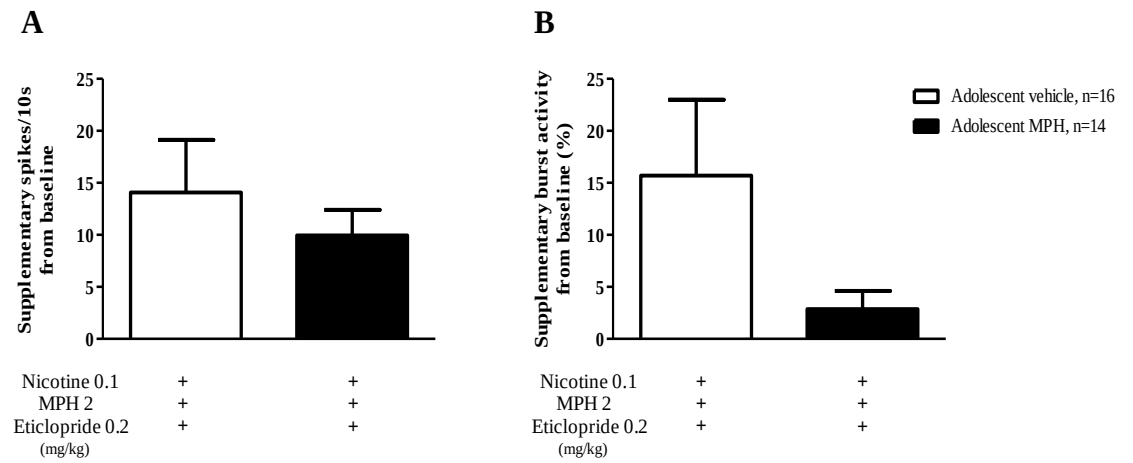


Figure 84: A possible tendency of adolescent methylphenidate exposure to induce long-lasting desensitisation to the excitatory component of MPH.

Adolescent exposure to MPH led to non-significantly decreased excitatory components of MPH in adulthood on the firing (**A**) and burst activities (**B**) of VTA dopaminergic neurons (respectively p=0.49 and p=0.12 *vs.* vehicles, unpaired Student's t-test).

VI-3-E- Nicotine normalises amphetamine-induced high rearing activities

Nicotine administration (100 µg/kg), in combination with D-amph (3 mg/kg), reduced D-amph-induced high rearing activities to similar levels observed with vehicle administration during the first 45 minutes, after which no further distinction could be seen between the two groups (D-amph *vs* D-amph combined with nicotine, Fig. 85). It is important to note that although the total number of rearing events of these animals was decreased (D-amph + nicotine), the time spent by each animal during such events was greatly enhanced, suggesting a tendency towards continuous rearing events. Nicotine alone does not induce any rearing activity, similarly to vehicle administration. These results suggest that nicotine may temporarily delay the effects of D-amphetamine on rearing behaviour.

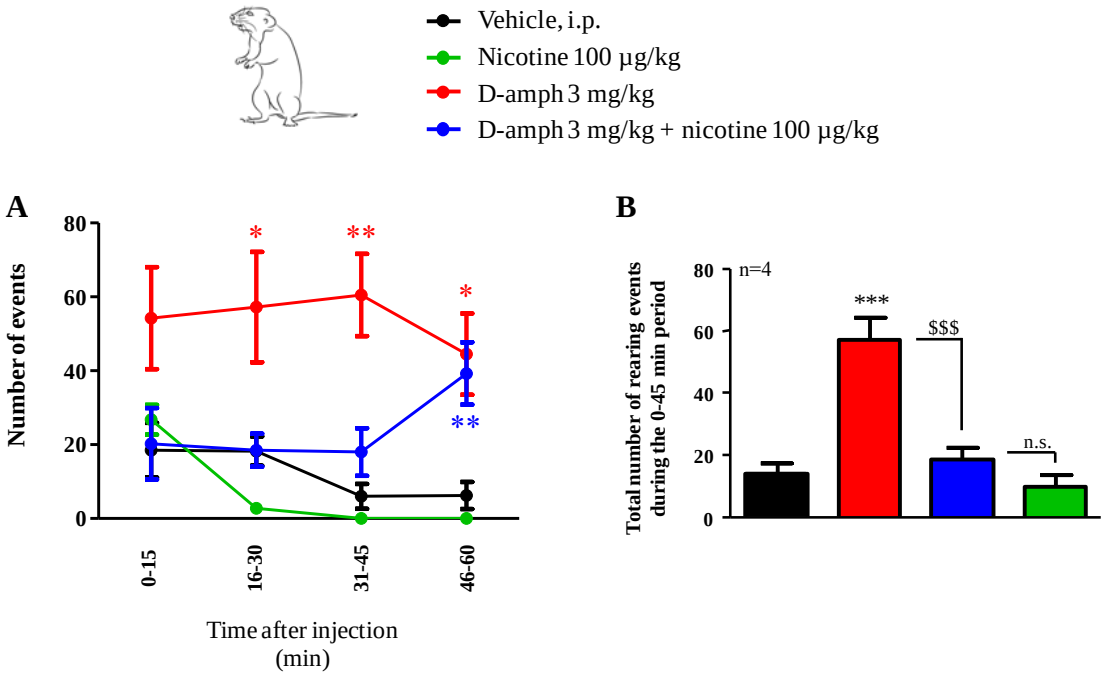


Figure 85: Nicotine normalises the rearing events induced by acute D-amphetamine exposure.
(**A**): Acute intraperitoneal exposure to 3 mg/kg of D-amphetamine increased rearing events in rats to levels significantly higher than 1 ml/kg of vehicle ($F_{(1,6)}$=12.97, p<0.01) or 100 µg/kg of nicotine ($F_{(1,6)}$=18.54, p<0.001). The adjunction of nicotine (100 µg/kg) to D-amphetamine (3 mg/kg) delayed the events induced by D-amph alone ($F_{(1,6)}$=5.91, p<0.05). *P<0.05, **P<0.01 *vs.* vehicle, Bonferroni tests after significant repeated measures two-way ANOVA. (**B**): Adjunction of nicotine to D-amphetamine significantly reduced the number of rearing events, to similar levels to those observed following vehicle exposure, but the time spent during each rearing event was greater (not scored). ***P<0.001 *vs.* vehicle, $$$P<0.001 *vs.* D-amph, Newman–Keuls after significant one-way ANOVA. n.s., non-significant. n=4 in each group.

VI-4- Discussion

First, the present study observed the lack of effects of progressive caffeine exposures on VTA dopamine neurons (Fig. 75). This suggests that adenosine receptor antagonism does not influence the electrical discharges of midbrain dopaminergic neurons. However, a study by Stoner in 1988 revealed that caffeine strongly decreases neuronal activities in such neurons (Stoner *et al.*, 1988), an effect that we only observed at its minimum. The authors also highlighted the importance of dopamine D_2 receptors in the mediation of such effects, as haloperidol administration (dopamine D_2 receptor antagonist) recovered the caffeine-induced firing rate depression, an effect that we never observed. Some studies suggested that caffeine (10 mg/kg, i.p.) triggers dopamine release in the nucleus accumbens *via* blockade of adenosine A_1, but not A_2 receptors, which can explain the amphetamine-like effects of caffeine in inducing firing rate decreases (Quarta *et al.*, 2004; Solinas *et al.*, 2002). Our results stand in contrast to these studies. Indeed, a cumulative dose of 10 mg/kg of caffeine failed to produce any significant change in the firing activities of VTA dopamine neurons, even with progressive 2 mg/kg increments (Fig. 75). Moreover, following caffeine administration, a subsequent dopamine D_2 receptor antagonism (eticlopride 0.2 mg/kg) failed to reveal any hidden excitatory or inhibitory component of caffeine in all of the VTA midbrain dopamine neurons recorded this way (6/6 neurons, Fig. 76C). Such differences can be explained by the different sensitivities to caffeine of the VTA and nucleus accumbens. Indeed, intermediate doses of caffeine (2.5-5 mg/kg) induce neuronal activation in the VTA, the thalamus and the amygdala, while higher doses (10 mg/kg) activate the shell of the nucleus accumbens, responsible for reward pathways (Nehlig *et al.*, 2000). Moreover, caffeine could stimulate meso-accumbens neurons, as suggested before (Solinas *et al.*, 2002). In the present study, we did not observe any increased firing rate activities in midbrain dopamine neurons after caffeine administration. In another study, caffeine was found to prevent cocaine-induced dopamine release in the nucleus accumbens (Malave *et al.*, 2014). Therefore, different mechanisms may take place in the VTA and nucleus accumbens, which could explain our results.

Acute caffeine pre-administration slightly enhanced the hidden excitatory effects of MPH on VTA dopaminergic neurons (Fig. 76-77). Indeed, we found that caffeine pre-treatment tends to increase the excitatory effect of MPH/eticlopride. Moreover, in 6

out of 6 neurons (100%), the firing and burst activities were significantly greater following the drug combination compared to neurons that did not receive caffeine pre-treatment (13/23, 57%). Cross-sensitisation between acute caffeine and MPH is therefore unlikely using our experimental protocol, but chronic caffeine consumption is known to induce such an effect (Boeck *et al.*, 2009; Holtzman, 1987). Co-localisation and functional interaction between adenosine A_{2A} receptors and dopamine D_2 receptors have been observed in rat striata (Ferre *et al.*, 1994; Ferre *et al.*, 2008; Fuxe *et al.*, 2005; Torvinen *et al.*, 2005). Dopamine D_1 and adenosine A_1 receptors also have antagonistic effects (Ferre *et al.*, 1996; Lillrank *et al.*, 1999; Rimondini *et al.*, 1998), and can sometimes co-localise (Gines *et al.*, 2000). If the excitatory effects of MPH on VTA dopamine neurons is triggered through dopamine D_1 receptors, then one could assume that interactions between dopamine D_1 receptors and adenosine A_2 receptors could mediate such greater excitatory inputs following caffeine exposure. In other words, adenosine A_2 receptor blockade by caffeine could promote dopamine D_1 receptor activation that may be responsible for the excitatory inputs of MPH onto VTA dopamine neurons. Interestingly, the adenosine A_1 receptor does not seem to be involved in the rewarding effects of caffeine (Sturgess *et al.*, 2010). Finally, we did not observe any *in vitro* cross-sensitisation of caffeine and MPH in inducing tritiated dopamine release from cortical and striatal slices (not shown).

Nicotine is known to increase firing and burst activities of ventral tegmental area dopamine neurons. In rodents, such effects start at 30-50 µg/kg of intravenous nicotine and at 0.5-1 mg/kg of intraperitoneal nicotine (Grenhoff *et al.*, 1986; Mameli-Engvall *et al.*, 2006; Marti *et al.*, 2011; Maskos *et al.*, 2005; Panin *et al.*, 2014). Taking the neuronal population as a whole (20 neurons), no firing rate or burst activities modifications were observed following nicotine intravenous administration in our study (100 µg/kg, Fig. 78A,B). To our knowledge, only very few studies have reported different possible modulations of dopamine neurons by nicotine, while the majority of studies seem to avoid mentioning neurons that were either insensitive to nicotine or that responded by a decrease of activity. However, two studies have carried out such an objective analysis and in remarkable details. Like in the recent studies by Eddine and Zhang, we found that while some neurons are activated by nicotine, some display clear firing rate reduction, suggesting different neuronal populations (Eddine *et al.*, 2015; Zhang *et al.*, 2012b) and confirming our current observations (Fig. 78C,D). Such

inhibitory effects of nicotine *via* dopamine release were found to be triggered by indirect activation of the inhibitory dopamine D_2 autoreceptor by nicotine because of the efficiency of dopamine D_2 antagonism pre-treatment to prevent such inhibitory effects, but not the excitatory effects (Eddine *et al.*, 2015). As a matter of fact, we have also observed nicotine-induced firing rate inhibition following D_2 antagonism in 5 out of 8 neurons (62%), which is not in agreement with Eddine's study. In addition, if nicotine had significantly inhibited the firing activity of dopaminergic neurons by stimulation of dopamine release, one would have expected to find a potentiation of the inhibitory effect of MPH, which was not found in our study. To explain this discrepancy, we suggest that partial activation of excitatory nicotinic receptors onto GABAergic interneurons can occur. In mice, the majority of neurons inhibited by nicotine were found to be located within the medial part of the VTA (Eddine *et al.*, 2015), suggesting a distinct segregation, according to their responses to nicotine, of the dopamine neurons within this area. Besides, the VTA is populated by very heterogeneous neurons, responding differently to addictive drugs, such as nicotine (Lammel *et al.*, 2014; Luscher *et al.*, 2011). One can argue that these 3 neuronal populations (firing rate activated or inhibited by nicotine, or even no change of firing rate) can be distinguished by different receptor compositions. On the other hand, according to our own unpublished data as well as many studies, the excitatory effects of nicotine on some VTA dopamine neurons is thought to be mediated by direct stimulation of nicotinic acetylcholine receptors, most probably *via* $\alpha_4\beta_2$ receptors, but not α_7 receptors, as the potent nicotine acetylcholine receptor antagonist mecamylamine prevented nicotine-induced firing rate activation, but not the alpha-7 receptor antagonist methyllycaconitine (Chen *et al.*, 2012; Chen *et al.*, 2003b; Grenhoff *et al.*, 1986; McGranahan *et al.*, 2011; Yin *et al.*, 2000). In humans, pharmacotherapies for smoking cessation specifically target nicotine acetylcholine receptors containing $\alpha_4\beta_2$ subunits (Picciotto *et al.*, 2013). One study has reported that VTA dopamine neurons that are activated by nicotine (50 µg/kg) display an initial firing rate decrease, an effect that is only temporary (Erhardt *et al.*, 2002). Interestingly, iontophoretic application of nicotine has only marginal effects on the firing of dopamine neurons, while it powerfully stimulates GABA neurons (unpublished observations from our laboratory).

Here, we also show that pre-administration of nicotine (100 µg/kg) does not induce sensitisation nor desensitisation to subsequent MPH and eticlopride administrations (Fig. 79-80). Moreover, nicotine pre-treatment does not alter the excitatory component of MPH following dopamine D_2 receptor antagonism (Fig. 80C,D). Besides, adolescent exposure to MPH fails to induce sensitisation to nicotine exposure in later life, although adolescent MPH exposure tends to decrease the excitatory effects of MPH following either pre- or post-administration of nicotine and eticlopride (Fig. 81-83). These results corroborate a previous observation in rodents where locomotor cross-sensitisation between chronic adolescent MPH and acute nicotine did not occur (Justo *et al.*, 2010). In our study, we lack results due to the diversity of the responses observed in midbrain dopaminergic neurons. Therefore, further experiments should be performed to acquire a reasonable number of neurons in each population. Nevertheless, interactions between MPH and nicotine have been demonstrated in humans (Bron *et al.*, 2013; Rush *et al.*, 2005; Vansickel *et al.*, 2011), suggesting great differences between species in the responses to both drugs. However, other clinical studies have reported no association (or beneficial effects) between nicotine and methylphenidate (Covey *et al.*, 2010; Gehricke *et al.*, 2011; Hammerness *et al.*, 2013).

When administered intraperitoneally, 100 µg/kg of nicotine did not induce any rearing activity (Fig. 85), as suggested before. Indeed, high doses of nicotine (ranging from 0.03 up to 1 mg/kg) are known to inhibit rearing behaviour in rodents (Chalabi-Yani *et al.*, 2015; Iwamoto, 1984; Ksir, 1994; Rodgers, 1979). When administered in combination with D-amphetamine (3 mg/kg), nicotine (100 µg/kg) strongly reduced D-amph-induced rearing activities (Fig. 85). This result differs slightly from a previous study, where both drugs had additive effects on rearing behaviour (Jutkiewicz *et al.*, 2008), although we observed in our study that animals spent more time during the rearing event itself. This puzzling result could be explained by a shift towards continuous stereotypy induced by the combination of D-amph and nicotine. Another study also observed similar results, where nicotine (0.1 mg/kg), given as a pre-treatment 2 hours before D-amph (0.32 mg/kg), increased D-amph-induced rearing activity as well as total locomotion (Kim *et al.*, 2011a). These differences, however, can be explained by the use of different doses as well as different genders and strains.

In this final chapter, we have shown that caffeine and nicotine do not appear to dramatically alter the responses of VTA dopamine neurons to MPH and eticlopride challenges. However, subtle adaptations may settle in midbrain dopamine neurons in response to MPH, caffeine or nicotine exposure, or a combination of the above. While caffeine is devoid of action on baseline spike generation and burst activity, nicotine induces either firing rate enhancement, firing rate reduction, or has no consequences on the firing rates. According to our electrophysiological study, chronic MPH exposure during adolescence does not seem to induce cross-sensitisation to either nicotine or MPH in adulthood. However, it can induce subtle but persistent neuronal adaptations. Our results showing decreased sensitivity to nicotine following adolescent exposure to methylphenidate is of great interest. These results may explain the greater nicotine intake in previously-treated ADHD patients (Krause *et al.*, 2006; Vansickel *et al.*, 2011). Finally, ADHD patients with a history of chronic nicotine use display lower striatal dopamine transporter densities compared to non-smoking ADHD patients (Krause *et al.*, 2003).

Chapter VII – Concluding remarks and perspectives.

The global aims of this study were to examine the pharmacology of ADHD drugs and the long-term consequences, if any, of chronic psychostimulant exposure. To some extent, we have partly achieved such objectives. The conduction of this study has enhanced our understanding of the underlying mechanisms of the three majorly prescribed ADHD drugs. One must remember that 2.7 million children are prescribed ADHD medication per year (Spiller *et al.*, 2013), with an average cost of 1500-2200$ (United States Dollars) per patient, depending on which type of medication is used (Marchetti *et al.*, 2001).

Results from Chapter I indicate that atomoxetine triggers a massive dopamine efflux in the striatum. Such strong neurotransmitter efflux induced by ATX can be explained by the ability of the drug to bind to other targets rather than the norepinephrine reuptake transporter itself, which contradicts its specificity, claimed by the manufacturing company. In addition, we also observed significant dopamine outflow starting at 10 µM, which is close to some regimens used in clinics. Clinical studies have shown the usefulness of ATX in ADHD treatment (Garnock-Jones *et al.*, 2009; Harfterkamp *et al.*, 2012; Svanborg *et al.*, 2009; Wietecha *et al.*, 2013a), but one can wonder if such therapeutic effects could be solely attributed to the blockade of the norepinephrine transporter. One could assume that the drug will bind to intracellular targets, which need to be formally identified. As a matter of fact, the addition of ATX to SSRIs (selective serotonin reuptake inhibitors) has been proven efficient in reducing depression levels in patients with or without ADHD (Berigan, 2004; Kratochvil *et al.*, 2005). Many antidepressants have been shown to interact with various potassium channels (Budni *et al.*, 2012; Donato *et al.*, 2015; Duncan *et al.*, 2007; Lee *et al.*, 2014; Terstappen *et al.*, 2003; Thomas *et al.*, 2002), particularly with $K_v1.1$ and K_v7 (Lodge *et al.*, 2008; Yeung *et al.*, 1999), which control cell excitability (Edwards *et al.*, 1995; Humphries *et al.*, 2015; Johnston *et al.*, 2010; Misonou, 2010; Pongs, 2008). In our experiments, atomoxetine promoted vesicular neurotransmitter efflux, which seem to

mimick vesicular release following enhancement of neuronal excitability. Although we did not observe that this effect was dependent on K_{IR} channels, which have been shown to interact with ATX (Kobayashi *et al.*, 2010; Scherer *et al.*, 2009), other potassium channels may be involved in this dopamine releasing process by ATX, a hypothesis that has, to our current knowledge, never yet been studied. One study has found that electrically-evoked striatal dopamine release is strongly modulated by Kv_1 potassium channels (voltage-gated), while both K_{IR} and K_{ATP} channels (respectively inwardly rectifying and ATP-gated potassium channels) have very limited impact on the dopamine releasing process (Martel *et al.*, 2011). These results can partially corroborate our own observations, where blockade of K_{IR} channels, using SCH23390, did not alter ATX-induced dopamine efflux. Besides, ATX induced specific dopamine outflows, while norepinephrine (partly shown) and serotonin outflows (not shown) were respectively mildly affected and not affected by ATX superfusions. We have also found that ATX increased the NMDA-induced electrical activities of the prefrontal cortex, a result that is contradictory to a previous *in vitro* study showing blockade of NMDA receptors by ATX (Ludolph *et al.*, 2010).

Results from Chapter II show that all three ADHD drugs (methylphenidate, atomoxetine and D-amphetamine) increased the NMDA-induced electrical activities of prefrontal cortex pyramidal neurons, which can be interpreted as a possible common mechanism between these three drugs. The present study confirms the role of glutamate neurotransmission in ADHD. Impairments of such neurotransmission have been suspected in the physiopathology of ADHD (Carlsson, 2000; Chang *et al.*, 2014). Here, we have shown that ADHD drugs improve glutamate-related neurotransmission, assessed using two very different techniques: electrophysiology and western blotting. At clinically relevant concentrations, these drugs may have a facilitating effect on NMDA neurotransmission, therefore enhancing the altered striatal connectivity observed in ADHD patients when attention deficits occur (Bush *et al.*, 2013; Carrey *et al.*, 2002). Enhancement of glutamatergic functions by ADHD drugs may not be without risk when used chronically. Indeed, high glutamate concentrations induce neurotoxicity (Choi *et al.*, 1987; Sandhu *et al.*, 2003) *via* calcium-dependent mechanisms (Choi, 1985), but such a neurotoxicity only occurs during very high intra-synaptic glutamate concentrations, as high glutamate concentrations cannot spread through the interstitial fluid (Obrenovitch *et al.*, 1997). Moreover, clinical studies have shown that recreational

use of amphetamine derivatives (MDMA, methamphetamine and amphetamine) can induce neurotoxicity, especially when poly-drug use occurs (Gouzoulis-Mayfrank *et al.*, 2009). There is a strong need to further investigate whether long-term enhancement of NMDA neurotransmission can be deleterious in chronically treated ADHD patients. In our study, such increases of NMDA neurotransmission by acute ADHD drug administration could be due to the indirect activation of dopamine D_1 receptors by ADHD drugs, as dopamine D_1 agonists have been shown to increase NMDA currents in isolated PFC pyramidal neurons (Chen *et al.*, 2004) and striatal neurons (Hallett *et al.*, 2006).

Interesting results from Chapter IV suggest that, following chronic methylphenidate, the increase of NMDA neurotransmission observed after acute ADHD drug exposure tends to disappear, as striatal NMDA neurotransmission was significantly decreased following adult treatment, assessed by both quantitative electrophysiology and western blotting.

Our study demonstrates that methylphenidate can exert a hidden excitatory effects on midbrain dopamine neurons when dopamine D_2 autoreceptor function is inhibited (Chapter III). Such an effect may help to maintain adequate dopaminergic neurotransmission, by counteracting a possible dampening of dopamine neuron activity induced by the indirect action of methylphenidate at dopamine D_2 inhibitory autoreceptors. Interestingly, this effect persists after chronic treatment with methylphenidate, at which point dopamine reuptake transporters became down-regulated. Indeed, we found that in the midbrain, chronic MPH exposure induces tolerance to subsequent MPH administration, which could be attributed to some degree of plasticity of dopamine reuptake transporters. Synaptic reorganisations of dopamine transporters have been observed in clinical studies (Wang *et al.*, 2013). How long this effect persists after the cessation of psychostimulant treatments, providing some therapeutic benefits, will be an interesting question to address. However, our results from Chapter V show no evidence of DAT down-regulation when the rats reach adulthood.

Further research is needed to understand the impact of increased NR_{2B} protein expression following acute psychostimulant exposure. Indeed, although the total NR_{2B} protein expression increased in some regions, regulations of the functions and the activities of NR_{2B} receptors also depend on receptor phosphorylation (Guo *et al.*, 2002). Amongst all possible NMDA receptor phosphorylations, NR_{2B} phosphorylation on tyrosine residues is crucial for receptor function and long term potentiation (Rosenblum *et al.*, 1996; Rostas *et al.*, 1996). Unfortunately, time constraints did not allow us to study the phosphorylation of NMDA receptors, although we have put forward the hypothesis that total NR_{2B} protein expression can vary proportionately with receptor phosphorylation, as observed with increased expression of total as well as phosphorylated glutamate receptors (respectively Glu_{A1} and Glu_{A1}-$pSer^{845}$) following chronic cocaine exposure (Nic Dhonnchadha *et al.*, 2013). It was also interesting to observe that this change in $NMDA_{2B}$ receptor expression occurred within a relatively short time interval. Whether this is associated with an increase in the total number of NR_{2B} receptors present in the neurons or only a sub-cellular redistribution of the receptor needs to be further assessed. It is uncertain if increases in the transcription of new receptors could lead to the transposition of these receptors at the membrane surface within less than one hour. Following the activation of intracellular signals indirectly triggered by psychostimulants, additional receptors may rather arise from some intracellular pools and could therefore insert relatively quickly into the synaptic membrane. However, in 2013, Urban *et al* witnessed decreased total, intracellular as well as surface NR_{2B} protein expressions following a single 1 mg/kg MPH administration (Urban *et al.*, 2013b), suggesting down-regulation of NR_{2B} through protein degradation. A similar protocol to the one used by Urban *et al* could be of great interest to determine precisely if NR_{2B} redistribution occurs following psychostimulant exposure, or if receptor transcription increases. Alternatively, these additional receptors may have been synthesised following activation of RNA transcription by some psychostimulant-dependent pathways. Such a hypothesis has been validated by some *in vitro* experiments. Indeed, immediate (occurring after only 10 minutes of exposure) increases of NR_{2B} expressions were observed after cocaine (1 µM) or SKF-81297 (D_1 agonist, 10 µM) applications, which was totally prevented by anisomycin (protein synthesis inhibitor) pre-treatments (Hu *et al.*, 2010; Schilstrom *et al.*, 2006).

In one batch of animals, daily intraperitoneal injections for 15 days resulted in decreased weight gain. Although chronic treatment with methylphenidate did not significantly affect body weight gain, clinical studies have shown that chronic MPH use can induce loss of appetite (Wolraich *et al.*, 2007). Attentive monitoring of possible side effects occurring during psychostimulant therapy is therefore required, especially for children and adolescents. As a matter of fact, a recent European guideline has been developed for the monitoring and the managing of such adverse effects (Graham *et al.*, 2011), which can be as extreme as sudden cardiac deaths or suicides (Alapati *et al.*, 2013; James *et al.*, 2004).

Our observation that adolescent exposure to methylphenidate induces an increased behavioural sensitivity to D-amphetamine challenge in adulthood is of great interest (Chapter V). Evidence of cross-sensitisation (hypersensitivity) with other psychostimulants has been observed, using clinically-relevant doses (Valvassori *et al.*, 2007; Yang *et al.*, 2011; Yang *et al.*, 2003). Here, we present evidence showing that adolescent exposure to methylphenidate can induce partial dopamine D_2 autoreceptor desensitisation in adulthood, suggesting that ADHD patients chronically treated with psychostimulants may be at higher risks of drug abuse compared to the background population. Moreover, this partial desensitisation of D_2 autoreceptors following adolescent exposure to MPH may induce the hypersensitivity observed after D-amphetamine challenge. These results are in total agreement with a previous study showing hypersensitivity to acute cocaine following adolescent methylphenidate exposure (Bello *et al.*, 2011). Furthermore, we have also shown that MPH exposure may be used to prevent the effects of D-amphetamine (Chapter I), which indicates a possible useful effect of psychostimulants (preventing overdose). Some clinical studies have suggested the usefulness of psychostimulants (mainly MPH) to treat chronic substance abuse in patients (Khantzian, 1983; Khantzian *et al.*, 1984; Miles *et al.*, 2013) as well as ADHD patients with substance use disorder (Imbert *et al.*, 2014; Levin *et al.*, 1998; Mariani *et al.*, 2007; Somoza *et al.*, 2004). However, this was contested by some other clinical studies, where ADHD pharmacotherapy using psychostimulants is not likely to induce substance abuse during later life (Biederman *et al.*, 1999; Lambert *et al.*, 1998; Wilens, 2004; Wilens *et al.*, 2003).

A correlation between daily sugar intake and ADHD has been hypothesised before (Johnson *et al.*, 2011; Kim *et al.*, 2011b; Millichap *et al.*, 2012; Woo *et al.*, 2014). According to these studies, increased sugar consumption, originating from fizzy drinks and snacks, is directly associated with higher risk of ADHD, suggesting either altered food-seeking behaviour or altered metabolisms in these patients, which may constitute risk factors for addiction. Sugars are known to induce dopaminergic activation, triggering strong responses in reward-related brain regions (Avena *et al.*, 2008; Hajnal *et al.*, 2004; Rada *et al.*, 2005; Stice *et al.*, 2013). Interestingly, addiction to sugar can be found in rats (Avena *et al.*, 2008; Hoebel *et al.*, 2009; Peters, 2011), but not in humans, although one study showed plausible arguments for sugar addiction in humans under continuous exposure to foods containing high sugar concentrations (Ahmed *et al.*, 2013). Finally, ADHD patients also display significant increases in their caffeine consumption (Dosh *et al.*, 2010; Pereira *et al.*, 2004). Nicotine also seems to be positively associated with ADHD in adolescents and adults (Lambert *et al.*, 1998; McClernon *et al.*, 2008; Milberger *et al.*, 1997; Molina *et al.*, 2003; Pomerleau *et al.*, 1995; Tercyak *et al.*, 2002; Whalen *et al.*, 2002). Moreover, some studies have shown that nicotine may target striatal dopamine transporters in ADHD patients (Krause *et al.*, 2006; Krause *et al.*, 2002).

Our behavioural study on sucrose preference did not find that a chronic treatment with methylphenidate during adolescence can cause a depressive phenotype in adulthood. Nevertheless, as previously mentioned, we found the electrical characteristics of the serotonin neurons to be persistently altered. As serotonin neurons have a very important role in the control of mood and impulsivity, as well as in the therapeutic effects of antidepressant treatments, the behavioural correlates and the molecular mechanisms involved in this change in bursting activity needs to be addressed in more detail. Clinical studies can now focus on the interaction between methylphenidate treatment and depression in ADHD patients. Therefore, if methylphenidate induces depressive traits, concerns can arise from its chronic use, as observed in a 7-year-old patient (Lakic, 2012). When MPH is used as a recreational drug, depressive symptoms can occur (Garland, 1998; Jaffe, 1991; Morton *et al.*, 2000).

We found no evidence for cross-sensitisation between nicotine and methylphenidate in our electrophysiological investigations. This lack of results may be the consequence of the very heterogeneous effects of nicotine on different populations of dopamine neurons. Indeed, acute nicotine (100 µg/kg) exerted an excitatory effect in 35% of all neurons (7/20), an inhibitory effect in 45% of all neurons (9/20) or even no effect in the remaining 20% of all neurons (4/20). The fact that the present study observed significant firing rate reductions following nicotine exposure is very interesting. Indeed, only one study observed and explained such effects (Eddine *et al.*, 2015), while a strong consensus seems to persist in the literature, stating that nicotine exposure only induces activation of midbrain dopamine neurons. However, the study by Eddine in 2015 explained such nicotine-induced firing rate reductions by a D_2-dependent mechanism, a result that is in opposition to what we observed within the present study (as we observed nicotine-induced firing rate reduction even following D_2 receptor antagonism, using eticlopride). The fact that current literature has not taken into account such firing rate decreases following nicotine exposure remains very surprising and needs to be further clarified. Hopefully, future work will allow definite conclusions to be drawn regarding psychostimulant exposure and smoking. To further assess any possible cross-sensitisation between activation of acetylcholine receptors (by nicotine) and dopamine neurotransmission, future animal studies might focus on the impact of chronic nicotine exposure on dopaminergic neurotransmission. Indeed, one study has reported that dopamine clearance is activated by nicotine (Middleton *et al.*, 2004). Another study has observed that nicotine reward is dependent upon $\alpha_4\beta_2$ receptors located on dopamine neurons, suggesting a dialogue between dopaminergic and nicotinic pathways (McGranahan *et al.*, 2011). Moreover, in the striatum, long-term depression depends upon interactions between dopamine and acetylcholine receptors (Partridge *et al.*, 2002). Dopamine and norepinephrine release from hippocampal slices also depends on nicotine acetylcholine receptors (Cao *et al.*, 2005). Finally, we have also observed that nicotine was able to interact with D-amphetamine-induced effects on locomotor activity. Nicotine may precipitate some of the motor effects of D-amphetamine by inducing enhanced stereotypical events, while reducing locomotor agitation (rearing events). Whether it can be partially explained by the aforementioned effects of nicotine on dopamine transporters would be of great interest in the future.

Publications

Research articles

From the present laboratory

Di Miceli M, Gronier B (2015). Psychostimulants and atomoxetine alter the electrophysiological activity of prefrontal cortex neurons, interaction with catecholamine and glutamate NMDA receptors. *Psychopharmacology (Berl)* **232**(12)**:** 2191-2205.

Gronier B, Savignac H, **Di Miceli M**, Idriss S, Tzortzis G, Anthony D, Burnet P (2016). The prebiotic, B-GOS, increases cortical NMDA receptors responses and cognitive flexibility in rats: the potential role of peptide YY. *PLoS One* (submitted).

From a previous laboratory

Michalak Z, Lebrun A, **Di Miceli M**, Rousset MC, Crespel A, Coubes P, *et al.* (2012). IgG leakage may contribute to neuronal dysfunction in drug-refractory epilepsies with blood-brain barrier disruption. *Journal of neuropathology and experimental neurology* **71**(9)**:** 826-838.

Research posters

Di Miceli M, Gronier B (2014a). Attention-Deficit Hyperactivity Disorder drugs: effects on in vitro dopamine release and in vivo electrophysiology. *Eur Neuropsychopharmacol* **24**(S1)**:** S24–S25.

Di Miceli M, Gronier B (2016). Effect of chronic methylphenidate administration on glutamate N-methyl-D-aspartate receptor function in prefrontal cortex and striatum. *Eur Neuropsychopharmacol*. (epub ahead of print).

Di Miceli M, Gronier B (2015). Excitatory glutamate component involved in the electrophysiological response of ventral tegmental area dopamine neurons to methylphenidate. *Eur Neuropsychopharmacol* **25**(S2)**:** S271–S272.

Di Miceli M, Gronier B (2014b). Voluntary chronic methylphenidate oral intake in rats, effect on ventral tegmental area dopamine neurons. *Eur Neuropsychopharmacol* **24**(S2)**:** S719–S720.

Gronier B, **Di Miceli M** (2013). Differential electrophysiological effects of atomoxetine and methylphenidate on prefrontal cortex and midbrain dopamine neurons. *Eur Neuropsychopharmacol* **23**(S2)**:** S598.

References

Aantaa R, Marjamaki A, Scheinin M (1995). Molecular pharmacology of alpha 2-adrenoceptor subtypes. *Annals of medicine* **27**(4)**:** 439-449.

Abenavoli L, Milic N, Rouabhia S, Addolorato G (2014). Pharmacotherapy of acute alcoholic hepatitis in clinical practice. *World journal of gastroenterology* **20**(9)**:** 2159-2167.

Achat-Mendes C, Anderson KL, Itzhak Y (2003). Methylphenidate and MDMA adolescent exposure in mice: long-lasting consequences on cocaine-induced reward and psychomotor stimulation in adulthood. *Neuropharmacology* **45**(1)**:** 106-115.

Ackerman JM, Johansen PA, Clark D, White FJ (1993). Electrophysiological effects of putative autoreceptor-selective dopamine agonists on A10 dopamine neurons. *J Pharmacol Exp Ther* **265**(2)**:** 963-970.

Adams J, Crosbie J, Wigg K, Ickowicz A, Pathare T, Roberts W, *et al.* (2004). Glutamate receptor, ionotropic, N-methyl D-aspartate 2A (GRIN2A) gene as a positional candidate for attention-deficit/hyperactivity disorder in the 16p13 region. *Mol Psychiatry* **9**(5)**:** 494-499.

Addolorato G, Ancona C, Capristo E, Gasbarrini G (2003). Metadoxine in the treatment of acute and chronic alcoholism: a review. *International journal of immunopathology and pharmacology* **16**(3)**:** 207-214.

Adesman AR (2001). The Diagnosis and Management of Attention-Deficit/Hyperactivity Disorder in Pediatric Patients. *Primary care companion to the Journal of clinical psychiatry* **3**(2)**:** 66-77.

Adinoff B (2004). Neurobiologic processes in drug reward and addiction. *Harvard review of psychiatry* **12**(6)**:** 305-320.

Adler A, Katabi S, Finkes I, Prut Y, Bergman H (2013). Different correlation patterns of cholinergic and GABAergic interneurons with striatal projection neurons. *Frontiers in systems neuroscience* **7:** 47.

Adler EM, Augustine GJ, Duffy SN, Charlton MP (1991). Alien intracellular calcium chelators attenuate neurotransmitter release at the squid giant synapse. *J Neurosci* **11**(6)**:** 1496-1507.

Adriani W, Caprioli A, Granstrem O, Carli M, Laviola G (2003a). The spontaneously hypertensive-rat as an animal model of ADHD: evidence for impulsive and non-impulsive subpopulations. *Neurosci Biobehav Rev* **27**(7)**:** 639-651.

Adriani W, Laviola G (2003b). Elevated levels of impulsivity and reduced place conditioning with d-amphetamine: two behavioral features of adolescence in mice. *Behavioral neuroscience* **117**(4)**:** 695-703.

Adriani W, Leo D, Guarino M, Natoli A, Di Consiglio E, De Angelis G, *et al.* (2006). Short-term effects of adolescent methylphenidate exposure on brain striatal gene expression and sexual/endocrine parameters in male rats. *Ann N Y Acad Sci* **1074:** 52-73.

Ago Y, Umehara M, Higashino K, Hasebe S, Fujita K, Takuma K, *et al.* (2014). Atomoxetine-Induced Increases in Monoamine Release in the Prefrontal Cortex are Similar in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats. *Neurochem Res*.

Aguilar-Rivera MI, Casanova JP, Gatica RI, Quirk GJ, Fuentealba JA (2015). Amphetamine sensitization is accompanied by an increase in prelimbic cortex activity. *Neuroscience* **288:** 1-9.

Ahmann PA, Waltonen SJ, Olson KA, Theye FW, Van Erem AJ, LaPlant RJ (1993). Placebo-controlled evaluation of Ritalin side effects. *Pediatrics* **91**(6)**:** 1101-1106.

Ahmed SH, Guillem K, Vandaele Y (2013). Sugar addiction: pushing the drug-sugar analogy to the limit. *Current opinion in clinical nutrition and metabolic care* **16**(4)**:** 434-439.

Ahn S, Phillips AG (2013). Daily monitoring of dopamine efflux reveals a short-lasting occlusion of the dopamine agonist properties of d-amphetamine by dopamine transporter blockers GBR 12909 and methylphenidate. *ACS chemical neuroscience* **4**(5)**:** 817-824.

Ahn S, Phillips AG (2002). Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. *J Neurosci* **22**(24)**:** 10958-10965.

Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Verdi AA, *et al.* (2007). Induction of diabetes by Streptozotocin in rats. *Indian journal of clinical biochemistry : IJCB* **22**(2)**:** 60-64.

Akutagava-Martins GC, Salatino-Oliveira A, Genro JP, Contini V, Polanczyk G, Zeni C, *et al.* (2014). Glutamatergic copy number variants and their role in attention-deficit/hyperactivity disorder. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **165B**(6)**:** 502-509.

al-Khatib IM, Dokmeci I, Fujiwara M (1995). Differential role of nucleus accumbens and caudate-putamen in mediating the effect of nomifensine and methamphetamine on ambulation and rearing of rats in the open-field test. *Japanese journal of pharmacology* **67**(1)**:** 69-77.

Alam N, Najam R, Khan SS (2015). Attenuation of methylphenidate-induced tolerance on cognition by buspirone co-administration. *Pakistan journal of pharmaceutical sciences* **28**(5)**:** 1601-1605.

Alapati S, Strobel N, Hashmi S, Bricker JT, Gupta-Malhotra M (2013). Sudden unexplained cardiac arrest in apparently healthy children: a single-center experience. *Pediatric cardiology* **34**(3)**:** 639-645.

Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, *et al.* (2012). Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. *Neuropharmacology* **62**(2)**:** 1152-1161.

Allers KA, Sharp T (2003). Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience* **122**(1)**:** 193-204.

Almodovar-Fabregas LJ, Segarra O, Colon N, Dones JG, Mercado M, Mejias-Aponte CA, *et al.* (2002). Effects of cocaine administration on VTA cell activity in response to prefrontal cortex stimulation. *Ann N Y Acad Sci* **965:** 157-171.

Alsop B (2007). Problems with spontaneously hypertensive rats (SHR) as a model of attention-deficit/hyperactivity disorder (AD/HD). *Journal of neuroscience methods* **162**(1-2)**:** 42-48.

Alvarez VA, Sabatini BL (2007). Anatomical and physiological plasticity of dendritic spines. *Annual review of neuroscience* **30:** 79-97.

Alves R, Barbosa de Carvalho JG, Benedito MA (2005). High and low rearing subgroups of rats selected in the open field differ in the activity of K+-stimulated p-nitrophenylphosphatase in the hippocampus. *Brain research* **1058**(1-2)**:** 178-182.

American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders. (V).

Amini B, Yang PB, Swann AC, Dafny N (2004). Differential locomotor responses in male rats from three strains to acute methylphenidate. *The International journal of neuroscience* **114**(9)**:** 1063-1084.

Amitai N, Markou A (2010). Disruption of performance in the five-choice serial reaction time task induced by administration of N-methyl-D-aspartate receptor antagonists: relevance to cognitive dysfunction in schizophrenia. *Biol Psychiatry* **68**(1)**:** 5-16.

Andersen SL (2003). Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev* **27**(1-2)**:** 3-18.

Anderson AL, Reid MS, Li SH, Holmes T, Shemanski L, Slee A, *et al.* (2009). Modafinil for the treatment of cocaine dependence. *Drug Alcohol Depend* **104**(1-2)**:** 133-139.

Andrade R, Aghajanian GK (1985). Opiate- and alpha 2-adrenoceptor-induced hyperpolarizations of locus ceruleus neurons in brain slices: reversal by cyclic adenosine 3':5'-monophosphate analogues. *J Neurosci* **5**(9)**:** 2359-2364.

Andrews GD, Lavin A (2006). Methylphenidate increases cortical excitability via activation of alpha-2 noradrenergic receptors. *Neuropsychopharmacology* **31**(3)**:** 594-601.

Anker JJ, Gliddon LA, Carroll ME (2008). Impulsivity on a Go/No-go task for intravenous cocaine or food in male and female rats selectively bred for high and low saccharin intake. *Behavioural pharmacology* **19**(5-6)**:** 615-629.

Antoniou K, Kafetzopoulos E, Papadopoulou-Daifoti Z, Hyphantis T, Marselos M (1998). D-amphetamine, cocaine and caffeine: a comparative study of acute effects on locomotor activity and behavioural patterns in rats. *Neurosci Biobehav Rev* **23**(2)**:** 189-196.

Arai A, Tomiyama M, Kannari K, Kimura T, Suzuki C, Watanabe M, *et al.* (2008). Reuptake of L-DOPA-derived extracellular DA in the striatum of a rodent model of Parkinson's disease via norepinephrine transporter. *Synapse* **62**(8)**:** 632-635.

Arnold HM, Fadel J, Sarter M, Bruno JP (2001). Amphetamine-stimulated cortical acetylcholine release: role of the basal forebrain. *Brain research* **894**(1)**:** 74-87.

Arns M, de Ridder S, Strehl U, Breteler M, Coenen A (2009). Efficacy of neurofeedback treatment in ADHD: the effects on inattention, impulsivity and hyperactivity: a meta-analysis. *Clin EEG Neurosci* **40**(3)**:** 180-189.

Arnsten AF, Dudley AG (2005). Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct* **1**(1)**:** 2.

Asser A, Taba P (2015). Psychostimulants and movement disorders. *Frontiers in neurology* **6:** 75.

Atcha Z, Rourke C, Neo AH, Goh CW, Lim JS, Aw CC, *et al.* (2010). Alternative method of oral dosing for rats. *Journal of the American Association for Laboratory Animal Science : JAALAS* **49**(3)**:** 335-343.

Avena NM, Rada P, Hoebel BG (2008). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci Biobehav Rev* **32**(1)**:** 20-39.

Ayache DC, Junior RF (2001). Methylphenidate in a patient with depression and respiratory insufficiency. *International journal of psychiatry in medicine* **31**(4)**:** 443-449.

Ayata C, Shimizu-Sasamata M, Lo EH, Noebels JL, Moskowitz MA (2000). Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the alpha1A subunit of P/Q type calcium channels. *Neuroscience* **95**(3)**:** 639-645.

Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG (2002). Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behavior genetics* **32**(6)**:** 435-443.

Backstrom P, Etelalahti TJ, Hyytia P (2011). Attenuation of reinforcing and psychomotor stimulant effects of amphetamine by aripiprazole. *Addiction biology* **16**(1)**:** 55-63.

Bado P, Madeira C, Vargas-Lopes C, Moulin TC, Wasilewska-Sampaio AP, Maretti L, *et al.* (2011). Effects of low-dose D-serine on recognition and working memory in mice. *Psychopharmacology (Berl)* **218**(3)**:** 461-470.

Baik JH (2013). Dopamine signaling in food addiction: role of dopamine D2 receptors. *BMB reports* **46**(11)**:** 519-526.

Balazs J, Dallos G, Kereszteny A, Czobor P, Gadoros J (2011). Methylphenidate treatment and dyskinesia in children with attention-deficit/hyperactivity disorder. *Journal of child and adolescent psychopharmacology* **21**(2)**:** 133-138.

Balcioglu A, Ren JQ, McCarthy D, Spencer TJ, Biederman J, Bhide PG (2009). Plasma and brain concentrations of oral therapeutic doses of methylphenidate and their impact on brain monoamine content in mice. *Neuropharmacology* **57**(7-8)**:** 687-693.

Banaschewski T, Becker K, Scherag S, Franke B, Coghill D (2010). Molecular genetics of attention-deficit/hyperactivity disorder: an overview. *Eur Child Adolesc Psychiatry* **19**(3)**:** 237-257.

Barkley RA, Fischer M, Smallish L, Fletcher K (2003). Does the treatment of attention-deficit/hyperactivity disorder with stimulants contribute to drug use/abuse? A 13-year prospective study. *Pediatrics* **111**(1)**:** 97-109.

Bastiaens L (2007). Effectiveness and tolerability of atomoxetine in a real-world ADHD population: nonrandomized comparison with stimulants. *Psychiatry* **4**(12)**:** 44-48.

Bean AJ, Roth RH (1991). Extracellular dopamine and neurotensin in rat prefrontal cortex in vivo: effects of median forebrain bundle stimulation frequency, stimulation pattern, and dopamine autoreceptors. *J Neurosci* **11**(9)**:** 2694-2702.

Beani L, Bianchi C, Siniscalchi A, Sivilotti L, Tanganelli S, Veratti E (1984). Different approaches to study acetylcholine release: endogenous ACh versus tritium efflux. *Naunyn-Schmiedeberg's archives of pharmacology* **328**(2)**:** 119-126.

Beckstead RM, Domesick VB, Nauta WJ (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain research* **175**(2)**:** 191-217.

Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, *et al.* (2008). BDNF is essential to promote persistence of long-term memory storage. *Proc Natl Acad Sci U S A* **105**(7)**:** 2711-2716.

Bell RD, Alexander GM, Schwartzman RJ, Yu J (1982). The methylphenidate-induced stereotypy in the awake rat: local cerebral metabolism. *Neurology* **32**(4)**:** 377-381.

Belle AM, Owesson-White C, Herr NR, Carelli RM, Wightman RM (2013). Controlled iontophoresis coupled with fast-scan cyclic voltammetry/electrophysiology in awake, freely moving animals. *ACS chemical neuroscience* **4**(5)**:** 761-771.

Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ, *et al.* (2011). Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nature neuroscience* **14**(8)**:** 1033-1038.

Bellot A, Guivernau B, Tajes M, Bosch-Morato M, Valls-Comamala V, Munoz FJ (2014). The structure and function of actin cytoskeleton in mature glutamatergic dendritic spines. *Brain research* **1573:** 1-16.

Bennett S, Gronier B (2007). Effects of antipsychotic treatments and D-serine supplementation on the electrophysiological activation of midbrain dopamine neurons induced by the noncompetitive NMDA antagonist MK 801. *Synapse* **61**(8)**:** 679-688.

Benraiss A, Chmielnicki E, Lerner K, Roh D, Goldman SA (2001). Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J Neurosci* **21**(17)**:** 6718-6731.

Bergeron R, Meyer TM, Coyle JT, Greene RW (1998). Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proc Natl Acad Sci U S A* **95**(26)**:** 15730-15734.

Bergstrom BP, Garris PA (2003). "Passive stabilization" of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. *Journal of neurochemistry* **87**(5)**:** 1224-1236.

Bergstrom KA, Tupala E, Tiihonen J (2001). Dopamine transporter in vitro binding and in vivo imaging in the brain. *Pharmacology & toxicology* **88**(6)**:** 287-293.

Berigan TR (2004). Atomoxetine Used Adjunctively With Selective Serotonin Reuptake Inhibitors to Treat Depression. *Primary care companion to the Journal of clinical psychiatry* **6**(2)**:** 93-94.

Berman S, O'Neill J, Fears S, Bartzokis G, London ED (2008). Abuse of amphetamines and structural abnormalities in the brain. *Ann N Y Acad Sci* **1141:** 195-220.

Berman SM, Kuczenski R, McCracken JT, London ED (2009a). Potential adverse effects of amphetamine treatment on brain and behavior: a review. *Mol Psychiatry* **14**(2)**:** 123-142.

Berman SM, Kuczenski R, McCracken JT, London ED (2009b). Potential adverse effects of amphetamine treatment on brain and behavior: a review. *Mol Psychiatry* **14**(2)**:** 123-142.

Bernacer J, Prensa L, Gimenez-Amaya JM (2007). Cholinergic interneurons are differentially distributed in the human striatum. *PloS one* **2**(11)**:** e1174.

Berquin PC, Giedd JN, Jacobsen LK, Hamburger SD, Krain AL, Rapoport JL, *et al.* (1998). Cerebellum in attention-deficit hyperactivity disorder: a morphometric MRI study. *Neurology* **50**(4)**:** 1087-1093.

Berridge CW (2006). Neural substrates of psychostimulant-induced arousal. *Neuropsychopharmacology* **31**(11)**:** 2332-2340.

Berridge CW, Devilbiss DM (2011). Psychostimulants as cognitive enhancers: the prefrontal cortex, catecholamines, and attention-deficit/hyperactivity disorder. *Biol Psychiatry* **69**(12)**:** e101-111.

Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B, *et al.* (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol Psychiatry* **60**(10)**:** 1111-1120.

Berridge CW, Stratford TL, Foote SL, Kelley AE (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse* **27**(3)**:** 230-241.

Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Herve D, Valjent E, *et al.* (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J Neurosci* **28**(22)**:** 5671-5685.

Beyer C, Staunton C, Moodley K (2014). The implications of methylphenidate use by healthy medical students and doctors in South Africa. *BMC medical ethics* **15:** 20.

Bezdjian S, Baker LA, Lozano DI, Raine A (2009). Assessing inattention and impulsivity in children during the Go/NoGo task. *The British journal of developmental psychology* **27**(Pt 2)**:** 365-383.

Biederman J, Faraone SV, Monuteaux MC, Plunkett EA, Gifford J, Spencer T (2003). Growth deficits and attention-deficit/hyperactivity disorder revisited: impact of gender, development, and treatment. *Pediatrics* **111**(5 Pt 1)**:** 1010-1016.

Biederman J, Spencer TJ, Monuteaux MC, Faraone SV (2010). A naturalistic 10-year prospective study of height and weight in children with attention-deficit hyperactivity disorder grown up: sex and treatment effects. *The Journal of pediatrics* **157**(4)**:** 635-640, 640 e631.

Biederman J, Wilens T, Mick E, Spencer T, Faraone SV (1999). Pharmacotherapy of attention-deficit/hyperactivity disorder reduces risk for substance use disorder. *Pediatrics* **104**(2)**:** e20.

Boeck CR, Marques VB, Valvassori SS, Constantino LC, Rosa DV, Lima FF, *et al.* (2009). Early long-term exposure with caffeine induces cross-sensitization to methylphenidate with involvement of DARPP-32 in adulthood of rats. *Neurochem Int* **55**(5)**:** 318-322.

Bokor G, Anderson PD (2014). Attention-Deficit/Hyperactivity Disorder. *Journal of pharmacy practice* **27**(4)**:** 336-349.

Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ (2003). Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry* **54**(12)**:** 1317-1329.

Bollmann S, Ghisleni C, Poil SS, Martin E, Ball J, Eich-Hochli D, *et al.* (2015). Developmental changes in gamma-aminobutyric acid levels in attention-deficit/hyperactivity disorder. *Translational psychiatry* **5:** e589.

Bonasera SJ, Schenk AK, Luxenberg EJ, Tecott LH (2008). A novel method for automatic quantification of psychostimulant-evoked route-tracing stereotypy: application to Mus musculus. *Psychopharmacology (Berl)* **196**(4)**:** 591-602.

Bono G, Sinforiani E, Merlo P, Belloni G, Soldati M, Gelso E (1991). Alcoholic abstinence syndrome: short-term treatment with metadoxine. *International journal of clinical pharmacology research* **11**(1)**:** 35-40.

Boyko M, Kutz R, Grinshpun J, Zvenigorodsky V, Gruenbaum SE, Gruenbaum BF, *et al.* (2015). Establishment of an animal model of depression contagion. *Behavioural brain research* **281:** 358-363.

Brandon CL, Marinelli M, Baker LK, White FJ (2001). Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology* **25**(5)**:** 651-661.

Brandon CL, Marinelli M, White FJ (2003). Adolescent exposure to methylphenidate alters the activity of rat midbrain dopamine neurons. *Biol Psychiatry* **54**(12)**:** 1338-1344.

Braz BY, Galinanes GL, Taravini IR, Belforte JE, Murer MG (2015). Altered Corticostriatal Connectivity and Exploration/Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction. *Neuropsychopharmacology* **40**(11)**:** 2576-2587.

Briones A, Gagno S, Martisova E, Dobarro M, Aisa B, Solas M, *et al.* (2012). Stress-induced anhedonia is associated with an increase in Alzheimer's disease-related markers. *British journal of pharmacology* **165**(4)**:** 897-907.

Bristow LJ, Thorn L, Tricklebank MD, Hutson PH (1994). Competitive NMDA receptor antagonists attenuate the behavioural and neurochemical effects of amphetamine in mice. *Eur J Pharmacol* **264**(3)**:** 353-359.

Brittain MK, Brustovetsky T, Brittain JM, Khanna R, Cummins TR, Brustovetsky N (2012). Ifenprodil, a NR2B-selective antagonist of NMDA receptor, inhibits reverse Na+/Ca2+ exchanger in neurons. *Neuropharmacology* **63**(6)**:** 974-982.

Bro SP, Kjaersgaard MI, Parner ET, Sorensen MJ, Olsen J, Bech BH, *et al.* (2015). Adverse pregnancy outcomes after exposure to methylphenidate or atomoxetine during pregnancy. *Clinical epidemiology* **7:** 139-147.

Broderick P, Benjamin AB (2004). Caffeine and psychiatric symptoms: a review. *J Okla State Med Assoc* **97**(12)**:** 538-542.

Bron TI, Bijlenga D, Kasander MV, Spuijbroek AT, Beekman AT, Kooij JJ (2013). Long-term relationship between methylphenidate and tobacco consumption and nicotine craving in adults with ADHD in a prospective cohort study. *Eur Neuropsychopharmacol* **23**(6)**:** 542-554.

Brookshire BR, Jones SR (2012). Chronic methylphenidate administration in mice produces depressive-like behaviors and altered responses to fluoxetine. *Synapse* **66**(9)**:** 844-847.

Brown AM (1999). A methodology for simulating biological systems using Microsoft Excel. *Computer methods and programs in biomedicine* **58**(2)**:** 181-190.

Brown AM (2000). Simulation of axonal excitability using a Spreadsheet template created in Microsoft Excel. *Computer methods and programs in biomedicine* **63**(1)**:** 47-54.

Brown GR, Nemes C (2008). The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? *Behavioural processes* **78**(3)**:** 442-448.

Brown JM, Hanson GR, Fleckenstein AE (2000). Methamphetamine rapidly decreases vesicular dopamine uptake. *Journal of neurochemistry* **74**(5)**:** 2221-2223.

Brunk I, Sanchis-Segura C, Blex C, Perreau-Lenz S, Bilbao A, Spanagel R, *et al.* (2010). Amphetamine regulates NR2B expression in Go2alpha knockout mice and thereby sustains behavioral sensitization. *Journal of neurochemistry* **115**(1)**:** 234-246.

Bucci MP, Seassau M, Larger S, Bui-Quoc E, Gerard CL (2014). Effect of visual attention on postural control in children with attention-deficit/hyperactivity disorder. *Research in developmental disabilities* **35**(6)**:** 1292-1300.

Buderath P, Gartner K, Frings M, Christiansen H, Schoch B, Konczak J, *et al.* (2009). Postural and gait performance in children with attention deficit/hyperactivity disorder. *Gait & posture* **29**(2)**:** 249-254.

Budni J, Freitas AE, Binfare RW, Rodrigues AL (2012). Role of potassium channels in the antidepressant-like effect of folic acid in the forced swimming test in mice. *Pharmacol Biochem Behav* **101**(1)**:** 148-154.

Bunney BS, Walters JR, Roth RH, Aghajanian GK (1973). Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. *J Pharmacol Exp Ther* **185**(3)**:** 560-571.

Bunney EB, Appel SB, Brodie MS (2001). Electrophysiological effects of cocaethylene, cocaine, and ethanol on dopaminergic neurons of the ventral tegmental area. *J Pharmacol Exp Ther* **297**(2)**:** 696-703.

Buoli M, Serati M, Cahn W (2016). Alternative pharmacological strategies for adult ADHD treatment: a systematic review. *Expert review of neurotherapeutics* **16**(2)**:** 131-144.

Bush G, Holmes J, Shin LM, Surman C, Makris N, Mick E, *et al.* (2013). Atomoxetine increases fronto-parietal functional MRI activation in attention-deficit/hyperactivity disorder: a pilot study. *Psychiatry Res* **211**(1)**:** 88-91.

Bushe CJ, Savill NC (2014). Systematic review of atomoxetine data in childhood and adolescent attention-deficit hyperactivity disorder 2009-2011: focus on clinical efficacy and safety. *J Psychopharmacol* **28**(3)**:** 204-211.

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, *et al.* (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology* **27**(5)**:** 699-711.

Caballeria J, Pares A, Bru C, Mercader J, Garcia Plaza A, Caballeria L, *et al.* (1998). Metadoxine accelerates fatty liver recovery in alcoholic patients: results of a randomized double-blind, placebo-control trial. Spanish Group for the Study of Alcoholic Fatty Liver. *Journal of hepatology* **28**(1)**:** 54-60.

Caballero M, Nunez F, Ahern S, Cuffi ML, Carbonell L, Sanchez S, *et al.* (2011). Caffeine improves attention deficit in neonatal 6-OHDA lesioned rats, an animal model of attention deficit hyperactivity disorder (ADHD). *Neurosci Lett* **494**(1)**:** 44-48.

Cachope R, Mateo Y, Mathur BN, Irving J, Wang HL, Morales M, *et al.* (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell reports* **2**(1)**:** 33-41.

Cador M, Bjijou Y, Cailhol S, Stinus L (1999). D-amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. *Neuroscience* **94**(3)**:** 705-721.

Calipari ES, Ferris MJ, Salahpour A, Caron MG, Jones SR (2013). Methylphenidate amplifies the potency and reinforcing effects of amphetamines by increasing dopamine transporter expression. *Nature communications* **4:** 2720.

Calipari ES, Sun H, Eldeeb K, Luessen DJ, Feng X, Howlett AC, *et al.* (2014). Amphetamine Self-Administration Attenuates Dopamine D2 Autoreceptor Function. *Neuropsychopharmacology*.

Camenisch G, Alsenz J, van de Waterbeemd H, Folkers G (1998). Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* **6**(4)**:** 317-324.

Cao YJ, Surowy CS, Puttfarcken PS (2005). Nicotinic acetylcholine receptor-mediated [3H]dopamine release from hippocampus. *J Pharmacol Exp Ther* **312**(3)**:** 1298-1304.

Caprioli D, Jupp B, Hong YT, Sawiak SJ, Ferrari V, Wharton L, *et al.* (2015). Dissociable rate-dependent effects of oral methylphenidate on impulsivity and D2/3 receptor availability in the striatum. *J Neurosci* **35**(9)**:** 3747-3755.

Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* **292**(5526)**:** 2499-2501.

Carlezon WA, Jr., Konradi C (2004). Understanding the neurobiological consequences of early exposure to psychotropic drugs: linking behavior with molecules. *Neuropharmacology* **47 Suppl 1:** 47-60.

Carli M, Invernizzi RW (2014). Serotoninergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the 5-choice serial reaction time task. *Frontiers in neural circuits* **8:** 58.

Carlsson ML (2000). On the role of cortical glutamate in obsessive-compulsive disorder and attention-deficit hyperactivity disorder, two phenomenologically antithetical conditions. *Acta psychiatrica Scandinavica* **102**(6)**:** 401-413.

Carlsson ML (2001). On the role of prefrontal cortex glutamate for the antithetical phenomenology of obsessive compulsive disorder and attention deficit hyperactivity disorder. *Progress in neuro-psychopharmacology & biological psychiatry* **25**(1)**:** 5-26.

Carr DB, Sesack SR (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci* **20**(10)**:** 3864-3873.

Carrey N, MacMaster FP, Sparkes SJ, Khan SC, Kusumakar V (2002). Glutamatergic changes with treatment in attention deficit hyperactivity disorder: a preliminary case series. *Journal of child and adolescent psychopharmacology* **12**(4)**:** 331-336.

Carrey NJ, MacMaster FP, Gaudet L, Schmidt MH (2007). Striatal creatine and glutamate/glutamine in attention-deficit/hyperactivity disorder. *Journal of child and adolescent psychopharmacology* **17**(1)**:** 11-17.

Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS, *et al.* (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *Jama* **288**(14)**:** 1740-1748.

Castells X, Ramos-Quiroga JA, Bosch R, Nogueira M, Casas M (2011). Amphetamines for Attention Deficit Hyperactivity Disorder (ADHD) in adults. *Cochrane Database Syst Rev*(6)**:** CD007813.

Catala-Lopez F, Hutton B, Nunez-Beltran A, Mayhew AD, Page MJ, Ridao M, *et al.* (2015). The pharmacological and non-pharmacological treatment of attention deficit hyperactivity disorder in children and adolescents: protocol for a systematic review and network meta-analysis of randomized controlled trials. *Systematic reviews* **4:** 19.

Catterall WA (2002). Molecular mechanisms of gating and drug block of sodium channels. *Novartis Foundation symposium* **241:** 206-218; discussion 218-232.

Centonze D, Usiello A, Gubellini P, Pisani A, Borrelli E, Bernardi G, *et al.* (2002). Dopamine D2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D2L receptors. *Neuropsychopharmacology* **27**(5)**:** 723-726.

Cepeda C, Levine MS (1998). Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. *Developmental neuroscience* **20**(1)**:** 1-18.

Cepeda C, Peacock W, Levine MS, Buchwald NA (1991). Iontophoretic application of NMDA produces different types of excitatory responses in developing human cortical and caudate neurons. *Neurosci Lett* **126**(2)**:** 167-171.

Cerrillo-Urbina AJ, Garcia-Hermoso A, Sanchez-Lopez M, Pardo-Guijarro MJ, Santos Gomez JL, Martinez-Vizcaino V (2015). The effects of physical exercise in children with attention deficit hyperactivity disorder: a systematic review and meta-analysis of randomized control trials. *Child: care, health and development* **41**(6)**:** 779-788.

Chacko A, Pelham WE, Gnagy EM, Greiner A, Vallano G, Bukstein O, *et al.* (2005). Stimulant medication effects in a summer treatment program among young children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* **44**(3)**:** 249-257.

Chalabi-Yani D, Sahraei H, Meftahi GH, Hosseini SB, Sadeghi-Gharajehdaghi S, Ali Beig H, *et al.* (2015). Effect of Transient Inactivation of Ventral Tegmental Area on the Expression and Acquisition of Nicotine-Induced Conditioned Place Preference in Rats. *Iranian biomedical journal* **19**(4)**:** 214-219.

Chang JP, Lane HY, Tsai GE (2014). Attention deficit hyperactivity disorder and N-methyl-D-aspartate (NMDA) dysregulation. *Current pharmaceutical design* **20**(32)**:** 5180-5185.

Chao LL, Knight RT (1998). Contribution of human prefrontal cortex to delay performance. *Journal of cognitive neuroscience* **10**(2)**:** 167-177.

Charach A, Figueroa M, Chen S, Ickowicz A, Schachar R (2006). Stimulant treatment over 5 years: effects on growth. *Journal of the American Academy of Child and Adolescent Psychiatry* **45**(4)**:** 415-421.

Charlety PJ, Grenhoff J, Chergui K, De la Chapelle B, Buda M, Svensson TH, *et al.* (1991). Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate. *Acta physiologica Scandinavica* **142**(1)**:** 105-112.

Chatterjee-Chakrabarty S, Miller BT, Collins TJ, Nagamani M (2005). Adverse effects of methylphenidate on the reproductive axis of adolescent female rats. *Fertility and sterility* **84 Suppl 2:** 1131-1138.

Chavez AE, Singer JH, Diamond JS (2006). Fast neurotransmitter release triggered by Ca influx through AMPA-type glutamate receptors. *Nature* **443**(7112)**:** 705-708.

Chefer VI, Thompson AC, Zapata A, Shippenberg TS (2009). Overview of brain microdialysis. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* **Chapter 7:** Unit7 1.

Chen G, Greengard P, Yan Z (2004). Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. *Proc Natl Acad Sci U S A* **101**(8)**:** 2596-2600.

Chen HT, Kandasamy SB (1996). Effect of chloral hydrate on in vivo KCl-induced striatal dopamine release in the rat. *Neurochem Res* **21**(6)**:** 695-700.

Chen L, Muhlhauser M, Yang CR (2003a). Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. *Journal of neurophysiology* **89**(2)**:** 691-703.

Chen TY, Zhang D, Dragomir A, Akay YM, Akay M (2011). Complexity of VTA DA neural activities in response to PFC transection in nicotine treated rats. *Journal of neuroengineering and rehabilitation* **8:** 13.

Chen Y, Broad LM, Phillips KG, Zwart R (2012). Partial agonists for alpha4beta2 nicotinic receptors stimulate dopaminergic neuron firing with relatively enhanced maximal effects. *British journal of pharmacology* **165**(4)**:** 1006-1016.

Chen Y, Sharples TJ, Phillips KG, Benedetti G, Broad LM, Zwart R, *et al.* (2003b). The nicotinic alpha 4 beta 2 receptor selective agonist, TC-2559, increases dopamine neuronal activity in the ventral tegmental area of rat midbrain slices. *Neuropharmacology* **45**(3)**:** 334-344.

Cheng J, Xiong Z, Duffney LJ, Wei J, Liu A, Liu S, *et al.* (2014). Methylphenidate exerts dose-dependent effects on glutamate receptors and behaviors. *Biol Psychiatry* **76**(12)**:** 953-962.

Chenu F, El Mansari M, Blier P (2013). Electrophysiological effects of repeated administration of agomelatine on the dopamine, norepinephrine, and serotonin systems in the rat brain. *Neuropsychopharmacology* **38**(2)**:** 275-284.

Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, *et al.* (1993). Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. *The European journal of neuroscience* **5**(2)**:** 137-144.

Chieng B, Azriel Y, Mohammadi S, Christie MJ (2011). Distinct cellular properties of identified dopaminergic and GABAergic neurons in the mouse ventral tegmental area. *The Journal of physiology* **589**(Pt 15)**:** 3775-3787.

Cho AK, Schaffer JC, Fischer JF (1975). Accumulation of 4-hydroxyamphetamine by rat striatal homogenates. *Biochemical pharmacology* **24**(16)**:** 1540-1542.

Cho AK, Wright J (1978). Pathways of metabolism of amphetamine and related compounds. *Life sciences* **22**(5)**:** 363-372.

Choi DW (1985). Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neurosci Lett* **58**(3)**:** 293-297.

Choi DW, Maulucci-Gedde M, Kriegstein AR (1987). Glutamate neurotoxicity in cortical cell culture. *J Neurosci* **7**(2)**:** 357-368.

Choong KC, Shen RY (2004). Methylphenidate restores ventral tegmental area dopamine neuron activity in prenatal ethanol-exposed rats by augmenting dopamine neurotransmission. *J Pharmacol Exp Ther* **309**(2)**:** 444-451.

Chotibut T, Apple DM, Jefferis R, Salvatore MF (2012). Dopamine transporter loss in 6-OHDA Parkinson's model is unmet by parallel reduction in dopamine uptake. *PloS one* **7**(12)**:** e52322.

Christoph T, Schiene K, Englberger W, Parsons CG, Chizh BA (2006). The antiallodynic effect of NMDA antagonists in neuropathic pain outlasts the duration of the in vivo NMDA antagonism. *Neuropharmacology* **51**(1)**:** 12-17.

Chudasama Y, Passetti F, Rhodes SE, Lopian D, Desai A, Robbins TW (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behavioural brain research* **146**(1-2)**:** 105-119.

Chue P (2013). Glycine reuptake inhibition as a new therapeutic approach in schizophrenia: focus on the glycine transporter 1 (GlyT1). *Current pharmaceutical design* **19**(7)**:** 1311-1320.

Chuhma N, Tanaka KF, Hen R, Rayport S (2011). Functional connectome of the striatal medium spiny neuron. *J Neurosci* **31**(4)**:** 1183-1192.

Claussen CM, Chong SL, Dafny N (2014a). Nucleus accumbens neuronal activity correlates to the animal's behavioral response to acute and chronic methylphenidate. *Physiology & behavior* **129:** 85-94.

Claussen CM, Dafny N (2014b). Acute administration of methylphenidate alters the prefrontal cortex neuronal activity in a dose-response characteristic. *Journal of experimental pharmacology* **6:** 1-9.

Clemo HR, Keniston LP, Meredith MA (2012). Structural Basis of Multisensory Processing: Convergence. In: Murray MM, Wallace MT (ed)^(eds). *The Neural Bases of Multisensory Processes*, edn. Boca Raton (FL). p^pp.

Cohen JY, Amoroso MW, Uchida N (2015). Serotonergic neurons signal reward and punishment on multiple timescales. *eLife* **4**.

Collingridge GL, Volianskis A, Bannister N, France G, Hanna L, Mercier M, *et al.* (2013). The NMDA receptor as a target for cognitive enhancement. *Neuropharmacology* **64:** 13-26.

Collins SL, Levin FR, Foltin RW, Kleber HD, Evans SM (2006). Response to cocaine, alone and in combination with methylphenidate, in cocaine abusers with ADHD. *Drug Alcohol Depend* **82**(2)**:** 158-167.

Connor DF, Chartier KG, Preen EC, Kaplan RF (2010). Impulsive aggression in attention-deficit/hyperactivity disorder: symptom severity, co-morbidity, and attention-deficit/hyperactivity disorder subtype. *Journal of child and adolescent psychopharmacology* **20**(2)**:** 119-126.

Conrad SC, Nichols NL, Ritucci NA, Dean JB, Putnam RW (2009). Development of chemosensitivity in neurons from the nucleus tractus solitarii (NTS) of neonatal rats. *Respiratory physiology & neurobiology* **166**(1)**:** 4-12.

Cooper GM (2000). *The Cell: A Molecular Approach*. 2nd edition. edn.

Corbett A, McGowin A, Sieber S, Flannery T, Sibbitt B (2012). A method for reliable voluntary oral administration of a fixed dosage (mg/kg) of chronic daily medication to rats. *Laboratory animals* **46**(4)**:** 318-324.

Couceyro PR, Evans C, McKinzie A, Mitchell D, Dube M, Hagshenas L, *et al.* (2005). Cocaine- and amphetamine-regulated transcript (CART) peptides modulate the locomotor and motivational properties of psychostimulants. *J Pharmacol Exp Ther* **315**(3)**:** 1091-1100.

Couch Y, Anthony DC, Dolgov O, Revischin A, Festoff B, Santos AI, *et al.* (2013). Microglial activation, increased TNF and SERT expression in the prefrontal cortex define stress-altered behaviour in mice susceptible to anhedonia. *Brain, behavior, and immunity* **29:** 136-146.

Courtney KR (1975). Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA. *J Pharmacol Exp Ther* **195**(2)**:** 225-236.

Courvoisie H, Hooper SR, Fine C, Kwock L, Castillo M (2004). Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: preliminary findings. *The Journal of neuropsychiatry and clinical neurosciences* **16**(1)**:** 63-69.

Covey LS, Hu MC, Winhusen T, Weissman J, Berlin I, Nunes EV (2010). OROS-methylphenidate or placebo for adult smokers with attention deficit hyperactivity disorder: racial/ethnic differences. *Drug Alcohol Depend* **110**(1-2)**:** 156-159.

Crawford CA, Baella SA, Farley CM, Herbert MS, Horn LR, Campbell RH, *et al.* (2011). Early methylphenidate exposure enhances cocaine self-administration but not cocaine-induced conditioned place preference in young adult rats. *Psychopharmacology (Berl)* **213**(1)**:** 43-52.

Crawford CA, Der-Ghazarian T, Britt CE, Varela FA, Kozanian OO (2013). Novelty-induced conditioned place preference, sucrose preference, and elevated plus maze behavior in adult rats after repeated exposure to methylphenidate during the preweanling period. *Behavioural brain research* **246:** 29-35.

Crocq MA (2003). Alcohol, nicotine, caffeine, and mental disorders. *Dialogues in clinical neuroscience* **5**(2)**:** 175-185.

Crunelle CL, van den Brink W, Veltman DJ, van Emmerik-van Oortmerssen K, Dom G, Schoevers RA, *et al.* (2013). Low dopamine transporter occupancy by methylphenidate as a possible reason for reduced treatment effectiveness in ADHD patients with cocaine dependence. *Eur Neuropsychopharmacol* **23**(12)**:** 1714-1723.

Cubillo A, Halari R, Smith A, Taylor E, Rubia K (2012). A review of fronto-striatal and fronto-cortical brain abnormalities in children and adults with Attention Deficit Hyperactivity Disorder (ADHD) and new evidence for dysfunction in adults with ADHD during motivation and attention. *Cortex; a journal devoted to the study of the nervous system and behavior* **48**(2)**:** 194-215.

Cull-Candy S, Brickley S, Farrant M (2001). NMDA receptor subunits: diversity, development and disease. *Current opinion in neurobiology* **11**(3)**:** 327-335.

Cummings JL (1993). Frontal-subcortical circuits and human behavior. *Archives of neurology* **50**(8)**:** 873-880.

Cvetkovic-Lopes V, Eggermann E, Uschakov A, Grivel J, Bayer L, Jones BE, *et al.* (2010). Rat hypocretin/orexin neurons are maintained in a depolarized state by TRPC channels. *PloS one* **5**(12)**:** e15673.

Dackis CA, Kampman KM, Lynch KG, Pettinati HM, O'Brien CP (2005). A double-blind, placebo-controlled trial of modafinil for cocaine dependence. *Neuropsychopharmacology* **30**(1)**:** 205-211.

Dackis CA, Lynch KG, Yu E, Samaha FF, Kampman KM, Cornish JW, *et al.* (2003). Modafinil and cocaine: a double-blind, placebo-controlled drug interaction study. *Drug Alcohol Depend* **70**(1)**:** 29-37.

Daigre C, Roncero C, Grau-Lopez L, Martinez-Luna N, Prat G, Valero S, *et al.* (2013). Attention deficit hyperactivity disorder in cocaine-dependent adults: a psychiatric comorbidity analysis. *The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions* **22**(5)**:** 466-473.

Dalley JW, Cardinal RN, Robbins TW (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev* **28**(7)**:** 771-784.

Dalley JW, Roiser JP (2012). Dopamine, serotonin and impulsivity. *Neuroscience* **215:** 42-58.

Dalsgaard S, Mortensen PB, Frydenberg M, Thomsen PH (2014). ADHD, stimulant treatment in childhood and subsequent substance abuse in adulthood - a naturalistic long-term follow-up study. *Addict Behav* **39**(1)**:** 325-328.

Daly JW (2007). Caffeine analogs: biomedical impact. *Cellular and molecular life sciences : CMLS* **64**(16)**:** 2153-2169.

Davids E, Zhang K, Tarazi FI, Baldessarini RJ (2003). Animal models of attention-deficit hyperactivity disorder. *Brain Res Brain Res Rev* **42**(1)**:** 1-21.

Davis KL, Hollister LE, Tepper J (1978). Cholinergic inhibition of methylphenidate-induced stereotypy: oxotremorine. *Psychopharmacology (Berl)* **56**(1)**:** 1-4.

De-Carolis C, Boyd GA, Mancinelli L, Pagano S, Eramo S (2015). Methamphetamine abuse and "meth mouth" in Europe. *Med Oral Patol Oral Cir Bucal*.

de Diego-Otero Y, Calvo-Medina R, Quintero-Navarro C, Sanchez-Salido L, Garcia-Guirado F, del Arco-Herrera I, *et al.* (2014). A combination of ascorbic acid and alpha-tocopherol to test the effectiveness and safety in the fragile X syndrome: study protocol for a phase II, randomized, placebo-controlled trial. *Trials* **15:** 345.

de Guglielmo G, Melis M, De Luca MA, Kallupi M, Li HW, Niswender K, *et al.* (2015). PPARgamma activation attenuates opioid consumption and modulates mesolimbic dopamine transmission. *Neuropsychopharmacology* **40**(4)**:** 927-937.

de la Torre R, Farre M, Roset PN, Lopez CH, Mas M, Ortuno J, *et al.* (2000). Pharmacology of MDMA in humans. *Ann N Y Acad Sci* **914:** 225-237.

De Potter WP, Partoens P, Strecker S (1997). Noradrenaline storing vesicles in sympathetic neurons and their role in neurotransmitter release: an historical overview of controversial issues. *Neurochem Res* **22**(8)**:** 911-919.

De Sousa A, Kalra G (2012). Drug therapy of attention deficit hyperactivity disorder: current trends. *Mens sana monographs* **10**(1)**:** 45-69.

Deacon RM, Penny C, Rawlins JN (2003). Effects of medial prefrontal cortex cytotoxic lesions in mice. *Behavioural brain research* **139**(1-2)**:** 139-155.

dela Pena I, Gevorkiana R, Shi WX (2015). Psychostimulants affect dopamine transmission through both dopamine transporter-dependent and independent mechanisms. *Eur J Pharmacol* **764:** 562-570.

Demetrius L (2005). Of mice and men. When it comes to studying ageing and the means to slow it down, mice are not just small humans. *EMBO reports* **6 Spec No:** S39-44.

DeRossi R, Hermeto LC, Jardim PH, de Andrade Bicudo N, de Assis KT (2015). Postoperative pain control in cats: clinical trials with pre-emptive lidocaine epidural co-administered with morphine or methadone. *Journal of feline medicine and surgery*.

Deutch AY, Goldstein M, Roth RH (1986). Activation of the locus coeruleus induced by selective stimulation of the ventral tegmental area. *Brain research* **363**(2)**:** 307-314.

Devilbiss DM, Berridge CW (2008). Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biol Psychiatry* **64**(7)**:** 626-635.

Devoto P, Flore G (2006). On the origin of cortical dopamine: is it a co-transmitter in noradrenergic neurons? *Current neuropharmacology* **4**(2)**:** 115-125.

Devoto P, Flore G, Longu G, Pira L, Gessa GL (2003). Origin of extracellular dopamine from dopamine and noradrenaline neurons in the medial prefrontal and occipital cortex. *Synapse* **50**(3)**:** 200-205.

Devoto P, Flore G, Pani L, Gessa GL (2001). Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol Psychiatry* **6**(6)**:** 657-664.

Devoto P, Flore G, Pira L, Longu G, Gessa GL (2004). Alpha2-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex. *Journal of neurochemistry* **88**(4)**:** 1003-1009.

Diaz Martinez MC, Diaz Martinez A, Villamil Salcedo V, Cruz Fuentes C (2002). Efficacy of metadoxine in the management of acute alcohol intoxication. *The Journal of international medical research* **30**(1)**:** 44-51.

Ding L, Perkel DJ (2002). Dopamine modulates excitability of spiny neurons in the avian basal ganglia. *J Neurosci* **22**(12)**:** 5210-5218.

Ding X, Qiao Y, Piao C, Zheng X, Liu Z, Liang J (2014). N-methyl-D-aspartate receptor-mediated glutamate transmission in nucleus accumbens plays a more important role than that in dorsal striatum in cognitive flexibility. *Frontiers in behavioral neuroscience* **8:** 304.

Ding YS, Gatley SJ, Thanos PK, Shea C, Garza V, Xu Y, *et al.* (2004). Brain kinetics of methylphenidate (Ritalin) enantiomers after oral administration. *Synapse* **53**(3)**:** 168-175.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999). The glutamate receptor ion channels. *Pharmacological reviews* **51**(1)**:** 7-61.

Diogo LN, Faustino IV, Afonso RA, Pereira SA, Monteiro EC, Santos AI (2015). Voluntary Oral Administration of Losartan in Rats. *Journal of the American Association for Laboratory Animal Science : JAALAS* **54**(5)**:** 549-556.

Djordjevic J, Djordjevic A, Adzic M, Radojcic MB (2012). Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers. *Neuropsychobiology* **66**(2)**:** 112-119.

Donato F, Borges Filho C, Giacomeli R, Alvater EE, Del Fabbro L, Antunes Mda S, *et al.* (2015). Evidence for the Involvement of Potassium Channel Inhibition in the Antidepressant-Like Effects of Hesperidin in the Tail Suspension Test in Mice. *Journal of medicinal food* **18**(7)**:** 818-823.

Dorval KM, Wigg KG, Crosbie J, Tannock R, Kennedy JL, Ickowicz A, *et al.* (2007). Association of the glutamate receptor subunit gene GRIN2B with attention-deficit/hyperactivity disorder. *Genes Brain Behav* **6**(5)**:** 444-452.

Dos Santos Pereira M, Sathler MF, Valli Tda R, Marques RS, Ventura AL, Peccinalli NR, *et al.* (2015). Long Withdrawal of Methylphenidate Induces a Differential Response of the Dopaminergic System and Increases Sensitivity to Cocaine in the Prefrontal Cortex of Spontaneously Hypertensive Rats. *PloS one* **10**(10)**:** e0141249.

Dosh T, Helmbrecht T, Anestis J, Guenthner G, Kelly TH, Martin CA (2010). A comparison of the associations of caffeine and cigarette use with depressive and ADHD symptoms in a sample of young adult smokers. *J Addict Med* **4**(1)**:** 52-54.

Dougan DF, Duffield AM, Duffield PH, Wade DN (1986a). Stereoselective accumulation of hydroxylated metabolites of amphetamine in rat striatum and hypothalamus. *British journal of pharmacology* **88**(1)**:** 285-290.

Dougan DF, Labrie SL, Paull PD, Duffield PH, Wade DN (1986b). Evidence that alpha-methyl-p-tyramine is implicated in behavioural augmentation to amphetamine. *General pharmacology* **17**(4)**:** 453-456.

Dreyer JK, Herrik KF, Berg RW, Hounsgaard JD (2010). Influence of phasic and tonic dopamine release on receptor activation. *J Neurosci* **30**(42)**:** 14273-14283.

Duan L, Yang J, Slaughter MM (2009). Caffeine inhibition of ionotropic glycine receptors. *The Journal of physiology* **587**(Pt 16)**:** 4063-4075.

Duncan RS, McPate MJ, Ridley JM, Gao Z, James AF, Leishman DJ, *et al.* (2007). Inhibition of the HERG potassium channel by the tricyclic antidepressant doxepin. *Biochemical pharmacology* **74**(3)**:** 425-437.

Dupont J, Dupont JC, Milon H, Froment A (1975). [Spontaneous mortality and vascular lesions in 3 rat strains with different blood pressure levels]. *Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles* **280**(13)**:** 1637-1640.

Easton N, Steward C, Marshall F, Fone K, Marsden C (2007). Effects of amphetamine isomers, methylphenidate and atomoxetine on synaptosomal and synaptic vesicle accumulation and release of dopamine and noradrenaline in vitro in the rat brain. *Neuropharmacology* **52**(2)**:** 405-414.

Edden RA, Crocetti D, Zhu H, Gilbert DL, Mostofsky SH (2012). Reduced GABA concentration in attention-deficit/hyperactivity disorder. *Archives of general psychiatry* **69**(7)**:** 750-753.

Eddine R, Valverde S, Tolu S, Dautan D, Hay A, Morel C, *et al.* (2015). A concurrent excitation and inhibition of dopaminergic subpopulations in response to nicotine. *Scientific reports* **5:** 8184.

Edwards G, Weston AH (1995). The role of potassium channels in excitable cells. *Diabetes research and clinical practice* **28 Suppl:** S57-66.

Efron D, Jarman F, Barker M (1997). Side effects of methylphenidate and dexamphetamine in children with attention deficit hyperactivity disorder: a double-blind, crossover trial. *Pediatrics* **100**(4)**:** 662-666.

Egan TM, Henderson G, North RA, Williams JT (1983). Noradrenaline-mediated synaptic inhibition in rat locus coeruleus neurones. *The Journal of physiology* **345:** 477-488.

Eiden LE, Weihe E (2011). VMAT2: a dynamic regulator of brain monoaminergic neuronal function interacting with drugs of abuse. *Ann N Y Acad Sci* **1216:** 86-98.

Einhorn LC, Johansen PA, White FJ (1988). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci* **8**(1)**:** 100-112.

El-Kabbani O, Ishikura S, Darmanin C, Carbone V, Chung RP, Usami N, *et al.* (2004). Crystal structure of human L-xylulose reductase holoenzyme: probing the role of Asn107 with site-directed mutagenesis. *Proteins* **55**(3)**:** 724-732.

El Iskandrani KS, Oosterhof CA, El Mansari M, Blier P (2015). Impact of subanesthetic doses of ketamine on AMPA-mediated responses in rats: An in vivo electrophysiological study on monoaminergic and glutamatergic neurons. *J Psychopharmacol* **29**(7)**:** 792-801.

El Mansari M, Guiard BP, Chernoloz O, Ghanbari R, Katz N, Blier P (2010). Relevance of norepinephrine-dopamine interactions in the treatment of major depressive disorder. *CNS neuroscience & therapeutics* **16**(3)**:** e1-17.

El Yacoubi M, Ledent C, Menard JF, Parmentier M, Costentin J, Vaugeois JM (2000). The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. *British journal of pharmacology* **129**(7)**:** 1465-1473.

Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, Hadley D, *et al.* (2012). Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. *Nature genetics* **44**(1)**:** 78-84.

Ellinwood EH, Jr., Lee TH (1983). Effect of continuous systemic infusion of D-amphetamine on the sensitivity of nigral dopamine cells to apomorphine inhibition of firing rates. *Brain research* **273**(2)**:** 379-383.

Emmett-Oglesby MW, Taylor KE (1981). Role of dose interval in the acquisition of tolerance to methylphenidate. *Neuropharmacology* **20**(10)**:** 995-1002.

Ende G, Cackowski S, Van Eijk J, Sack M, Demirakca T, Kleindienst N, *et al.* (2015). Impulsivity and Aggression in Female BPD and ADHD Patients: Association with ACC Glutamate and GABA Concentrations. *Neuropsychopharmacology*.

Ende G, Cackowski S, Van Eijk J, Sack M, Demirakca T, Kleindienst N, *et al.* (2016). Impulsivity and Aggression in Female BPD and ADHD Patients: Association with ACC Glutamate and GABA Concentrations. *Neuropsychopharmacology* **41**(2)**:** 410-418.

Erhardt S, Schwieler L, Engberg G (2002). Excitatory and inhibitory responses of dopamine neurons in the ventral tegmental area to nicotine. *Synapse* **43**(4)**:** 227-237.

Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci U S A* **93**(10)**:** 5166-5171.

Ernst M, Zametkin AJ, Matochik J, Schmidt M, Jons PH, Liebenauer LL, *et al.* (1997). Intravenous dextroamphetamine and brain glucose metabolism. *Neuropsychopharmacology* **17**(6)**:** 391-401.

Eubig PA, Aguiar A, Schantz SL (2010). Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environmental health perspectives* **118**(12)**:** 1654-1667.

Evans SW, Pelham WE (1991). Psychostimulant effects on academic and behavioral measures for ADHD junior high school students in a lecture format classroom. *Journal of abnormal child psychology* **19**(5)**:** 537-552.

Fabiano GA, Pelham WE, Jr., Coles EK, Gnagy EM, Chronis-Tuscano A, O'Connor BC (2009). A meta-analysis of behavioral treatments for attention-deficit/hyperactivity disorder. *Clin Psychol Rev* **29**(2)**:** 129-140.

Fang TY, Tan SE (1998). The effects of methylphenidate and maturation on exploratory activity in rats. *The Chinese journal of physiology* **41**(1)**:** 53-58.

Faraone SV, Biederman J, Monuteaux M, Spencer T (2005). Long-term effects of extended-release mixed amphetamine salts treatment of attention- deficit/hyperactivity disorder on growth. *Journal of child and adolescent psychopharmacology* **15**(2)**:** 191-202.

Faraone SV, Biederman J, Morley CP, Spencer TJ (2008). Effect of stimulants on height and weight: a review of the literature. *Journal of the American Academy of Child and Adolescent Psychiatry* **47**(9)**:** 994-1009.

Fazelipour S, Jahromy MH, Tootian Z, Kiaei SB, Sheibani MT, Talaee N (2012). The effect of chronic administration of methylphenidate on morphometric parameters of testes and fertility in male mice. *Journal of reproduction & infertility* **13**(4)**:** 232-236.

Federici M, Geracitano R, Bernardi G, Mercuri NB (2005). Actions of methylphenidate on dopaminergic neurons of the ventral midbrain. *Biol Psychiatry* **57**(4)**:** 361-365.

Fellows LK, Boutelle MG, Fillenz M (1992). Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats. *Journal of neurochemistry* **59**(6)**:** 2141-2147.

Fernandez-Jaen A, Lopez-Martin S, Albert J, Fernandez-Mayoralas DM, Fernandez-Perrone AL, Tapia DQ, *et al.* (2014). Cortical thinning of temporal pole and orbitofrontal cortex in medication-naive children and adolescents with ADHD. *Psychiatry Res* **224**(1)**:** 8-13.

Ferre S, O'Connor WT, Snaprud P, Ungerstedt U, Fuxe K (1994). Antagonistic interaction between adenosine A2A receptors and dopamine D2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. *Neuroscience* **63**(3)**:** 765-773.

Ferre S, Popoli P, Tinner-Staines B, Fuxe K (1996). Adenosine A1 receptor-dopamine D1 receptor interaction in the rat limbic system: modulation of dopamine D1 receptor antagonist binding sites. *Neurosci Lett* **208**(2)**:** 109-112.

Ferre S, Quiroz C, Woods AS, Cunha R, Popoli P, Ciruela F, *et al.* (2008). An update on adenosine A2A-dopamine D2 receptor interactions: implications for the function of G protein-coupled receptors. *Current pharmaceutical design* **14**(15)**:** 1468-1474.

Ferreira PE, Palmini A, Bau CH, Grevet EH, Hoefel JR, Rohde LA, *et al.* (2009). Differentiating attention-deficit/hyperactivity disorder inattentive and combined types: a (1)H-magnetic resonance spectroscopy study of fronto-striato-thalamic regions. *Journal of neural transmission* **116**(5)**:** 623-629.

Ferrucci M, Giorgi FS, Bartalucci A, Busceti CL, Fornai F (2013). The effects of locus coeruleus and norepinephrine in methamphetamine toxicity. *Current neuropharmacology* **11**(1)**:** 80-94.

Fischer CW, Liebenberg N, Elfving B, Lund S, Wegener G (2012). Isolation-induced behavioural changes in a genetic animal model of depression. *Behavioural brain research* **230**(1)**:** 85-91.

Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A (2000). Glutamate receptors regulate actin-based plasticity in dendritic spines. *Nature neuroscience* **3**(9)**:** 887-894.

Flecknell PA, Roughan JV, Stewart R (1999). Use of oral buprenorphine ('buprenorphine jello') for postoperative analgesia in rats--a clinical trial. *Laboratory animals* **33**(2)**:** 169-174.

Fletcher PC, Shallice T, Dolan RJ (1998). The functional roles of prefrontal cortex in episodic memory. I. Encoding. *Brain : a journal of neurology* **121 (Pt 7):** 1239-1248.

Florence NT, Benoit MZ, Jonas K, Alexandra T, Desire DD, Pierre K, *et al.* (2014). Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. *Journal of ethnopharmacology* **151**(2)**:** 784-790.

Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, Calvert CR, Jokel ES, Fienberg AA, *et al.* (2002). Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. *Journal of neurophysiology* **88**(6)**:** 3010-3020.

Floresco SB (2013). Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Frontiers in neuroscience* **7:** 62.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature neuroscience* **6**(9)**:** 968-973.

Ford CP (2014). The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience* **282C:** 13-22.

Ford CP, Mark GP, Williams JT (2006). Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. *J Neurosci* **26**(10)**:** 2788-2797.

Fornai F, Grazia Alessandri M, Bonuccelli U, Scalori V, Corsini GU (1993). Effect of metadoxine on striatal dopamine levels in C57 black mice. *The Journal of pharmacy and pharmacology* **45**(5)**:** 476-478.

Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological reviews* **51**(1)**:** 83-133.

Fredholm BB, Hu PS (1993). Effect of an intracellular calcium chelator on the regulation of electrically evoked [3H]-noradrenaline release from rat hippocampal slices. *British journal of pharmacology* **108**(1)**:** 126-131.

French ED, Dillon K, Wu X (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport* **8**(3)**:** 649-652.

French ED, Mura A, Wang T (1993). MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists. *Synapse* **13**(2)**:** 108-116.

Friedmann E, Thomas SA, Liu F, Morton PG, Chapa D, Gottlieb SS, *et al.* (2006). Relationship of depression, anxiety, and social isolation to chronic heart failure outpatient mortality. *American heart journal* **152**(5)**:** 940 e941-948.

Fritz KM, O'Connor PJ (2016). Acute Exercise Improves Mood and Motivation in Young Men with ADHD Symptoms. *Medicine and science in sports and exercise* **48**(6)**:** 1153-1160.

Froehlich TE, Lanphear BP, Epstein JN, Barbaresi WJ, Katusic SK, Kahn RS (2007). Prevalence, recognition, and treatment of attention-deficit/hyperactivity disorder in a national sample of US children. *Arch Pediatr Adolesc Med* **161**(9)**:** 857-864.

Fuh MR, Haung CH, Wu TY, Lin SL, Pan WH (2004). Determination of amphetamine in rat brain by in vivo microdialysis and ion-pairing liquid chromatography with electrospray tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM* **18**(15)**:** 1711-1714.

Fujimori K, Benet H, Mehendale HM, Ho IK (1986). In vivo and in vitro synthesis, release, and uptake of [3-H]-dopamine in mouse striatal slices after in vivo exposure to chlordecone. *Journal of biochemical toxicology* **1**(4)**:** 1-12.

Fujisawa S, Buzsaki G (2011). A 4 Hz oscillation adaptively synchronizes prefrontal, VTA, and hippocampal activities. *Neuron* **72**(1)**:** 153-165.

Furlong TM, Leavitt LS, Keefe KA, Son JH (2016). Methamphetamine-, d-Amphetamine-, and p-Chloroamphetamine-Induced Neurotoxicity Differentially Effect Impulsive Responding on the Stop-Signal Task in Rats. *Neurotoxicity research* **29**(4)**:** 569-582.

Furman CA, Chen R, Guptaroy B, Zhang M, Holz RW, Gnegy M (2009). Dopamine and amphetamine rapidly increase dopamine transporter trafficking to the surface: live-cell imaging using total internal reflection fluorescence microscopy. *J Neurosci* **29**(10)**:** 3328-3336.

Fusar-Poli P, Rubia K, Rossi G, Sartori G, Balottin U (2012). Striatal dopamine transporter alterations in ADHD: pathophysiology or adaptation to psychostimulants? A meta-analysis. *The American journal of psychiatry* **169**(3)**:** 264-272.

Fuxe K, Ferre S, Canals M, Torvinen M, Terasmaa A, Marcellino D, *et al.* (2005). Adenosine A2A and dopamine D2 heteromeric receptor complexes and their function. *Journal of molecular neuroscience : MN* **26**(2-3)**:** 209-220.

Gadow KD, Sverd J, Sprafkin J, Nolan EE, Grossman S (1999). Long-term methylphenidate therapy in children with comorbid attention-deficit hyperactivity disorder and chronic multiple tic disorder. *Archives of general psychiatry* **56**(4)**:** 330-336.

Galati S, D'Angelo V, Scarnati E, Stanzione P, Martorana A, Procopio T, *et al.* (2008). In vivo electrophysiology of dopamine-denervated striatum: focus on the nitric oxide/cGMP signaling pathway. *Synapse* **62**(6)**:** 409-420.

Galvan A, Hu X, Smith Y, Wichmann T (2012). In vivo optogenetic control of striatal and thalamic neurons in non-human primates. *PloS one* **7**(11)**:** e50808.

Gamo NJ, Wang M, Arnsten AF (2010). Methylphenidate and atomoxetine enhance prefrontal function through alpha2-adrenergic and dopamine D1 receptors. *Journal of the American Academy of Child and Adolescent Psychiatry* **49**(10)**:** 1011-1023.

Gandal MJ, Anderson RL, Billingslea EN, Carlson GC, Roberts TP, Siegel SJ (2012). Mice with reduced NMDA receptor expression: more consistent with autism than schizophrenia? *Genes Brain Behav* **11**(6)**:** 740-750.

Gapin JI, Labban JD, Etnier JL (2011). The effects of physical activity on attention deficit hyperactivity disorder symptoms: the evidence. *Preventive medicine* **52 Suppl 1:** S70-74.

Garfinkel BD, Webster CD, Sloman L (1981). Responses to methylphenidate and varied doses of caffeine in children with attention deficit disorder. *Can J Psychiatry* **26**(6)**:** 395-401.

Gariano RF, Groves PM (1988). Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. *Brain research* **462**(1)**:** 194-198.

Garland EJ (1998). Intranasal abuse of prescribed methylphenidate. *Journal of the American Academy of Child and Adolescent Psychiatry* **37**(6)**:** 573-574.

Garnock-Jones KP, Keating GM (2009). Atomoxetine: a review of its use in attention-deficit hyperactivity disorder in children and adolescents. *Paediatr Drugs* **11**(3)**:** 203-226.

Gartside SE, Hajos-Korcsok E, Bagdy E, Harsing LG, Jr., Sharp T, Hajos M (2000). Neurochemical and electrophysiological studies on the functional significance of burst firing in serotonergic neurons. *Neuroscience* **98**(2)**:** 295-300.

Gaytan O, Nason R, Alagugurusamy R, Swann A, Dafny N (2000). MK-801 blocks the development of sensitization to the locomotor effects of methylphenidate. *Brain research bulletin* **51**(6)**:** 485-492.

Gearhardt AN, White MA, Potenza MN (2011). Binge eating disorder and food addiction. *Current drug abuse reviews* **4**(3)**:** 201-207.

Gehricke JG, Hong N, Wigal TL, Chan V, Doan A (2011). ADHD medication reduces cotinine levels and withdrawal in smokers with ADHD. *Pharmacol Biochem Behav* **98**(3)**:** 485-491.

Geisler S, Wise RA (2008). Functional implications of glutamatergic projections to the ventral tegmental area. *Reviews in the neurosciences* **19**(4-5)**:** 227-244.

Gentile JP, Atiq R, Gillig PM (2006). Adult ADHD: Diagnosis, Differential Diagnosis, and Medication Management. *Psychiatry* **3**(8)**:** 25-30.

Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, *et al.* (2000). Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *J Pharmacol Exp Ther* **295**(1)**:** 51-57.

Gerfen CR, Surmeier DJ (2011). Modulation of striatal projection systems by dopamine. *Annual review of neuroscience* **34:** 441-466.

Germann PG, Ockert D (1994). Granulomatous inflammation of the oropharyngeal cavity as a possible cause for unexpected high mortality in a Fischer 344 rat carcinogenicity study. *Laboratory animal science* **44**(4)**:** 338-343.

Germinario EA, Arcieri R, Bonati M, Zuddas A, Masi G, Vella S, *et al.* (2013). Attention-deficit/hyperactivity disorder drugs and growth: an Italian prospective observational study. *Journal of child and adolescent psychopharmacology* **23**(7)**:** 440-447.

Gill KE, Pierre PJ, Daunais J, Bennett AJ, Martelle S, Gage HD, *et al.* (2012). Chronic treatment with extended release methylphenidate does not alter dopamine systems or increase vulnerability for cocaine self-administration: a study in nonhuman primates. *Neuropsychopharmacology* **37**(12)**:** 2555-2565.

Gines S, Hillion J, Torvinen M, Le Crom S, Casado V, Canela EI, *et al.* (2000). Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. *Proc Natl Acad Sci U S A* **97**(15)**:** 8606-8611.

Ginsberg Y, Quintero J, Anand E, Casillas M, Upadhyaya HP (2014). Underdiagnosis of attention-deficit/hyperactivity disorder in adult patients: a review of the literature. *The primary care companion for CNS disorders* **16**(3).

Giorgetti M, Hotsenpiller G, Froestl W, Wolf ME (2002). In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. *Neuroscience* **109**(3)**:** 585-595.

Giros B, Wang YM, Suter S, McLeskey SB, Pifl C, Caron MG (1994). Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *The Journal of biological chemistry* **269**(23)**:** 15985-15988.

Gizer IR, Ficks C, Waldman ID (2009). Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet* **126**(1)**:** 51-90.

Glade MJ (2010). Caffeine-Not just a stimulant. *Nutrition* **26**(10)**:** 932-938.

Glowa JR, Fantegrossi WE, Lewis DB, Matecka D, Rice KC, Rothman RB (1996). Sustained decrease in cocaine-maintained responding in rhesus monkeys with 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-hydroxy-3-phenylpropyl) piperazinyl decanoate, a long-acting ester derivative of GBR 12909. *Journal of medicinal chemistry* **39**(24)**:** 4689-4691.

Gnegy ME, Khoshbouei H, Berg KA, Javitch JA, Clarke WP, Zhang M, *et al.* (2004). Intracellular Ca2+ regulates amphetamine-induced dopamine efflux and currents mediated by the human dopamine transporter. *Molecular pharmacology* **66**(1)**:** 137-143.

Gobbi G, Janiri L (2006). Sodium- and magnesium-valproate in vivo modulate glutamatergic and GABAergic synapses in the medial prefrontal cortex. *Psychopharmacology (Berl)* **185**(2)**:** 255-262.

Gobel B, Oltmanns KM, Chung M (2013). Linking neuronal brain activity to the glucose metabolism. *Theoretical biology & medical modelling* **10:** 50.

Goldman-Rakic PS (1996). The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **351**(1346)**:** 1445-1453.

Goldman-Rakic PS, Muly EC, 3rd, Williams GV (2000). D(1) receptors in prefrontal cells and circuits. *Brain Res Brain Res Rev* **31**(2-3)**:** 295-301.

Goldman RD (2010). ADHD stimulants and their effect on height in children. *Canadian family physician Medecin de famille canadien* **56**(2)**:** 145-146.

Gomez P, Ratcliff R, Perea M (2007). A model of the go/no-go task. *Journal of experimental psychology. General* **136**(3)**:** 389-413.

Gonon F (2009). The dopaminergic hypothesis of attention-deficit/hyperactivity disorder needs re-examining. *Trends Neurosci* **32**(1)**:** 2-8.

Gonon FG (1988). Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Neuroscience* **24**(1)**:** 19-28.

Gouzoulis-Mayfrank E, Daumann J (2009). Neurotoxicity of drugs of abuse--the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues in clinical neuroscience* **11**(3)**:** 305-317.

Grace AA, Bunney BS (1984). The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci* **4**(11)**:** 2877-2890.

Grace AA, Bunney BS (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons--1. Identification and characterization. *Neuroscience* **10**(2)**:** 301-315.

Grados MA, Atkins EB, Kovacikova GI, McVicar E (2015). A selective review of glutamate pharmacological therapy in obsessive-compulsive and related disorders. *Psychology research and behavior management* **8:** 115-131.

Graham DL, Herring NR, Schaefer TL, Vorhees CV, Williams MT (2010). Glucose and corticosterone changes in developing and adult rats following exposure to (+/-)-3,4-methylendioxymethamphetamine or 5-methoxydiisopropyltryptamine. *Neurotoxicology and teratology* **32**(2)**:** 152-157.

Graham J, Banaschewski T, Buitelaar J, Coghill D, Danckaerts M, Dittmann RW, *et al.* (2011). European guidelines on managing adverse effects of medication for ADHD. *Eur Child Adolesc Psychiatry* **20**(1)**:** 17-37.

Greenhill LL, Findling RL, Swanson JM, Group AS (2002). A double-blind, placebo-controlled study of modified-release methylphenidate in children with attention-deficit/hyperactivity disorder. *Pediatrics* **109**(3)**:** E39.

Greenwald MK, Lundahl LH, Steinmiller CL (2010). Sustained release d-amphetamine reduces cocaine but not 'speedball'-seeking in buprenorphine-maintained volunteers: a test of dual-agonist pharmacotherapy for cocaine/heroin polydrug abusers. *Neuropsychopharmacology* **35**(13)**:** 2624-2637.

Grenhoff J, Aston-Jones G, Svensson TH (1986). Nicotinic effects on the firing pattern of midbrain dopamine neurons. *Acta physiologica Scandinavica* **128**(3)**:** 351-358.

Grenhoff J, Nisell M, Ferre S, Aston-Jones G, Svensson TH (1993a). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *Journal of neural transmission. General section* **93**(1)**:** 11-25.

Grenhoff J, Svensson TH (1993b). Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *Eur J Pharmacol* **233**(1)**:** 79-84.

Griesar WS, Zajdel DP, Oken BS (2002). Nicotine effects on alertness and spatial attention in non-smokers. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* **4**(2)**:** 185-194.

Gronier B (2011). In vivo electrophysiological effects of methylphenidate in the prefrontal cortex: involvement of dopamine D1 and alpha 2 adrenergic receptors. *Eur Neuropsychopharmacol* **21**(2)**:** 192-204.

Gronier B, Aston J, Liauzun C, Zetterstrom T (2010). Age-dependent effects of methylphenidate in the prefrontal cortex: evidence from electrophysiological and Arc gene expression measurements. *J Psychopharmacol* **24**(12)**:** 1819-1827.

Gronier B, Waters S, Ponten H (2013). The dopaminergic stabilizer pridopidine increases neuronal activity of pyramidal neurons in the prefrontal cortex. *Journal of neural transmission* **120**(9)**:** 1281-1294.

Guan L, Wang B, Chen Y, Yang L, Li J, Qian Q, *et al.* (2009). A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: suggesting multiple susceptibility genes among Chinese Han population. *Mol Psychiatry* **14**(5)**:** 546-554.

Guerrini I, Gentili C, Nelli G, Guazzelli M (2006). A follow up study on the efficacy of metadoxine in the treatment of alcohol dependence. *Substance abuse treatment, prevention, and policy* **1:** 35.

Guiard BP, El Mansari M, Blier P (2008). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Molecular pharmacology* **74**(5)**:** 1463-1475.

Guillem AM, Martinez-Lozada Z, Hernandez-Kelly LC, Lopez-Bayghen E, Lopez-Bayghen B, Calleros OA, *et al.* (2015). Methylphenidate Increases Glutamate Uptake in Bergmann Glial Cells. *Neurochem Res* **40**(11)**:** 2317-2324.

Gulley JM, Zahniser NR (2003). Rapid regulation of dopamine transporter function by substrates, blockers and presynaptic receptor ligands. *Eur J Pharmacol* **479**(1-3)**:** 139-152.

Guo W, Zou S, Guan Y, Ikeda T, Tal M, Dubner R, *et al.* (2002). Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia. *J Neurosci* **22**(14)**:** 6208-6217.

Gupta D, Radhakrishnan M, Kurhe Y, Thangaraj D, Prabhakar V, Kanade P (2014). Antidepressant-like effects of a novel 5-HT3 receptor antagonist 6z in acute and chronic murine models of depression. *Acta pharmacologica Sinica* **35**(12)**:** 1493-1503.

Gutierrez-Arenas O, Eriksson O, Kotaleski JH (2014). Segregation and crosstalk of D1 receptor-mediated activation of ERK in striatal medium spiny neurons upon acute administration of psychostimulants. *PLoS computational biology* **10**(1)**:** e1003445.

Ha JS, Park SS (2006). Glutamate-induced oxidative stress, but not cell death, is largely dependent upon extracellular calcium in mouse neuronal HT22 cells. *Neurosci Lett* **393**(2-3)**:** 165-169.

Hajnal A, Smith GP, Norgren R (2004). Oral sucrose stimulation increases accumbens dopamine in the rat. *American journal of physiology. Regulatory, integrative and comparative physiology* **286**(1)**:** R31-37.

Hajos M, Allers KA, Jennings K, Sharp T, Charette G, Sik A, *et al.* (2007). Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. *The European journal of neuroscience* **25**(1)**:** 119-126.

Hajos M, Gartside SE, Villa AE, Sharp T (1995). Evidence for a repetitive (burst) firing pattern in a sub-population of 5-hydroxytryptamine neurons in the dorsal and median raphe nuclei of the rat. *Neuroscience* **69**(1)**:** 189-197.

Hajos M, Sharp T (1996). Burst-firing activity of presumed 5-HT neurones of the rat dorsal raphe nucleus: electrophysiological analysis by antidromic stimulation. *Brain research* **740**(1-2)**:** 162-168.

Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW (2006). Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. *J Neurosci* **26**(17)**:** 4690-4700.

Hammerness P, Biederman J, Petty C, Henin A, Moore CM (2012). Brain biochemical effects of methylphenidate treatment using proton magnetic spectroscopy in youth with attention-

deficit hyperactivity disorder: a controlled pilot study. *CNS neuroscience & therapeutics* **18**(1)**:** 34-40.

Hammerness P, Joshi G, Doyle R, Georgiopoulos A, Geller D, Spencer T, *et al.* (2013). Do stimulants reduce the risk for cigarette smoking in youth with attention-deficit hyperactivity disorder? A prospective, long-term, open-label study of extended-release methylphenidate. *The Journal of pediatrics* **162**(1)**:** 22-27 e22.

Han D, Sen CK, Roy S, Kobayashi MS, Tritschler HJ, Packer L (1997). Protection against glutamate-induced cytotoxicity in C6 glial cells by thiol antioxidants. *The American journal of physiology* **273**(5 Pt 2)**:** R1771-1778.

Han DD, Gu HH (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC pharmacology* **6:** 6.

Han DH, Bolo N, Daniels MA, Arenella L, Lyoo IK, Renshaw PF (2011). Brain activity and desire for Internet video game play. *Comprehensive psychiatry* **52**(1)**:** 88-95.

Hand DJ, Fox AT, Reilly MP (2009). Differential effects of d-amphetamine on impulsive choice in spontaneously hypertensive and Wistar-Kyoto rats. *Behavioural pharmacology* **20**(5-6)**:** 549-553.

Hardy SE (2009). Methylphenidate for the treatment of depressive symptoms, including fatigue and apathy, in medically ill older adults and terminally ill adults. *The American journal of geriatric pharmacotherapy* **7**(1)**:** 34-59.

Harfterkamp M, van de Loo-Neus G, Minderaa RB, van der Gaag RJ, Escobar R, Schacht A, *et al.* (2012). A randomized double-blind study of atomoxetine versus placebo for attention-deficit/hyperactivity disorder symptoms in children with autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* **51**(7)**:** 733-741.

Harstad EB, Weaver AL, Katusic SK, Colligan RC, Kumar S, Chan E, *et al.* (2014). ADHD, stimulant treatment, and growth: a longitudinal study. *Pediatrics* **134**(4)**:** e935-944.

Hart CL, Haney M, Vosburg SK, Rubin E, Foltin RW (2008). Smoked cocaine self-administration is decreased by modafinil. *Neuropsychopharmacology* **33**(4)**:** 761-768.

Hart H, Radua J, Nakao T, Mataix-Cols D, Rubia K (2013). Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: exploring task-specific, stimulant medication, and age effects. *JAMA Psychiatry* **70**(2)**:** 185-198.

Hasan MT, Hernandez-Gonzalez S, Dogbevia G, Trevino M, Bertocchi I, Gruart A, *et al.* (2013). Role of motor cortex NMDA receptors in learning-dependent synaptic plasticity of behaving mice. *Nature communications* **4:** 2258.

Hasbani MJ, Schlief ML, Fisher DA, Goldberg MP (2001). Dendritic spines lost during glutamate receptor activation reemerge at original sites of synaptic contact. *J Neurosci* **21**(7)**:** 2393-2403.

Hassani E, Mahoori A, Aghdashi MM, Pirnejad H (2015). Evaluating the quality of intravenous regional anesthesia following adding dexamethasone to lidocaine. *Saudi journal of anaesthesia* **9**(4)**:** 418-421.

Heal DJ, Cheetham SC, Smith SL (2009). The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety. *Neuropharmacology* **57**(7-8)**:** 608-618.

Hechtman L, Abikoff H, Klein RG, Weiss G, Respitz C, Kouri J, *et al.* (2004). Academic achievement and emotional status of children with ADHD treated with long-term methylphenidate and multimodal psychosocial treatment. *Journal of the American Academy of Child and Adolescent Psychiatry* **43**(7)**:** 812-819.

Hemrick-Luecke SK, Henderson MG, Fuller RW (1992). MK801 antagonism of the prolonged depletion of striatal dopamine by amphetamine in iprindole-treated rats. *Life sciences* **50**(6)**:** PL31-33.

Hendrickx MO (2015). Lidocaine use in pigs, cattle and horses. *The Veterinary record* **176**(24)**:** 630.

Henley JM, Wilkinson KA (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. *Dialogues in clinical neuroscience* **15**(1)**:** 11-27.

Hennessy MB, McCowan B, Jiang J, Capitanio JP (2014). Depressive-like behavioral response of adult male rhesus monkeys during routine animal husbandry procedure. *Frontiers in behavioral neuroscience* **8:** 309.

Hermeto LC, DeRossi R, Marques BC, Jardim PH (2015). Potentiation of epidural lidocaine by co-administering tramadol by either intramuscular or epidural route in cats. *Canadian journal of veterinary research = Revue canadienne de recherche veterinaire* **79**(3)**:** 214-220.

Herr NR, Daniel KB, Belle AM, Carelli RM, Wightman RM (2010). Probing presynaptic regulation of extracellular dopamine with iontophoresis. *ACS chemical neuroscience* **1**(9)**:** 627-638.

Hibi M, Shimizu T (2012). Development of the cerebellum and cerebellar neural circuits. *Developmental neurobiology* **72**(3)**:** 282-301.

Hill SL, El-Khayat RH, Sandilands EA, Thomas SH (2010). Electrocardiographic effects of methylphenidate overdose. *Clinical toxicology* **48**(4)**:** 342-346.

Hillman J, Aneman O, Anderson C, Sjogren F, Saberg C, Mellergard P (2005). A microdialysis technique for routine measurement of macromolecules in the injured human brain. *Neurosurgery* **56**(6)**:** 1264-1268; discussion 1268-1270.

Hilton DL, Watts C (2011). Pornography addiction: A neuroscience perspective. *Surgical neurology international* **2:** 19.

Hinerth MA, Collins HA, Baniecki M, Hanson RN, Waszczak BL (2000). Novel in vivo electrophysiological assay for the effects of cocaine and putative "cocaine antagonists" on dopamine transporter activity of substantia nigra and ventral tegmental area dopamine neurons. *Synapse* **38**(3)**:** 305-312.

Hnasko TS, Hjelmstad GO, Fields HL, Edwards RH (2012). Ventral tegmental area glutamate neurons: electrophysiological properties and projections. *J Neurosci* **32**(43)**:** 15076-15085.

Hodgkin AL, Huxley AF (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of physiology* **117**(4)**:** 500-544.

Hodgson K, Hutchinson AD, Denson L (2014). Nonpharmacological treatments for ADHD: a meta-analytic review. *J Atten Disord* **18**(4)**:** 275-282.

Hoebel BG, Avena NM, Bocarsly ME, Rada P (2009). Natural addiction: a behavioral and circuit model based on sugar addiction in rats. *J Addict Med* **3**(1)**:** 33-41.

Hoggatt AF, Hoggatt J, Honerlaw M, Pelus LM (2010). A spoonful of sugar helps the medicine go down: a novel technique to improve oral gavage in mice. *Journal of the American Association for Laboratory Animal Science : JAALAS* **49**(3)**:** 329-334.

Hole LD, Schjott J (2014). Myocardial injury in a 41-year-old male treated with methylphenidate: a case report. *BMC research notes* **7:** 480.

Holtzman SG (1987). Discriminative stimulus effects of caffeine: tolerance and cross-tolerance with methylphenidate. *Life sciences* **40**(4)**:** 381-389.

Honarmand A, Safavi M, Nemati K, Oghab P (2015). The efficacy of different doses of Midazolam added to Lidocaine for upper extremity Bier block on the sensory and motor block characteristics and postoperative pain. *Journal of research in pharmacy practice* **4**(3)**:** 160-166.

Hong SB, Harrison BJ, Fornito A, Sohn CH, Song IC, Kim JW (2015). Functional dysconnectivity of corticostriatal circuitry and differential response to methylphenidate in youth with attention-deficit/hyperactivity disorder. *J Psychiatry Neurosci* **40**(1)**:** 46-57.

Horn AS (1973). Structure-activity relations for the inhibition of catecholamine uptake into synaptosomes from noradrenaline and dopaminergic neurones in rat brain homogenates. *British journal of pharmacology* **47**(2)**:** 332-338.

Hove MJ, Zeffiro TA, Biederman J, Li Z, Schmahmann J, Valera EM (2015). Postural sway and regional cerebellar volume in adults with attention-deficit/hyperactivity disorder. *NeuroImage. Clinical* **8:** 422-428.

Howell LL, Negus SS (2014). Monoamine transporter inhibitors and substrates as treatments for stimulant abuse. *Advances in pharmacology* **69:** 129-176.

Hoza B, Smith AL, Shoulberg EK, Linnea KS, Dorsch TE, Blazo JA, *et al.* (2015). A randomized trial examining the effects of aerobic physical activity on attention-deficit/hyperactivity disorder symptoms in young children. *Journal of abnormal child psychology* **43**(4)**:** 655-667.

Hu JL, Liu G, Li YC, Gao WJ, Huang YQ (2010). Dopamine D1 receptor-mediated NMDA receptor insertion depends on Fyn but not Src kinase pathway in prefrontal cortical neurons. *Molecular brain* **3:** 20.

Hu WH, Bi YF, Zhang K, Meng FG, Zhang JG (2011). High-frequency electrical stimulation in the nucleus accumbens of morphine-treated rats suppresses neuronal firing in reward-related brain regions. *Medical science monitor : international medical journal of experimental and clinical research* **17**(6)**:** BR153-160.

Huang-Brown KM, Guhad FA (2002). Chocolate, an effective means of oral drug delivery in rats. *Lab animal* **31**(10)**:** 34-36.

Huang EJ, Reichardt LF (2001). Neurotrophins: roles in neuronal development and function. *Annual review of neuroscience* **24:** 677-736.

Hughes RN (1972). Methylphenidate induced inhibition of exploratory behaviour in rats. *Life sciences. Pt. 1: Physiology and pharmacology* **11**(4)**:** 161-167.

Humphries ES, Dart C (2015). Neuronal and Cardiovascular Potassium Channels as Therapeutic Drug Targets: Promise and Pitfalls. *Journal of biomolecular screening* **20**(9)**:** 1055-1073.

Hunt JM, Redman RS, Silinsky EM (1994). Reduction by intracellular calcium chelation of acetylcholine secretion without occluding the effects of adenosine at frog motor nerve endings. *British journal of pharmacology* **111**(3)**:** 753-758.

Hunter RE, Barrera CM, Dohanich GP, Dunlap WP (1990). Effects of uric acid and caffeine on A1 adenosine receptor binding in developing rat brain. *Pharmacol Biochem Behav* **35**(4)**:** 791-795.

Hurt RD, Ebbert JO, Croghan IT, Schroeder DR, Sood A, Hays JT (2011). Methylphenidate for treating tobacco dependence in non-attention deficit hyperactivity disorder smokers: a pilot randomized placebo-controlled trial. *J Negat Results Biomed* **10:** 1.

Hurtig T, Ebeling H, Taanila A, Miettunen J, Smalley SL, McGough JJ, *et al.* (2007). ADHD symptoms and subtypes: relationship between childhood and adolescent symptoms. *Journal of the American Academy of Child and Adolescent Psychiatry* **46**(12)**:** 1605-1613.

Imbert B, Labrune N, Lancon C, Simon N (2014). Use of extended-release methylphenidate (concerta) in treatment of cocaine dependence in a patient presenting with attention deficit hyperactivity disorder. *The Journal of neuropsychiatry and clinical neurosciences* **26**(3)**:** E49-50.

Ioannidis K, Chamberlain SR, Muller U (2014). Ostracising caffeine from the pharmacological arsenal for attention-deficit hyperactivity disorder--was this a correct decision? A literature review. *J Psychopharmacol* **28**(9)**:** 830-836.

Itier V, Bertrand D (2001). Neuronal nicotinic receptors: from protein structure to function. *FEBS letters* **504**(3)**:** 118-125.

Itokawa M, Lin Z, Uhl GR (2002). Dopamine efflux via wild-type and mutant dopamine transporters: alanine substitution for proline-572 enhances efflux and reduces dependence on extracellular dopamine, sodium and chloride concentrations. *Brain research. Molecular brain research* **108**(1-2)**:** 71-80.

Ivanov I, Bansal R, Hao X, Zhu H, Kellendonk C, Miller L, *et al.* (2010). Morphological abnormalities of the thalamus in youths with attention deficit hyperactivity disorder. *The American journal of psychiatry* **167**(4)**:** 397-408.

Ivanov I, Murrough JW, Bansal R, Hao X, Peterson BS (2014). Cerebellar morphology and the effects of stimulant medications in youths with attention deficit-hyperactivity disorder. *Neuropsychopharmacology* **39**(3)**:** 718-726.

Iwamoto ET (1984). An assessment of the spontaneous activity of rats administered morphine, phencyclidine, or nicotine using automated and observational methods. *Psychopharmacology (Berl)* **84**(3)**:** 374-382.

Jaffe SL (1991). Intranasal abuse of prescribed methylphenidate by an alcohol and drug abusing adolescent with ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry* **30**(5)**:** 773-775.

Jahng JW, Yoo SB, Ryu V, Lee JH (2012). Hyperphagia and depression-like behavior by adolescence social isolation in female rats. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience* **30**(1)**:** 47-53.

Jalabert M, Bourdy R, Courtin J, Veinante P, Manzoni OJ, Barrot M, *et al.* (2011). Neuronal circuits underlying acute morphine action on dopamine neurons. *Proc Natl Acad Sci U S A* **108**(39)**:** 16446-16450.

James A, Lai FH, Dahl C (2004). Attention deficit hyperactivity disorder and suicide: a review of possible associations. *Acta psychiatrica Scandinavica* **110**(6)**:** 408-415.

Jarrar SF, Obeid OA (2014). Timing of caffeine ingestion alters postprandial metabolism in rats. *Nutrition* **30**(1)**:** 107-111.

Jennings KA (2013). A comparison of the subsecond dynamics of neurotransmission of dopamine and serotonin. *ACS chemical neuroscience* **4**(5)**:** 704-714.

Jensen L, Pagsberg A, Dalhoff K (2014). Methylphenidate misuse in adult patients and the impact of therapeutic use. *Human & experimental toxicology*.

Jiang ZG, North RA (1991). Membrane properties and synaptic responses of rat striatal neurones in vitro. *The Journal of physiology* **443:** 533-553.

Johansen EB, Fonnum F, Lausund PL, Walaas SI, Baerland NE, Woien G, *et al.* (2014). Behavioral changes following PCB 153 exposure in the spontaneously hypertensive rat - an animal model of Attention-Deficit/Hyperactivity Disorder. *Behav Brain Funct* **10:** 1.

Johnson RJ, Gold MS, Johnson DR, Ishimoto T, Lanaspa MA, Zahniser NR, *et al.* (2011). Attention-deficit/hyperactivity disorder: is it time to reappraise the role of sugar consumption? *Postgraduate medicine* **123**(5)**:** 39-49.

Johnson SW, Seutin V, North RA (1992). Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. *Science* **258**(5082)**:** 665-667.

Johnston J, Forsythe ID, Kopp-Scheinpflug C (2010). Going native: voltage-gated potassium channels controlling neuronal excitability. *The Journal of physiology* **588**(Pt 17)**:** 3187-3200.

Jones BE, Moore RY (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain research* **127**(1)**:** 25-53.

Jones SR, Gainetdinov RR, Wightman RM, Caron MG (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J Neurosci* **18**(6)**:** 1979-1986.

Jones Z, Dafny N (2014). Acute and chronic dose-response effect of methylphenidate on ventral tegmental area neurons correlated with animal behavior. *Journal of neural transmission* **121**(3)**:** 327-345.

Jones Z, Dafny N (2013). Dose response effect of methylphenidate on ventral tegmental area neurons and animal behavior. *Brain research bulletin* **96:** 86-92.

Jordan CJ, Harvey RC, Baskin BB, Dwoskin LP, Kantak KM (2014). Cocaine-seeking behavior in a genetic model of attention-deficit/hyperactivity disorder following adolescent methylphenidate or atomoxetine treatments. *Drug Alcohol Depend* **140:** 25-32.

Jurado S (2014). The dendritic SNARE fusion machinery involved in AMPARs insertion during long-term potentiation. *Frontiers in cellular neuroscience* **8:** 407.

Justo CC, Carneiro-de-Oliveira PE, Delucia R, Aizenstein ML, Planeta CS (2010). Repeated exposure of adolescent rats to oral methylphenidate does not induce behavioral sensitization or cross-sensitization to nicotine. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.]* **43**(7)**:** 651-656.

Jutkiewicz EM, Nicolazzo DM, Kim MN, Gnegy ME (2008). Nicotine and amphetamine acutely cross-potentiate their behavioral and neurochemical responses in female Holtzman rats. *Psychopharmacology (Berl)* **200**(1)**:** 93-103.

Kahlig KM, Binda F, Khoshbouei H, Blakely RD, McMahon DG, Javitch JA, *et al.* (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proc Natl Acad Sci U S A* **102**(9)**:** 3495-3500.

Kahn BB, Shulman GI, DeFronzo RA, Cushman SW, Rossetti L (1991). Normalization of blood glucose in diabetic rats with phlorizin treatment reverses insulin-resistant glucose transport in adipose cells without restoring glucose transporter gene expression. *The Journal of clinical investigation* **87**(2)**:** 561-570.

Kalivas PW (2007). Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. *Dialogues in clinical neuroscience* **9**(4)**:** 389-397.

Kalivas PW (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Brain Res Rev* **18**(1)**:** 75-113.

Kalivas PW, Alesdatter JE (1993). Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther* **267**(1)**:** 486-495.

Kantor L, Hewlett GH, Gnegy ME (1999). Enhanced amphetamine- and K+-mediated dopamine release in rat striatum after repeated amphetamine: differential requirements for Ca2+- and calmodulin-dependent phosphorylation and synaptic vesicles. *J Neurosci* **19**(10)**:** 3801-3808.

Kantor L, Hewlett GH, Park YH, Richardson-Burns SM, Mellon MJ, Gnegy ME (2001). Protein kinase C and intracellular calcium are required for amphetamine-mediated dopamine release via the norepinephrine transporter in undifferentiated PC12 cells. *J Pharmacol Exp Ther* **297**(3)**:** 1016-1024.

Kantor L, Park YH, Wang KK, Gnegy M (2002). Enhanced amphetamine-mediated dopamine release develops in PC12 cells after repeated amphetamine treatment. *Eur J Pharmacol* **451**(1)**:** 27-35.

Kapoor A, Shandilya M, Kundu S (2011). Structural insight of dopamine beta-hydroxylase, a drug target for complex traits, and functional significance of exonic single nucleotide polymorphisms. *PloS one* **6**(10)**:** e26509.

Kasai H (1993). Cytosolic Ca2+ gradients, Ca2+ binding proteins and synaptic plasticity. *Neuroscience research* **16**(1)**:** 1-7.

Kash TL, Matthews RT, Winder DG (2008). Alcohol inhibits NR2B-containing NMDA receptors in the ventral bed nucleus of the stria terminalis. *Neuropsychopharmacology* **33**(6)**:** 1379-1390.

Kashani J, Ruha AM (2007). Isolated atomoxetine overdose resulting in seizure. *The Journal of emergency medicine* **32**(2)**:** 175-178.

Kasparbauer AM, Rujescu D, Riedel M, Pogarell O, Costa A, Meindl T, *et al.* (2015). Methylphenidate effects on brain activity as a function of SLC6A3 genotype and striatal dopamine transporter availability. *Neuropsychopharmacology* **40**(3)**:** 736-745.

Kauer JA, Malenka RC (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**(11)**:** 844-858.

Kawaguchi Y, Aosaki T, Kubota Y (1997). Cholinergic and GABAergic interneurons in the striatum. *Nihon shinkei seishin yakurigaku zasshi = Japanese journal of psychopharmacology* **17**(2)**:** 87-90.

Kayser AS, Allen DC, Navarro-Cebrian A, Mitchell JM, Fields HL (2012a). Dopamine, corticostriatal connectivity, and intertemporal choice. *J Neurosci* **32**(27)**:** 9402-9409.

Kayser C, Petkov CI, Remedios R, Logothetis NK (2012b). Multisensory Influences on Auditory Processing: Perspectives from fMRI and Electrophysiology. In: Murray MM, Wallace MT (ed)^(eds). *The Neural Bases of Multisensory Processes*, edn. Boca Raton (FL). p^pp.

Kebir O, Tabbane K, Sengupta S, Joober R (2009). Candidate genes and neuropsychological phenotypes in children with ADHD: review of association studies. *J Psychiatry Neurosci* **34**(2)**:** 88-101.

Keeler GD, Durdik JM, Stenken JA (2014). Comparison of microdialysis sampling perfusion fluid components on the foreign body reaction in rat subcutaneous tissue. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* **57:** 60-67.

Kemp JM, Powell TP (1971). The synaptic organization of the caudate nucleus. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **262**(845)**:** 403-412.

Kerr CW, Drake J, Milch RA, Brazeau DA, Skretny JA, Brazeau GA, *et al.* (2012). Effects of methylphenidate on fatigue and depression: a randomized, double-blind, placebo-controlled trial. *Journal of pain and symptom management* **43**(1)**:** 68-77.

Kessler D, Angstadt M, Welsh RC, Sripada C (2014). Modality-spanning deficits in attention-deficit/hyperactivity disorder in functional networks, gray matter, and white matter. *J Neurosci* **34**(50)**:** 16555-16566.

Kessler RC, Adler L, Barkley R, Biederman J, Conners CK, Demler O, *et al.* (2006). The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. *The American journal of psychiatry* **163**(4)**:** 716-723.

Kessler RC, Adler LA, Barkley R, Biederman J, Conners CK, Faraone SV, *et al.* (2005). Patterns and predictors of attention-deficit/hyperactivity disorder persistence into adulthood: results from the national comorbidity survey replication. *Biol Psychiatry* **57**(11)**:** 1442-1451.

Khan JS, Yousuf M, Victor JC, Sharma A, Siddiqui N (2015). An estimation for an appropriate end time for an intraoperative intravenous lidocaine infusion in bowel surgery: a comparative meta-analysis. *Journal of clinical anesthesia*.

Khantzian EJ (1983). An extreme case of cocaine dependence and marked improvement with methylphenidate treatment. *The American journal of psychiatry* **140**(6)**:** 784-785.

Khantzian EJ, Gawin F, Kleber HD, Riordan CE (1984). Methylphenidate (Ritalin) treatment of cocaine dependence--a preliminary report. *Journal of substance abuse treatment* **1**(2)**:** 107-112.

Kheramin S, Body S, Mobini S, Ho MY, Velazquez-Martinez DN, Bradshaw CM, *et al.* (2002). Effects of quinolinic acid-induced lesions of the orbital prefrontal cortex on inter-temporal choice: a quantitative analysis. *Psychopharmacology (Berl)* **165**(1)**:** 9-17.

Khvotchev M, Lonart G, Sudhof TC (2000). Role of calcium in neurotransmitter release evoked by alpha-latrotoxin or hypertonic sucrose. *Neuroscience* **101**(3)**:** 793-802.

Kieling C, Goncalves RR, Tannock R, Castellanos FX (2008). Neurobiology of attention deficit hyperactivity disorder. *Child and adolescent psychiatric clinics of North America* **17**(2)**:** 285-307, viii.

Kim JI, Ganesan S, Luo SX, Wu YW, Park E, Huang EJ, *et al.* (2015). Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons. *Science* **350**(6256)**:** 102-106.

Kim MN, Jutkiewicz EM, Zhang M, Gnegy ME (2011a). The sensitizing effect of acute nicotine on amphetamine-stimulated behavior and dopamine efflux requires activation of beta2 subunit-containing nicotinic acetylcholine receptors and glutamate N-methyl-D-aspartate receptors. *Neuropharmacology* **60**(7-8)**:** 1126-1134.

Kim Y, Chang H (2011b). Correlation between attention deficit hyperactivity disorder and sugar consumption, quality of diet, and dietary behavior in school children. *Nutrition research and practice* **5**(3)**:** 236-245.

Kim Y, Teylan MA, Baron M, Sands A, Nairn AC, Greengard P (2009). Methylphenidate-induced dendritic spine formation and DeltaFosB expression in nucleus accumbens. *Proc Natl Acad Sci U S A* **106**(8)**:** 2915-2920.

Kimko HC, Cross JT, Abernethy DR (1999). Pharmacokinetics and clinical effectiveness of methylphenidate. *Clin Pharmacokinet* **37**(6)**:** 457-470.

Kirkpatrick MG, Gunderson EW, Johanson CE, Levin FR, Foltin RW, Hart CL (2012). Comparison of intranasal methamphetamine and d-amphetamine self-administration by humans. *Addiction* **107**(4)**:** 783-791.

Kitai ST, Shepard PD, Callaway JC, Scroggs R (1999). Afferent modulation of dopamine neuron firing patterns. *Current opinion in neurobiology* **9**(6)**:** 690-697.

Kiyatkin EA, Rebec GV (1998). Heterogeneity of ventral tegmental area neurons: single-unit recording and iontophoresis in awake, unrestrained rats. *Neuroscience* **85**(4)**:** 1285-1309.

Klein RG, Landa B, Mattes JA, Klein DF (1988). Methylphenidate and growth in hyperactive children. A controlled withdrawal study. *Archives of general psychiatry* **45**(12)**:** 1127-1130.

Kobayashi T, Washiyama K, Ikeda K (2010). Inhibition of G-protein-activated inwardly rectifying K+ channels by the selective norepinephrine reuptake inhibitors atomoxetine and reboxetine. *Neuropsychopharmacology* **35**(7)**:** 1560-1569.

Koda K, Ago Y, Cong Y, Kita Y, Takuma K, Matsuda T (2010). Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice. *Journal of neurochemistry* **114**(1)**:** 259-270.

Koff JM, Shuster L, Miller LG (1994). Chronic cocaine administration is associated with behavioral sensitization and time-dependent changes in striatal dopamine transporter binding. *J Pharmacol Exp Ther* **268**(1)**:** 277-282.

Kolar D, Keller A, Golfinopoulos M, Cumyn L, Syer C, Hechtman L (2008). Treatment of adults with attention-deficit/hyperactivity disorder. *Neuropsychiatric disease and treatment* **4**(2)**:** 389-403.

Kollins SH, Anastopoulos AD, Lachiewicz AM, FitzGerald D, Morrissey-Kane E, Garrett ME, *et al.* (2008). SNPs in dopamine D2 receptor gene (DRD2) and norepinephrine transporter gene (NET) are associated with continuous performance task (CPT) phenotypes in ADHD children and their families. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **147B**(8)**:** 1580-1588.

Komatsu DE, Thanos PK, Mary MN, Janda HA, John CM, Robison L, *et al.* (2012). Chronic exposure to methylphenidate impairs appendicular bone quality in young rats. *Bone* **50**(6)**:** 1214-1222.

Konova AB, Moeller SJ, Tomasi D, Goldstein RZ (2015). Effects of chronic and acute stimulants on brain functional connectivity hubs. *Brain research*.

Konova AB, Moeller SJ, Tomasi D, Volkow ND, Goldstein RZ (2013). Effects of methylphenidate on resting-state functional connectivity of the mesocorticolimbic dopamine pathways in cocaine addiction. *JAMA Psychiatry* **70**(8)**:** 857-868.

Konstenius M, Jayaram-Lindstrom N, Beck O, Franck J (2010). Sustained release methylphenidate for the treatment of ADHD in amphetamine abusers: a pilot study. *Drug Alcohol Depend* **108**(1-2)**:** 130-133.

Koob GF, Volkow ND (2010). Neurocircuitry of addiction. *Neuropsychopharmacology* **35**(1)**:** 217-238.

Kooistra L, Ramage B, Crawford S, Cantell M, Wormsbecker S, Gibbard B, *et al.* (2009). Can attention deficit hyperactivity disorder and fetal alcohol spectrum disorder be differentiated by motor and balance deficits? *Human movement science* **28**(4)**:** 529-542.

Korinek M, Kapras V, Vyklicky V, Adamusova E, Borovska J, Vales K, *et al.* (2011). Neurosteroid modulation of N-methyl-D-aspartate receptors: molecular mechanism and behavioral effects. *Steroids* **76**(13)**:** 1409-1418.

Kostrzewa JP, Kostrzewa RA, Kostrzewa RM, Brus R, Nowak P (2015). Perinatal 6-Hydroxydopamine Modeling of ADHD. *Current topics in behavioral neurosciences*.

Kostrzewa RM, Brus R, Kalbfleisch JH, Perry KW, Fuller RW (1994). Proposed animal model of attention deficit hyperactivity disorder. *Brain research bulletin* **34**(2)**:** 161-167.

Koyama S, Mori M, Kanamaru S, Sazawa T, Miyazaki A, Terai H, *et al.* (2014). Obesity attenuates D2 autoreceptor-mediated inhibition of putative ventral tegmental area dopaminergic neurons. *Physiological reports* **2**(5)**:** e12004.

Kranke P, Jokinen J, Pace NL, Schnabel A, Hollmann MW, Hahnenkamp K, *et al.* (2015). Continuous intravenous perioperative lidocaine infusion for postoperative pain and recovery. *Cochrane Database Syst Rev* **7:** CD009642.

Kratochvil CJ, Newcorn JH, Arnold LE, Duesenberg D, Emslie GJ, Quintana H, *et al.* (2005). Atomoxetine alone or combined with fluoxetine for treating ADHD with comorbid depressive or anxiety symptoms. *Journal of the American Academy of Child and Adolescent Psychiatry* **44**(9)**:** 915-924.

Krause J, Krause KH, Dresel SH, la Fougere C, Ackenheil M (2006). ADHD in adolescence and adulthood, with a special focus on the dopamine transporter and nicotine. *Dialogues in clinical neuroscience* **8**(1)**:** 29-36.

Krause J, la Fougere C, Krause KH, Ackenheil M, Dresel SH (2005). Influence of striatal dopamine transporter availability on the response to methylphenidate in adult patients with ADHD. *European archives of psychiatry and clinical neuroscience* **255**(6)**:** 428-431.

Krause KH, Dresel SH, Krause J, Kung HF, Tatsch K (2000). Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. *Neurosci Lett* **285**(2)**:** 107-110.

Krause KH, Dresel SH, Krause J, Kung HF, Tatsch K, Ackenheil M (2002). Stimulant-like action of nicotine on striatal dopamine transporter in the brain of adults with attention deficit hyperactivity disorder. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* **5**(2)**:** 111-113.

Krause KH, Dresel SH, Krause J, la Fougere C, Ackenheil M (2003). The dopamine transporter and neuroimaging in attention deficit hyperactivity disorder. *Neurosci Biobehav Rev* **27**(7)**:** 605-613.

Kritis AA, Stamoula EG, Paniskaki KA, Vavilis TD (2015). Researching glutamate - induced cytotoxicity in different cell lines: a comparative/collective analysis/study. *Frontiers in cellular neuroscience* **9:** 91.

Kritzer MF, Goldman-Rakic PS (1995). Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefrontal cortex in the rhesus monkey. *The Journal of comparative neurology* **359**(1)**:** 131-143.

Kruse MS, Premont J, Krebs MO, Jay TM (2009). Interaction of dopamine D1 with NMDA NR1 receptors in rat prefrontal cortex. *Eur Neuropsychopharmacol* **19**(4)**:** 296-304.

Ksir C (1994). Acute and chronic nicotine effects on measures of activity in rats: a multivariate analysis. *Psychopharmacology (Berl)* **115**(1-2)**:** 105-109.

Kubera M, Filip M, Basta-Kaim A, Nowak E, Budziszewska B, Tetich M, *et al.* (2002). The effect of amphetamine sensitization on mouse immunoreactivity. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* **53**(2)**:** 233-242.

Kucyi A, Hove MJ, Biederman J, Van Dijk KR, Valera EM (2015). Disrupted functional connectivity of cerebellar default network areas in attention-deficit/hyperactivity disorder. *Hum Brain Mapp* **36**(9)**:** 3373-3386.

Kuczenski R, Segal DS (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *Journal of neurochemistry* **68**(5)**:** 2032-2037.

Kuczenski R, Segal DS (2002). Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci* **22**(16)**:** 7264-7271.

Kuriyama K, Honma M, Shimazaki M, Horie M, Yoshiike T, Koyama S, *et al.* (2011). An N-methyl-D-aspartate receptor agonist facilitates sleep-independent synaptic plasticity associated with working memory capacity enhancement. *Scientific reports* **1:** 127.

Kuster T, Zumkehr B, Hermann C, Theurillat R, Thormann W, Gottstein B, *et al.* (2012). Voluntary ingestion of antiparasitic drugs emulsified in honey represents an alternative to gavage in mice. *Journal of the American Association for Laboratory Animal Science : JAALAS* **51**(2)**:** 219-223.

Kuzhikandathil EV, Oxford GS (2002). Classic D1 dopamine receptor antagonist R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) directly inhibits G protein-coupled inwardly rectifying potassium channels. *Molecular pharmacology* **62**(1)**:** 119-126.

Labonte B, McLaughlin RJ, Dominguez-Lopez S, Bambico FR, Lucchino I, Ochoa-Sanchez R, *et al.* (2012). Adolescent amphetamine exposure elicits dose-specific effects on monoaminergic neurotransmission and behaviour in adulthood. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* **15**(9)**:** 1319-1330.

Lakhan SE, Kirchgessner A (2012). Prescription stimulants in individuals with and without attention deficit hyperactivity disorder: misuse, cognitive impact, and adverse effects. *Brain and behavior* **2**(5)**:** 661-677.

Lakic A (2012). Depressive symptoms as a side effect of the sustained release form of methylphenidate in a 7-year-old boy with attention-deficit hyperactivity disorder. *Vojnosanitetski pregled* **69**(2)**:** 201-204.

Lambert NM, Hartsough CS (1998). Prospective study of tobacco smoking and substance dependencies among samples of ADHD and non-ADHD participants. *Journal of learning disabilities* **31**(6)**:** 533-544.

Lamberti M, Italiano D, Guerriero L, D'Amico G, Siracusano R, Ingrassia M, *et al.* (2015). Evaluation of acute cardiovascular effects of immediate-release methylphenidate in children and adolescents with attention-deficit hyperactivity disorder. *Neuropsychiatric disease and treatment* **11:** 1169-1174.

Lambertucci AC, Lambertucci RH, Hirabara SM, Curi R, Moriscot AS, Alba-Loureiro TC, *et al.* (2012). Glutamine supplementation stimulates protein-synthetic and inhibits protein-degradative signaling pathways in skeletal muscle of diabetic rats. *PloS one* **7**(12)**:** e50390.

Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J (2008). Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* **57**(5)**:** 760-773.

Lammel S, Lim BK, Malenka RC (2014). Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology* **76 Pt B:** 351-359.

Lanau F, Zenner MT, Civelli O, Hartman DS (1997). Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D4 receptor. *Journal of neurochemistry* **68**(2)**:** 804-812.

Lasky-Su J, Anney RJ, Neale BM, Franke B, Zhou K, Maller JB, *et al.* (2008). Genome-wide association scan of the time to onset of attention deficit hyperactivity disorder. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **147B**(8)**:** 1355-1358.

Lauderdale K, Murphy T, Tung T, Davila D, Binder DK, Fiacco TA (2015). Osmotic Edema Rapidly Increases Neuronal Excitability Through Activation of NMDA Receptor-Dependent Slow Inward Currents in Juvenile and Adult Hippocampus. *ASN neuro* **7**(5).

Laurienti PJ, Wallace MT, Maldjian JA, Susi CM, Stein BE, Burdette JH (2003). Cross-modal sensory processing in the anterior cingulate and medial prefrontal cortices. *Hum Brain Mapp* **19**(4)**:** 213-223.

Lavretsky H, Reinlieb M, St Cyr N, Siddarth P, Ercoli LM, Senturk D (2015). Citalopram, methylphenidate, or their combination in geriatric depression: a randomized, double-blind, placebo-controlled trial. *The American journal of psychiatry* **172**(6)**:** 561-569.

Lecomte T, Masse M (2014). [Methamphetamine - just another stimulant or a more complex problem?]. *Sante Ment Que* **39**(2)**:** 133-148.

Ledbetter M (2006). Atomoxetine: a novel treatment for child and adult ADHD. *Neuropsychiatric disease and treatment* **2**(4)**:** 455-466.

Lee MJ, Swann AC, Dafny N (2008). Methylphenidate sensitization is prevented by prefrontal cortex lesion. *Brain research bulletin* **76**(1-2)**:** 131-140.

Lee SH, Sung MJ, Lee HM, Chu D, Hahn SJ, Jo SH, *et al.* (2014). Blockade of HERG human K+ channels by the antidepressant drug paroxetine. *Biological & pharmaceutical bulletin* **37**(9)**:** 1495-1504.

Lee TH, Gao WY, Davidson C, Ellinwood EH (1999). Altered activity of midbrain dopamine neurons following 7-day withdrawal from chronic cocaine abuse is normalized by D2 receptor stimulation during the early withdrawal phase. *Neuropsychopharmacology* **21**(1)**:** 127-136.

Lehmann A, Sandberg M (1990). In vivo substitution of choline for sodium evokes a selective osmoinsensitive increase of extracellular taurine in the rat hippocampus. *Journal of neurochemistry* **54**(1)**:** 126-129.

Leo D, Gainetdinov RR (2013). Transgenic mouse models for ADHD. *Cell and tissue research* **354**(1)**:** 259-271.

Lepelletier FX, Tauber C, Nicolas C, Solinas M, Castelnau P, Belzung C, *et al.* (2015). Prenatal exposure to methylphenidate affects the dopamine system and the reactivity to natural reward in adulthood in rats. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* **18**(4).

Lepore FE (1985). Diagnostic pharmacology of the pupil. *Clinical neuropharmacology* **8**(1)**:** 27-37.

Lerner M, Wigal T (2008). Long-term safety of stimulant medications used to treat children with ADHD. *Journal of psychosocial nursing and mental health services* **46**(8)**:** 38-48.

Lesch KP, Merker S, Reif A, Novak M (2013). Dances with black widow spiders: dysregulation of glutamate signalling enters centre stage in ADHD. *Eur Neuropsychopharmacol* **23**(6)**:** 479-491.

Levin ED, Lawrence SS, Petro A, Horton K, Rezvani AH, Seidler FJ, *et al.* (2007). Adolescent vs. adult-onset nicotine self-administration in male rats: duration of effect and differential nicotinic receptor correlates. *Neurotoxicology and teratology* **29**(4)**:** 458-465.

Levin FR, Evans SM, McDowell DM, Kleber HD (1998). Methylphenidate treatment for cocaine abusers with adult attention-deficit/hyperactivity disorder: a pilot study. *The Journal of clinical psychiatry* **59**(6)**:** 300-305.

Levy F (2009). Dopamine vs noradrenaline: inverted-U effects and ADHD theories. *Aust N Z J Psychiatry* **43**(2)**:** 101-108.

Lewis BL, O'Donnell P (2000). Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cereb Cortex* **10**(12)**:** 1168-1175.

Leza JC, Lizasoain I, Lorenzo P (1991). Effects of antihistaminics on locomotor activity in mice. Comparison with opiate and amphetamine-induced hyperactivity. *General pharmacology* **22**(2)**:** 293-296.

Li CS, Morgan PT, Matuskey D, Abdelghany O, Luo X, Chang JL, *et al.* (2010). Biological markers of the effects of intravenous methylphenidate on improving inhibitory control in cocaine-dependent patients. *Proc Natl Acad Sci U S A* **107**(32)**:** 14455-14459.

Li X, Cao Q, Pu F, Li D, Fan Y, An L, *et al.* (2015a). Abnormalities of structural covariance networks in drug-naive boys with attention deficit hyperactivity disorder. *Psychiatry Res*.

Li X, Xu F, Xie L, Ji Y, Cheng K, Zhou Q, *et al.* (2013). Depression-like behavioral phenotypes by social and social plus visual isolation in the adult female Macaca fascicularis. *PloS one* **8**(9)**:** e73293.

Li Y, Mou X, Jiang W, Yang Z, Shen X, Jin Z, *et al.* (2015b). A comparative study of anhedonia components between major depression and schizophrenia in Chinese populations. *Annals of general psychiatry* **14:** 24.

Li Y, Zhong W, Wang D, Feng Q, Liu Z, Zhou J, *et al.* (2016). Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nature communications* **7:** 10503.

Liang BF, Huang F, Wang HT, Wang GH, Yuan X, Zhang MZ, *et al.* (2015). Involvement of norepinephrine and serotonin system in antidepressant-like effects of hederagenin in the rat model of unpredictable chronic mild stress-induced depression. *Pharmaceutical biology* **53**(3)**:** 368-377.

Lienert GA, Huber HP (1966). Differential effects of coffee on speed and power tests. *The Journal of psychology* **63**(2)**:** 269-274.

Lile JA, Stoops WW, Durell TM, Glaser PE, Rush CR (2006). Discriminative-stimulus, self-reported, performance, and cardiovascular effects of atomoxetine in methylphenidate-trained humans. *Exp Clin Psychopharmacol* **14**(2)**:** 136-147.

Lillrank SM, Lipska BK, Weinberger DR, Fredholm BB, Fuxe K, Ferre S (1999). Adenosine and dopamine receptor antagonist binding in the rat ventral and dorsal striatum: lack of changes after a neonatal bilateral lesion of the ventral hippocampus. *Neurochem Int* **34**(3)**:** 235-244.

Lim SA, Kang UJ, McGehee DS (2014). Striatal cholinergic interneuron regulation and circuit effects. *Frontiers in synaptic neuroscience* **6:** 22.

Linden DE (2014). Neurofeedback and networks of depression. *Dialogues in clinical neuroscience* **16**(1)**:** 103-112.

Linderholm KR, Andersson A, Olsson S, Olsson E, Snodgrass R, Engberg G, *et al.* (2007). Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: a pharmacological analysis. *Neuropharmacology* **53**(8)**:** 918-924.

Linner L, Endersz H, Ohman D, Bengtsson F, Schalling M, Svensson TH (2001). Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. *J Pharmacol Exp Ther* **297**(2)**:** 540-546.

Lipkin PH, Goldstein IJ, Adesman AR (1994). Tics and dyskinesias associated with stimulant treatment in attention-deficit hyperactivity disorder. *Arch Pediatr Adolesc Med* **148**(8)**:** 859-861.

Liston C, Malter Cohen M, Teslovich T, Levenson D, Casey BJ (2011). Atypical prefrontal connectivity in attention-deficit/hyperactivity disorder: pathway to disease or pathological end point? *Biol Psychiatry* **69**(12)**:** 1168-1177.

Liu CL, Gao M, Jin GZ, Zhen X (2012a). GABA neurons in the ventral tegmental area responding to peripheral sensory input. *PloS one* **7**(12)**:** e51507.

Liu WH, Wang LZ, Zhao SH, Ning YP, Chan RC (2012b). Anhedonia and emotional word memory in patients with depression. *Psychiatry Res* **200**(2-3)**:** 361-367.

Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH, *et al.* (2006). Modulation of D2R-NR2B interactions in response to cocaine. *Neuron* **52**(5)**:** 897-909.

Llinas R, Steinberg IZ, Walton K (1981). Relationship between presynaptic calcium current and postsynaptic potential in squid giant synapse. *Biophysical journal* **33**(3)**:** 323-351.

Llinas R, Sugimori M, Simon SM (1982). Transmission by presynaptic spike-like depolarization in the squid giant synapse. *Proc Natl Acad Sci U S A* **79**(7)**:** 2415-2419.

Lodge DJ (2011). The medial prefrontal and orbitofrontal cortices differentially regulate dopamine system function. *Neuropsychopharmacology* **36**(6)**:** 1227-1236.

Lodge NJ, Li YW (2008). Ion channels as potential targets for the treatment of depression. *Current opinion in drug discovery & development* **11**(5)**:** 633-641.

Loftis JM, Janowsky A (2003). The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharmacology & therapeutics* **97**(1)**:** 55-85.

Loghin C, Haber H, Beasley CM, Jr., Kothare PA, Kauffman L, April J, *et al.* (2013). Effects of atomoxetine on the QT interval in healthy CYP2D6 poor metabolizers. *British journal of clinical pharmacology* **75**(2)**:** 538-549.

Lopez-Larson MP, King JB, Terry J, McGlade EC, Yurgelun-Todd D (2012). Reduced insular volume in attention deficit hyperactivity disorder. *Psychiatry Res* **204**(1)**:** 32-39.

Lou HC, Henriksen L, Bruhn P, Borner H, Nielsen JB (1989). Striatal dysfunction in attention deficit and hyperkinetic disorder. *Archives of neurology* **46**(1)**:** 48-52.

Love T, Laier C, Brand M, Hatch L, Hajela R (2015). Neuroscience of Internet Pornography Addiction: A Review and Update. *Behavioral sciences* **5**(3)**:** 388-433.

Loweth JA, Svoboda R, Austin JD, Guillory AM, Vezina P (2009). The PKC inhibitor Ro31-8220 blocks acute amphetamine-induced dopamine overflow in the nucleus accumbens. *Neurosci Lett* **455**(2)**:** 88-92.

Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT (2001). Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* **29**(1)**:** 243-254.

Ludolph AG, Udvardi PT, Schaz U, Henes C, Adolph O, Weigt HU, *et al.* (2010). Atomoxetine acts as an NMDA receptor blocker in clinically relevant concentrations. *British journal of pharmacology* **160**(2)**:** 283-291.

Luscher C, Malenka RC (2011). Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron* **69**(4)**:** 650-663.

Luscher C, Malenka RC (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harbor perspectives in biology* **4**(6).

Machens CK, Romo R, Brody CD (2010). Functional, but not anatomical, separation of "what" and "when" in prefrontal cortex. *J Neurosci* **30**(1)**:** 350-360.

Mackie S, Shaw P, Lenroot R, Pierson R, Greenstein DK, Nugent TF, 3rd, *et al.* (2007). Cerebellar development and clinical outcome in attention deficit hyperactivity disorder. *The American journal of psychiatry* **164**(4)**:** 647-655.

MacMaster FP, Carrey N, Sparkes S, Kusumakar V (2003). Proton spectroscopy in medication-free pediatric attention-deficit/hyperactivity disorder. *Biol Psychiatry* **53**(2)**:** 184-187.

Macpherson T, Morita M, Hikida T (2014). Striatal direct and indirect pathways control decision-making behavior. *Frontiers in psychology* **5:** 1301.

MacVane SH, Housman ST, Nicolau DP (2014). In vitro microdialysis membrane efficiency of broad-spectrum antibiotics in combination and alone. *Clinical pharmacology : advances and applications* **6:** 97-101.

Magara F, Ricceri L, Wolfer DP, Lipp HP (2000). The acallosal mouse strain I/LnJ: a putative model of ADHD? *Neurosci Biobehav Rev* **24**(1)**:** 45-50.

Magnoni S, Tedesco C, Carbonara M, Pluderi M, Colombo A, Stocchetti N (2012). Relationship between systemic glucose and cerebral glucose is preserved in patients with severe traumatic brain injury, but glucose delivery to the brain may become limited when oxidative metabolism is impaired: implications for glycemic control. *Critical care medicine* **40**(6)**:** 1785-1791.

Mah L, Arnold MC, Grafman J (2004). Impairment of social perception associated with lesions of the prefrontal cortex. *The American journal of psychiatry* **161**(7)**:** 1247-1255.

Malave LB, Broderick PA (2014). Caffeine's Attenuation of Cocaine-Induced Dopamine Release by Inhibition of Adenosine. *Journal of caffeine research* **4**(2)**:** 35-40.

Malenka RC, Nicoll RA (1993). NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci* **16**(12)**:** 521-527.

Malin DH, Goyarzu P (2009). Rodent models of nicotine withdrawal syndrome. *Handbook of experimental pharmacology*(192)**:** 401-434.

Mallet N, Ballion B, Le Moine C, Gonon F (2006). Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. *J Neurosci* **26**(14)**:** 3875-3884.

Mallet N, Le Moine C, Charpier S, Gonon F (2005). Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo. *J Neurosci* **25**(15)**:** 3857-3869.

Maltezos S, Horder J, Coghlan S, Skirrow C, O'Gorman R, Lavender TJ, *et al.* (2014). Glutamate/glutamine and neuronal integrity in adults with ADHD: a proton MRS study. *Translational psychiatry* **4:** e373.

Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, *et al.* (2006). Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* **50**(6)**:** 911-921.

Manahan-Vaughan D, Wildforster V, Thomsen C (2008). Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). *The European journal of neuroscience* **28**(7)**:** 1342-1350.

Manepalli S, Surratt CK, Madura JD, Nolan TL (2012). Monoamine transporter structure, function, dynamics, and drug discovery: a computational perspective. *The AAPS journal* **14**(4)**:** 820-831.

Manjunath S, Kugali SN, Deodurg PM (2009). Effect of clonidine on blood glucose levels in euglycemic and alloxan-induced diabetic rats and its interaction with glibenclamide. *Indian journal of pharmacology* **41**(5)**:** 218-220.

Manor I, Ben-Hayun R, Aharon-Peretz J, Salomy D, Weizman A, Daniely Y, *et al.* (2012). A randomized, double-blind, placebo-controlled, multicenter study evaluating the efficacy, safety, and tolerability of extended-release metadoxine in adults with attention-deficit/hyperactivity disorder. *The Journal of clinical psychiatry* **73**(12)**:** 1517-1523.

Manor I, Newcorn JH, Faraone SV, Adler LA (2013). Efficacy of metadoxine extended release in patients with predominantly inattentive subtype attention-deficit/hyperactivity disorder. *Postgraduate medicine* **125**(4)**:** 181-190.

Manor I, Rubin J, Daniely Y, Adler LA (2014). Attention benefits after a single dose of metadoxine extended release in adults with predominantly inattentive ADHD. *Postgraduate medicine* **126**(5)**:** 7-16.

Manos MJ (2008). Pharmacologic treatment of ADHD: road conditions in driving patients to successful outcomes. *Medscape journal of medicine* **10**(1)**:** 5.

Manta S, Dong J, Debonnel G, Blier P (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J Psychiatry Neurosci* **34**(4)**:** 272-280.

Mao AR, Findling RL (2014). Comorbidities in adult attention-deficit/hyperactivity disorder: a practical guide to diagnosis in primary care. *Postgraduate medicine* **126**(5)**:** 42-51.

Mao LM, Wang W, Chu XP, Zhang GC, Liu XY, Yang YJ, *et al.* (2009). Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nature neuroscience* **12**(5)**:** 602-610.

Marchetti A, Magar R, Lau H, Murphy EL, Jensen PS, Conners CK, *et al.* (2001). Pharmacotherapies for attention-deficit/hyperactivity disorder: expected-cost analysis. *Clin Ther* **23**(11)**:** 1904-1921.

Marco EM, Adriani W, Ruocco LA, Canese R, Sadile AG, Laviola G (2011). Neurobehavioral adaptations to methylphenidate: the issue of early adolescent exposure. *Neurosci Biobehav Rev* **35**(8)**:** 1722-1739.

Margolis EB, Lock H, Hjelmstad GO, Fields HL (2006). The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *The Journal of physiology* **577**(Pt 3)**:** 907-924.

Margolis EB, Mitchell JM, Ishikawa J, Hjelmstad GO, Fields HL (2008). Midbrain dopamine neurons: projection target determines action potential duration and dopamine D(2) receptor inhibition. *J Neurosci* **28**(36)**:** 8908-8913.

Mariani JJ, Levin FR (2007). Treatment strategies for co-occurring ADHD and substance use disorders. *The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions* **16 Suppl 1:** 45-54; quiz 55-46.

Marinelli M, McCutcheon JE (2014). Heterogeneity of dopamine neuron activity across traits and states. *Neuroscience* **282:** 176-197.

Markowitz JS, Patrick KS (2008). Differential pharmacokinetics and pharmacodynamics of methylphenidate enantiomers: does chirality matter? *Journal of clinical psychopharmacology* **28**(3 Suppl 2)**:** S54-61.

Marsteller DA, Gerasimov MR, Schiffer WK, Geiger JM, Barnett CR, Schaich Borg J, *et al.* (2002). Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. *Neuropsychopharmacology* **27**(2)**:** 163-170.

Martel P, Leo D, Fulton S, Berard M, Trudeau LE (2011). Role of Kv1 potassium channels in regulating dopamine release and presynaptic D2 receptor function. *PloS one* **6**(5)**:** e20402.

Martelle SE, Porrino LJ, Nader MA (2013). Effects of chronic methylphenidate in adolescence on later methylphenidate self-administration in rhesus monkeys. *Behavioural pharmacology* **24**(5-6)**:** 478-481.

Marti F, Arib O, Morel C, Dufresne V, Maskos U, Corringer PJ, *et al.* (2011). Smoke extracts and nicotine, but not tobacco extracts, potentiate firing and burst activity of ventral tegmental area dopaminergic neurons in mice. *Neuropsychopharmacology* **36**(11)**:** 2244-2257.

Martignoni M, Groothuis GM, de Kanter R (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert opinion on drug metabolism & toxicology* **2**(6)**:** 875-894.

Martin CA, Cook C, Woodring JH, Burkhardt G, Guenthner G, Omar HA, *et al.* (2008). Caffeine use: association with nicotine use, aggression, and other psychopathology in psychiatric and pediatric outpatient adolescents. *ScientificWorldJournal* **8:** 512-516.

Martin JH (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neurosci Lett* **127**(2)**:** 160-164.

Martina M, Gorfinkel Y, Halman S, Lowe JA, Periyalwar P, Schmidt CJ, *et al.* (2004). Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. *The Journal of physiology* **557**(Pt 2)**:** 489-500.

Martinez-Raga J, Knecht C, Szerman N, Martinez MI (2013). Risk of serious cardiovascular problems with medications for attention-deficit hyperactivity disorder. *CNS Drugs* **27**(1)**:** 15-30.

Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, *et al.* (2005). Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* **436**(7047)**:** 103-107.

Masuo Y, Shibato J, Rakwal R (2012). ADHD animal model characterization: transcriptomics and proteomics analyses. *Methods in molecular biology* **829:** 505-530.

Matamales M, Gotz J, Bertran-Gonzalez J (2016). Quantitative Imaging of Cholinergic Interneurons Reveals a Distinctive Spatial Organization and a Functional Gradient across the Mouse Striatum. *PloS one* **11**(6)**:** e0157682.

Mateus-Pinheiro A, Patricio P, Alves ND, Machado-Santos AR, Morais M, Bessa JM, *et al.* (2014). The Sweet Drive Test: refining phenotypic characterization of anhedonic behavior in rodents. *Frontiers in behavioral neuroscience* **8:** 74.

Matochik JA, Nordahl TE, Gross M, Semple WE, King AC, Cohen RM, *et al.* (1993). Effects of acute stimulant medication on cerebral metabolism in adults with hyperactivity. *Neuropsychopharmacology* **8**(4)**:** 377-386.

Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, *et al.* (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J Neurosci* **29**(2)**:** 444-453.

Matsumoto M, Hagio M, Inoue R, Mitani T, Yajima M, Hara H, *et al.* (2014). Long-term oral feeding of lutein-fortified milk increases voluntary running distance in rats. *PloS one* **9**(4)**:** e93529.

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**(6993)**:** 761-766.

Matthys F, Soyez V, van den Brink W, Joostens P, Tremmery S, Sabbe B (2014). Barriers to implementation of treatment guidelines for ADHD in adults with substance use disorder. *Journal of dual diagnosis* **10**(3)**:** 130-138.

Mattiuz EL, Ponsler GD, Barbuch RJ, Wood PG, Mullen JH, Shugert RL, *et al.* (2003). Disposition and metabolic fate of atomoxetine hydrochloride: pharmacokinetics, metabolism, and excretion in the Fischer 344 rat and beagle dog. *Drug Metab Dispos* **31**(1)**:** 88-97.

McClernon FJ, Kollins SH (2008). ADHD and smoking: from genes to brain to behavior. *Ann N Y Acad Sci* **1141:** 131-147.

McClure I (2013). ADHD is a behavioural construct, not a psychiatric condition. *Bmj* **347:** f7071.

McCutcheon JE, Marinelli M (2009). Age matters. *The European journal of neuroscience* **29**(5)**:** 997-1014.

McDonald JC, Spruyt O, Alhatem A (2015). Control of intractable pruritus in a patient with cutaneous T-cell lymphoma using a continuous subcutaneous infusion of lidocaine. *Journal of pain and symptom management* **49**(4)**:** e1-3.

McGehee DS, Role LW (1995). Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annual review of physiology* **57:** 521-546.

McGinty VB, Grace AA (2008). Selective activation of medial prefrontal-to-accumbens projection neurons by amygdala stimulation and Pavlovian conditioned stimuli. *Cereb Cortex* **18**(8)**:** 1961-1972.

McGranahan TM, Patzlaff NE, Grady SR, Heinemann SF, Booker TK (2011). alpha4beta2 nicotinic acetylcholine receptors on dopaminergic neurons mediate nicotine reward and anxiety relief. *J Neurosci* **31**(30)**:** 10891-10902.

McLaughlin KA, Sheridan MA, Winter W, Fox NA, Zeanah CH, Nelson CA (2014). Widespread reductions in cortical thickness following severe early-life deprivation: a neurodevelopmental pathway to attention-deficit/hyperactivity disorder. *Biol Psychiatry* **76**(8)**:** 629-638.

McNamara RK, Logue A, Stanford K, Xu M, Zhang J, Richtand NM (2006). Dose-response analysis of locomotor activity and stereotypy in dopamine D3 receptor mutant mice following acute amphetamine. *Synapse* **60**(5)**:** 399-405.

Mejias-Aponte CA, Ye C, Bonci A, Kiyatkin EA, Morales M (2015). A subpopulation of neurochemically-identified ventral tegmental area dopamine neurons is excited by intravenous cocaine. *J Neurosci* **35**(5)**:** 1965-1978.

Meneses A, Perez-Garcia G, Ponce-Lopez T, Tellez R, Gallegos-Cari A, Castillo C (2011). Spontaneously hypertensive rat (SHR) as an animal model for ADHD: a short overview. *Reviews in the neurosciences* **22**(3)**:** 365-371.

Mercuri NB, Saiardi A, Bonci A, Picetti R, Calabresi P, Bernardi G, *et al.* (1997). Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. *Neuroscience* **79**(2)**:** 323-327.

Mereu G, Lilliu V, Casula A, Vargiu PF, Diana M, Musa A, *et al.* (1997). Spontaneous bursting activity of dopaminergic neurons in midbrain slices from immature rats: role of N-methyl-D-aspartate receptors. *Neuroscience* **77**(4)**:** 1029-1036.

Michelson D, Faries D, Wernicke J, Kelsey D, Kendrick K, Sallee FR, *et al.* (2001a). Atomoxetine in the treatment of children and adolescents with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled, dose-response study. *Pediatrics* **108**(5)**:** E83.

Michelson D, Faries D, Wernicke J, Kelsey D, Kendrick K, Sallee FR, *et al.* (2001b). Atomoxetine in the treatment of children and adolescents with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled, dose-response study. *Pediatrics* **108**(5)**:** E83.

Micoulaud-Franchi JA, MacGregor A, Fond G (2014). A preliminary study on cognitive enhancer consumption behaviors and motives of French Medicine and Pharmacology students. *European review for medical and pharmacological sciences* **18**(13)**:** 1875-1878.

Middleton LS, Cass WA, Dwoskin LP (2004). Nicotinic receptor modulation of dopamine transporter function in rat striatum and medial prefrontal cortex. *J Pharmacol Exp Ther* **308**(1)**:** 367-377.

Milberger S, Biederman J, Faraone SV, Chen L, Jones J (1997). ADHD is associated with early initiation of cigarette smoking in children and adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry* **36**(1)**:** 37-44.

Miles SW, Sheridan J, Russell B, Kydd R, Wheeler A, Walters C, *et al.* (2013). Extended-release methylphenidate for treatment of amphetamine/methamphetamine dependence: a randomized, double-blind, placebo-controlled trial. *Addiction* **108**(7)**:** 1279-1286.

Mileva GR, Bielajew C (2015). Environmental manipulation affects depressive-like behaviours in female Wistar-Kyoto rats. *Behavioural brain research* **293:** 208-216.

Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A, *et al.* (2001). S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* **298**(2)**:** 565-580.

Miller EM, Pomerleau F, Huettl P, Gerhardt GA, Glaser PE (2014). Aberrant glutamate signaling in the prefrontal cortex and striatum of the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl)* **231**(15)**:** 3019-3029.

Millichap JG (2008). Etiologic classification of attention-deficit/hyperactivity disorder. *Pediatrics* **121**(2)**:** e358-365.

Millichap JG, Yee MM (2012). The diet factor in attention-deficit/hyperactivity disorder. *Pediatrics* **129**(2)**:** 330-337.

Milstein JA, Elnabawi A, Vinish M, Swanson T, Enos JK, Bailey AM, *et al.* (2013). Olanzapine treatment of adolescent rats causes enduring specific memory impairments and alters cortical development and function. *PloS one* **8**(2)**:** e57308.

Misonou H (2010). Homeostatic regulation of neuronal excitability by K(+) channels in normal and diseased brains. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **16**(1)**:** 51-64.

Mok MH, Fricker AC, Weil A, Kew JN (2009). Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels. *Neuropharmacology* **57**(3)**:** 242-249.

Molina BS, Pelham WE, Jr. (2003). Childhood predictors of adolescent substance use in a longitudinal study of children with ADHD. *Journal of abnormal psychology* **112**(3)**:** 497-507.

Monaco SA, Gulchina Y, Gao WJ (2015). NR2B subunit in the prefrontal cortex: A double-edged sword for working memory function and psychiatric disorders. *Neurosci Biobehav Rev* **56:** 127-138.

Moore CM, Biederman J, Wozniak J, Mick E, Aleardi M, Wardrop M, *et al.* (2006). Differences in brain chemistry in children and adolescents with attention deficit hyperactivity disorder with

and without comorbid bipolar disorder: a proton magnetic resonance spectroscopy study. *The American journal of psychiatry* **163**(2)**:** 316-318.

Moore DA, Richardson M, Gwernan-Jones R, Thompson-Coon J, Stein K, Rogers M, *et al.* (2015). Non-Pharmacological Interventions for ADHD in School Settings: An Overarching Synthesis of Systematic Reviews. *J Atten Disord*.

Moore KE, Sawdy LC, Shaul SR (1965). Effects of D-Amphetamine on Blood Glucose and Tissue Glycogen Levels of Isolated and Aggregated Mice. *Biochemical pharmacology* **14:** 197-204.

Moran-Gates T, Zhang K, Baldessarini RJ, Tarazi FI (2005). Atomoxetine blocks motor hyperactivity in neonatal 6-hydroxydopamine-lesioned rats: implications for treatment of attention-deficit hyperactivity disorder. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* **8**(3)**:** 439-444.

Morgaz J, Munoz-Rascon P, Serrano-Rodriguez JM, Navarrete R, Dominguez JM, Fernandez-Sarmiento JA, *et al.* (2014). Effectiveness of pre-peritoneal continuous wound infusion with lidocaine for pain control following ovariohysterectomy in dogs. *Veterinary journal* **202**(3)**:** 522-526.

Moron JA, Brockington A, Wise RA, Rocha BA, Hope BT (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**(2)**:** 389-395.

Morris C, Lecar H (1981). Voltage oscillations in the barnacle giant muscle fiber. *Biophysical journal* **35**(1)**:** 193-213.

Morris SA, Kelso ML, Liput DJ, Marshall SA, Nixon K (2010). Similar withdrawal severity in adolescents and adults in a rat model of alcohol dependence. *Alcohol* **44**(1)**:** 89-98.

Morton WA, Stockton GG (2000). Methylphenidate Abuse and Psychiatric Side Effects. *Primary care companion to the Journal of clinical psychiatry* **2**(5)**:** 159-164.

Mostofsky SH, Cooper KL, Kates WR, Denckla MB, Kaufmann WE (2002). Smaller prefrontal and premotor volumes in boys with attention-deficit/hyperactivity disorder. *Biol Psychiatry* **52**(8)**:** 785-794.

Mostofsky SH, Reiss AL, Lockhart P, Denckla MB (1998). Evaluation of cerebellar size in attention-deficit hyperactivity disorder. *Journal of child neurology* **13**(9)**:** 434-439.

Motaghinejad M, Motevalian M, Ebrahimzadeh A, Larijani SF, Khajehamedi Z (2015). Reduction of Methylphenidate Induced Anxiety, Depression and Cognition Impairment by Various doses of Venlafaxine in Rat. *International journal of preventive medicine* **6:** 52.

Moyer JT, Halterman BL, Finkel LH, Wolf JA (2014). Lateral and feedforward inhibition suppress asynchronous activity in a large, biophysically-detailed computational model of the striatal network. *Frontiers in computational neuroscience* **8:** 152.

Mraz J, Cross H, Gescher A, Threadgill MD, Flek J (1989). Differences between rodents and humans in the metabolic toxification of N,N-dimethylformamide. *Toxicology and applied pharmacology* **98**(3)**:** 507-516.

Mueller AK, Fuermaier AB, Koerts J, Tucha L (2012). Stigma in attention deficit hyperactivity disorder. *Attention deficit and hyperactivity disorders* **4**(3)**:** 101-114.

Mueller K (1993). Locomotor stereotypy is produced by methylphenidate and amfonelic acid and reduced by haloperidol but not clozapine or thioridazine. *Pharmacol Biochem Behav* **45**(1)**:** 71-76.

Muir JL, Everitt BJ, Robbins TW (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb Cortex* **6**(3)**:** 470-481.

Mulkey DK, Henderson RA, 3rd, Putnam RW, Dean JB (2003). Hyperbaric oxygen and chemical oxidants stimulate CO2/H+-sensitive neurons in rat brain stem slices. *Journal of applied physiology* **95**(3)**:** 910-921.

Munk K, Gormsen L, Kim WY, Andersen NH (2015). Cardiac Arrest following a Myocardial Infarction in a Child Treated with Methylphenidate. *Case reports in pediatrics* **2015:** 905097.

Murase S, Grenhoff J, Chouvet G, Gonon FG, Svensson TH (1993). Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. *Neurosci Lett* **157**(1)**:** 53-56.

Murray JE, Belin-Rauscent A, Simon M, Giuliano C, Benoit-Marand M, Everitt BJ, *et al.* (2015). Basolateral and central amygdala differentially recruit and maintain dorsolateral striatum-dependent cocaine-seeking habits. *Nature communications* **6:** 10088.

Nagai N, Ito Y, Taga A (2013). Comparison of the enhancement of plasma glucose levels in type 2 diabetes Otsuka Long-Evans Tokushima Fatty rats by oral administration of sucrose or maple syrup. *Journal of oleo science* **62**(9)**:** 737-743.

Nagai T, Delay RJ, Welton J, Roper SD (1998). Uptake and release of neurotransmitter candidates, [3H]serotonin, [3H]glutamate, and [3H]gamma-aminobutyric acid, in taste buds of the mudpuppy, Necturus maculosus. *The Journal of comparative neurology* **392**(2)**:** 199-208.

Nagamitsu S, Yamashita Y, Tanigawa H, Chiba H, Kaida H, Ishibashi M, *et al.* (2015). Upregulated GABA Inhibitory Function in ADHD Children with Child Behavior Checklist-Dysregulation Profile: 123I-Iomazenil SPECT Study. *Frontiers in psychiatry* **6:** 84.

Nagy J (2004). The NR2B subtype of NMDA receptor: a potential target for the treatment of alcohol dependence. *Current drug targets. CNS and neurological disorders* **3**(3)**:** 169-179.

Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience* **152**(4)**:** 1024-1031.

Najib J (2009). The efficacy and safety profile of lisdexamfetamine dimesylate, a prodrug of d-amphetamine, for the treatment of attention-deficit/hyperactivity disorder in children and adults. *Clin Ther* **31**(1)**:** 142-176.

Nakanishi S (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science* **258**(5082)**:** 597-603.

Neef NA, Marckel J, Ferreri SJ, Bicard DF, Endo S, Aman MG, *et al.* (2005). Behavioral assessment of impulsivity: a comparison of children with and without attention deficit hyperactivity disorder. *Journal of applied behavior analysis* **38**(1)**:** 23-37.

Negus SS, Mello NK, Kimmel HL, Howell LL, Carroll FI (2009). Effects of the monoamine uptake inhibitors RTI-112 and RTI-113 on cocaine- and food-maintained responding in rhesus monkeys. *Pharmacol Biochem Behav* **91**(3)**:** 333-338.

Nehlig A (2010). Is caffeine a cognitive enhancer? *Journal of Alzheimer's disease : JAD* **20 Suppl 1:** S85-94.

Nehlig A, Boyet S (2000). Dose-response study of caffeine effects on cerebral functional activity with a specific focus on dependence. *Brain research* **858**(1)**:** 71-77.

Nehlig A, Daval JL, Debry G (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Brain Res Rev* **17**(2)**:** 139-170.

Nehlin C, Nyberg F, Oster C (2014). The Patient's Perspective on the Link Between ADHD and Substance Use: A Qualitative Interview Study. *J Atten Disord*.

Nelson AB, Hammack N, Yang CF, Shah NM, Seal RP, Kreitzer AC (2014). Striatal cholinergic interneurons Drive GABA release from dopamine terminals. *Neuron* **82**(1)**:** 63-70.

Newman-Tancredi A, Audinot-Bouchez V, Gobert A, Millan MJ (1997). Noradrenaline and adrenaline are high affinity agonists at dopamine D4 receptors. *Eur J Pharmacol* **319**(2-3)**:** 379-383.

Ng CG, Wong SK, Loh HS, Yee A (2014). Anhedonia among patients with Major Depressive Disorder: A comparison between patients on escitalopram and healthy controls. *La Clinica terapeutica* **165**(6)**:** e384-390.

Nic Dhonnchadha BA, Lin A, Leite-Morris KA, Kaplan GB, Man HY, Kantak KM (2013). Alterations in expression and phosphorylation of GluA1 receptors following cocaine-cue extinction learning. *Behavioural brain research* **238:** 119-123.

Nickell JR, Siripurapu KB, Vartak A, Crooks PA, Dwoskin LP (2014). The vesicular monoamine transporter-2: an important pharmacological target for the discovery of novel therapeutics to treat methamphetamine abuse. *Advances in pharmacology* **69:** 71-106.

Niddam R, Arbilla S, Scatton B, Dennis T, Langer SZ (1985). Amphetamine induced release of endogenous dopamine in vitro is not reduced following pretreatment with reserpine. *Naunyn-Schmiedeberg's archives of pharmacology* **329**(2)**:** 123-127.

Niederhofer H (2010). Duloxetine may improve some symptoms of attention-deficit/hyperactivity disorder. *Primary care companion to the Journal of clinical psychiatry* **12**(2).

Nikiforuk A, Kos T, Rafa D, Behl B, Bespalov A, Popik P (2011). Blockade of glycine transporter 1 by SSR-504734 promotes cognitive flexibility in glycine/NMDA receptor-dependent manner. *Neuropharmacology* **61**(1-2)**:** 262-267.

Nimitvilai S, Arora DS, Brodie MS (2012). Reversal of dopamine inhibition of dopaminergic neurons of the ventral tegmental area is mediated by protein kinase C. *Neuropsychopharmacology* **37**(2)**:** 543-556.

Nishi A, Kuroiwa M, Shuto T (2011). Mechanisms for the modulation of dopamine d(1) receptor signaling in striatal neurons. *Frontiers in neuroanatomy* **5:** 43.

Nissbrandt H, Elverfors A, Engberg G (1994). Pharmacologically induced cessation of burst activity in nigral dopamine neurons: significance for the terminal dopamine efflux. *Synapse* **17**(4)**:** 217-224.

Noda T, Shimizu W, Satomi K, Suyama K, Kurita T, Aihara N, *et al.* (2004). Classification and mechanism of Torsade de Pointes initiation in patients with congenital long QT syndrome. *European heart journal* **25**(23)**:** 2149-2154.

Nomikos GG, Damsma G, Wenkstern D, Fibiger HC (1990). In vivo characterization of locally applied dopamine uptake inhibitors by striatal microdialysis. *Synapse* **6**(1)**:** 106-112.

O'Keefe LM, Doran SJ, Mwilambwe-Tshilobo L, Conti LH, Venna VR, McCullough LD (2014). Social isolation after stroke leads to depressive-like behavior and decreased BDNF levels in mice. *Behavioural brain research* **260:** 162-170.

Oades RD, Lasky-Su J, Christiansen H, Faraone SV, Sonuga-Barke EJ, Banaschewski T, *et al.* (2008). The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. *Behav Brain Funct* **4:** 48.

Obrenovitch TP, Urenjak J, Zilkha E (1997). Effects of increased extracellular glutamate levels on the local field potential in the brain of anaesthetized rats. *British journal of pharmacology* **122**(2)**:** 372-378.

Okuma Y, Osumi Y (1986). KCl-induced calcium-independent release of endogenous dopamine from rat brain slices. *Brain research* **363**(1)**:** 47-52.

Olateju T, Begley J, Green DJ, Kerr D (2015). Physiological and glycemic responses following acute ingestion of a popular functional drink in patients with type 1 diabetes. *Canadian journal of diabetes* **39**(1)**:** 78-82.

Omelchenko N, Sesack SR (2007). Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. *Neuroscience* **146**(3)**:** 1259-1274.

Onnink AM, Zwiers MP, Hoogman M, Mostert JC, Kan CC, Buitelaar J, *et al.* (2014). Brain alterations in adult ADHD: effects of gender, treatment and comorbid depression. *Eur Neuropsychopharmacol* **24**(3)**:** 397-409.

Oosterhof CA, Mansari ME, Bundgaard C, Blier P (2015). Brexpiprazole Alters Monoaminergic Systems following Repeated Administration: an in Vivo Electrophysiological Study. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*.

Oster A, Faure P, Gutkin BS (2015). Mechanisms for multiple activity modes of VTA dopamine neurons. *Frontiers in computational neuroscience* **9:** 95.

Ou XM, Partoens PM, Wang JM, Walker JH, Danks K, Vaughan PF, *et al.* (1998). The storage of noradrenaline, neuropeptide Y and chromogranins in and stoichiometric release from large dense cored vesicles of the undifferentiated human neuroblastoma cell line SH-SY5Y. *International journal of molecular medicine* **1**(1)**:** 105-112.

Outram SM (2010). The use of methylphenidate among students: the future of enhancement? *Journal of medical ethics* **36**(4)**:** 198-202.

Overstreet DH (2012). Modeling depression in animal models. *Methods in molecular biology* **829:** 125-144.

Overton PG, Clark D (1997). Burst firing in midbrain dopaminergic neurons. *Brain Res Brain Res Rev* **25**(3)**:** 312-334.

Ozaki S, Wada K (2006). Characteristics of methylphenidate dependence syndrome in psychiatric hospital settings. *Nihon Arukoru Yakubutsu Igakkai zasshi = Japanese journal of alcohol studies & drug dependence* **41**(2)**:** 89-99.

Pachmerhiwala R, Bhide N, Straiko M, Gudelsky GA (2010). Role of serotonin and/or norepinephrine in the MDMA-induced increase in extracellular glucose and glycogenolysis in the rat brain. *Eur J Pharmacol* **644**(1-3)**:** 67-72.

Paladini CA, Robinson S, Morikawa H, Williams JT, Palmiter RD (2003). Dopamine controls the firing pattern of dopamine neurons via a network feedback mechanism. *Proc Natl Acad Sci U S A* **100**(5)**:** 2866-2871.

Paladini CA, Roeper J (2014). Generating bursts (and pauses) in the dopamine midbrain neurons. *Neuroscience* **282:** 109-121.

Paladini CA, Williams JT (2004). Noradrenergic inhibition of midbrain dopamine neurons. *J Neurosci* **24**(19)**:** 4568-4575.

Pandolfo P, Machado NJ, Kofalvi A, Takahashi RN, Cunha RA (2013). Caffeine regulates frontocorticostriatal dopamine transporter density and improves attention and cognitive deficits in an animal model of attention deficit hyperactivity disorder. *Eur Neuropsychopharmacol* **23**(4)**:** 317-328.

Panin F, Cathala A, Piazza PV, Spampinato U (2012). Coupled intracerebral microdialysis and electrophysiology for the assessment of dopamine neuron function in vivo. *Journal of pharmacological and toxicological methods* **65**(2)**:** 83-92.

Panin F, Lintas A, Diana M (2014). Nicotine-induced increase of dopaminergic mesoaccumbal neuron activity is prevented by acute restraint stress. In vivo electrophysiology in rats. *Eur Neuropsychopharmacol* **24**(7)**:** 1175-1180.

Papapetrou P, Kumar AJ, Muppuri R, Chakrabortty S (2015). Intravenous Lidocaine Infusion to Treat Chemotherapy-Induced Peripheral Neuropathy. *A & A case reports* **5**(9)**:** 154-155.

Papke RL, Dwoskin LP, Crooks PA (2007). The pharmacological activity of nicotine and nornicotine on nAChRs subtypes: relevance to nicotine dependence and drug discovery. *Journal of neurochemistry* **101**(1)**:** 160-167.

Pardey MC, Kumar NN, Goodchild AK, Clemens KJ, Homewood J, Cornish JL (2012). Long-term effects of chronic oral Ritalin administration on cognitive and neural development in adolescent wistar kyoto rats. *Brain sciences* **2**(3)**:** 375-404.

Park S, Jung SW, Kim BN, Cho SC, Shin MS, Kim JW, *et al.* (2013). Association between the GRM7 rs3792452 polymorphism and attention deficit hyperacitiveity disorder in a Korean sample. *Behav Brain Funct* **9:** 1.

Park S, Kim BN, Cho SC, Kim JW, Kim JI, Shin MS, *et al.* (2014). The metabotropic glutamate receptor subtype 7 rs3792452 polymorphism is associated with the response to methylphenidate in children with attention-deficit/hyperactivity disorder. *Journal of child and adolescent psychopharmacology* **24**(4)**:** 223-227.

Partilla JS, Dempsey AG, Nagpal AS, Blough BE, Baumann MH, Rothman RB (2006). Interaction of amphetamines and related compounds at the vesicular monoamine transporter. *J Pharmacol Exp Ther* **319**(1)**:** 237-246.

Partridge JG, Apparsundaram S, Gerhardt GA, Ronesi J, Lovinger DM (2002). Nicotinic acetylcholine receptors interact with dopamine in induction of striatal long-term depression. *J Neurosci* **22**(7)**:** 2541-2549.

Pascoli V, Besnard A, Herve D, Pages C, Heck N, Girault JA, *et al.* (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol Psychiatry* **69**(3)**:** 218-227.

Paterson NE, Ricciardi J, Wetzler C, Hanania T (2011). Sub-optimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and d-amphetamine, but unaffected by the COMT inhibitor tolcapone. *Neuroscience research* **69**(1)**:** 41-50.

Patkar AA, Masand PS, Pae CU, Peindl K, Hooper-Wood C, Mannelli P, *et al.* (2006). A randomized, double-blind, placebo-controlled trial of augmentation with an extended release formulation of methylphenidate in outpatients with treatment-resistant depression. *Journal of clinical psychopharmacology* **26**(6)**:** 653-656.

Patrick KS, Gonzalez MA, Straughn AB, Markowitz JS (2005). New methylphenidate formulations for the treatment of attention-deficit/hyperactivity disorder. *Expert opinion on drug delivery* **2**(1)**:** 121-143.

Pattij T, Vanderschuren LJ (2008). The neuropharmacology of impulsive behaviour. *Trends in pharmacological sciences* **29**(4)**:** 192-199.

Patton MH, Bizup BT, Grace AA (2013). The infralimbic cortex bidirectionally modulates mesolimbic dopamine neuron activity via distinct neural pathways. *J Neurosci* **33**(43)**:** 16865-16873.

Paul K, Venkitaramani DV, Cox CL (2013). Dampened dopamine-mediated neuromodulation in prefrontal cortex of fragile X mice. *The Journal of physiology* **591**(4)**:** 1133-1143.

Paulus MP, Geyer MA (1991). A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. *Psychopharmacology (Berl)* **104**(1)**:** 6-16.

Paxinos G, Watson C (1997). *The Rat Brain in Stereotaxic Coordinates*. 3rd edn. Academic Press: San Diego.

Pearl RG, Seiden LS (1976). The existence of tolerance to and cross-tolerance between d-amphetamine and methylphenidate for their effects on milk consumption and on differential-reinforcement-of-low-rate performance in the rat. *J Pharmacol Exp Ther* **198**(3)**:** 635-647.

Pehlivanidis A (2012). [Awareness of adult attention-deficit/hyperactivity disorder (ADHD) in Greece]. *Psychiatrike = Psychiatriki* **23 Suppl 1:** 60-65.

Pelham WE, Jr., Carlson C, Sams SE, Vallano G, Dixon MJ, Hoza B (1993). Separate and combined effects of methylphenidate and behavior modification on boys with attention deficit-hyperactivity disorder in the classroom. *Journal of consulting and clinical psychology* **61**(3)**:** 506-515.

Pencea V, Bingaman KD, Wiegand SJ, Luskin MB (2001). Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J Neurosci* **21**(17)**:** 6706-6717.

Penner MR, McFadyen MP, Carrey N, Brown RE (2001). Effects of chronic and acute methylphenidate hydrochloride (Ritalin) administration on locomotor activity, ultrasonic vocalizations, and neuromotor development in 3- to 11-day-old CD-1 mouse pups. *Developmental psychobiology* **39**(3)**:** 216-228.

Peraile I, Torres E, Mayado A, Izco M, Lopez-Jimenez A, Lopez-Moreno JA, *et al.* (2010). Dopamine transporter down-regulation following repeated cocaine: implications for 3,4-methylenedioxymethamphetamine-induced acute effects and long-term neurotoxicity in mice. *British journal of pharmacology* **159**(1)**:** 201-211.

Pereira RR, van de Wetering BJ (2004). [Caffeine, cannabis and cocaine: from automedication to 'automutilation' in adults with ADHD]. *Nederlands tijdschrift voor geneeskunde* **148**(52)**:** 2573-2576.

Perera H, Fernando SM, Yasawardena AD, Karunaratne I (2009). Prevalence of attention deficit hyperactivity disorder (ADHD) in children presenting with self-inserted nasal and aural foreign bodies. *Int J Pediatr Otorhinolaryngol* **73**(10)**:** 1362-1364.

Perlov E, Philipsen A, Hesslinger B, Buechert M, Ahrendts J, Feige B, *et al.* (2007). Reduced cingulate glutamate/glutamine-to-creatine ratios in adult patients with attention deficit/hyperactivity disorder -- a magnet resonance spectroscopy study. *Journal of psychiatric research* **41**(11)**:** 934-941.

Perlov E, Philipsen A, Tebartz van Elst L, Ebert D, Henning J, Maier S, *et al.* (2008). Hippocampus and amygdala morphology in adults with attention-deficit hyperactivity disorder. *J Psychiatry Neurosci* **33**(6)**:** 509-515.

Perlov E, Tebarzt van Elst L, Buechert M, Maier S, Matthies S, Ebert D, *et al.* (2010). H(1)-MR-spectroscopy of cerebellum in adult attention deficit/hyperactivity disorder. *Journal of psychiatric research* **44**(14)**:** 938-943.

Perniola A, Magnuson A, Axelsson K, Gupta A (2014). Intraperitoneal local anesthetics have predominant local analgesic effect: a randomized, double-blind study. *Anesthesiology* **121**(2)**:** 352-361.

Perry JL, Stairs DJ, Bardo MT (2008). Impulsive choice and environmental enrichment: effects of d-amphetamine and methylphenidate. *Behavioural brain research* **193**(1)**:** 48-54.

Peters A (2011). Does sugar addiction really cause obesity? *Frontiers in neuroenergetics* **3:** 11.

Petroff OA (2002). GABA and glutamate in the human brain. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **8**(6)**:** 562-573.

Pfortner TK, Hublet A, Schnohr CW, Rathmann K, Moor I, de Looze M, *et al.* (2015). Socioeconomic inequalities in the impact of tobacco control policies on adolescent smoking. A multilevel study in 29 European countries. *Addict Behav* **53:** 58-66.

Picciotto MR, Kenny PJ (2013). Molecular mechanisms underlying behaviors related to nicotine addiction. *Cold Spring Harbor perspectives in medicine* **3**(1)**:** a012112.

Pillidge K, Porter AJ, Young JW, Stanford SC (2016). Perseveration by NK1R-/- ('knockout') mice is blunted by doses of methylphenidate that affect neither other aspects of their cognitive performance nor the behaviour of wild-type mice in the 5-Choice Continuous Performance Test. *J Psychopharmacol* **30**(9)**:** 837-847.

Pitcher TM, Piek JP, Hay DA (2003). Fine and gross motor ability in males with ADHD. *Developmental medicine and child neurology* **45**(8)**:** 525-535.

Pizzi WJ, Rode EC, Barnhart JE (1987). Differential effects of methylphenidate on the growth of neonatal and adolescent rats. *Neurotoxicology and teratology* **9**(2)**:** 107-111.

Pizzi WJ, Rode EC, Barnhart JE (1986). Methylphenidate and growth: demonstration of a growth impairment and a growth-rebound phenomenon. *Developmental pharmacology and therapeutics* **9**(5)**:** 361-368.

Platkiewicz J, Brette R (2010). A threshold equation for action potential initiation. *PLoS computational biology* **6**(7)**:** e1000850.

Plessen KJ, Bansal R, Zhu H, Whiteman R, Amat J, Quackenbush GA, *et al.* (2006). Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. *Archives of general psychiatry* **63**(7)**:** 795-807.

Pliszka S, Issues AWGoQ (2007). Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* **46**(7)**:** 894-921.

Pomerleau OF, Downey KK, Stelson FW, Pomerleau CS (1995). Cigarette smoking in adult patients diagnosed with attention deficit hyperactivity disorder. *Journal of substance abuse* **7**(3)**:** 373-378.

Poncin Y, Sukhodolsky DG, McGuire J, Scahill L (2007). Drug and non-drug treatments of children with ADHD and tic disorders. *Eur Child Adolesc Psychiatry* **16 Suppl 1:** 78-88.

Pongs O (2008). Regulation of excitability by potassium channels. *Results and problems in cell differentiation* **44:** 145-161.

Ponizovsky AM, Marom E, Fitoussi I (2014). Trends in attention deficit hyperactivity disorder drugs consumption, Israel, 2005-2012. *Pharmacoepidemiol Drug Saf.*

Pontifex MB, Saliba BJ, Raine LB, Picchietti DL, Hillman CH (2013). Exercise improves behavioral, neurocognitive, and scholastic performance in children with attention-deficit/hyperactivity disorder. *The Journal of pediatrics* **162**(3)**:** 543-551.

Posner J, Siciliano F, Wang Z, Liu J, Sonuga-Barke E, Greenhill L (2014). A multimodal MRI study of the hippocampus in medication-naive children with ADHD: what connects ADHD and depression? *Psychiatry Res* **224**(2)**:** 112-118.

Potenza MN, Fiellin DA, Heninger GR, Rounsaville BJ, Mazure CM (2002). Gambling: an addictive behavior with health and primary care implications. *Journal of general internal medicine* **17**(9)**:** 721-732.

Pothos EN, Przedborski S, Davila V, Schmitz Y, Sulzer D (1998). D2-Like dopamine autoreceptor activation reduces quantal size in PC12 cells. *J Neurosci* **18**(15)**:** 5575-5585.

Potter AS, Newhouse PA (2008). Acute nicotine improves cognitive deficits in young adults with attention-deficit/hyperactivity disorder. *Pharmacol Biochem Behav* **88**(4)**:** 407-417.

Poulton A (2005). Growth on stimulant medication; clarifying the confusion: a review. *Arch Dis Child* **90**(8)**:** 801-806.

Poulton A, Cowell CT (2003). Slowing of growth in height and weight on stimulants: a characteristic pattern. *Journal of paediatrics and child health* **39**(3)**:** 180-185.

Poulton AS, Melzer E, Tait PR, Garnett SP, Cowell CT, Baur LA, *et al.* (2013). Growth and pubertal development of adolescent boys on stimulant medication for attention deficit hyperactivity disorder. *The Medical journal of Australia* **198**(1)**:** 29-32.

Powell SG, Frydenberg M, Thomsen PH (2015). The effects of long-term medication on growth in children and adolescents with ADHD: an observational study of a large cohort of real-life patients. *Child and adolescent psychiatry and mental health* **9:** 50.

Powers RL, Marks DJ, Miller CJ, Newcorn JH, Halperin JM (2008). Stimulant treatment in children with attention-deficit/hyperactivity disorder moderates adolescent academic outcome. *Journal of child and adolescent psychopharmacology* **18**(5)**:** 449-459.

Pramod AB, Foster J, Carvelli L, Henry LK (2013). SLC6 transporters: structure, function, regulation, disease association and therapeutics. *Molecular aspects of medicine* **34**(2-3)**:** 197-219.

Prasad V, Brogan E, Mulvaney C, Grainge M, Stanton W, Sayal K (2013). How effective are drug treatments for children with ADHD at improving on-task behaviour and academic achievement in the school classroom? A systematic review and meta-analysis. *Eur Child Adolesc Psychiatry* **22**(4)**:** 203-216.

Prosser J, Hughes CW, Sheikha S, Kowatch RA, Kramer GL, Rosenbarger N, *et al.* (1997). Plasma GABA in children and adolescents with mood, behavior, and comorbid mood and behavior disorders: a preliminary study. *Journal of child and adolescent psychopharmacology* **7**(3)**:** 181-199.

Purkayastha P, Malapati A, Yogeeswari P, Sriram D (2015). A Review on GABA/Glutamate Pathway for Therapeutic Intervention of ASD and ADHD. *Current medicinal chemistry*.

Purper-Ouakil D, Fourneret P, Wohl M, Reneric JP (2005). [Atomoxetine: a new treatment for Attention Deficit/Hyperactivity Disorder (ADHD) in children and adolescents]. *L'Encephale* **31**(3)**:** 337-348.

Puumala T, Ruotsalainen S, Jakala P, Koivisto E, Riekkinen P, Jr., Sirvio J (1996). Behavioral and pharmacological studies on the validation of a new animal model for attention deficit hyperactivity disorder. *Neurobiology of learning and memory* **66**(2)**:** 198-211.

Quarta D, Ferre S, Solinas M, You ZB, Hockemeyer J, Popoli P, *et al.* (2004). Opposite modulatory roles for adenosine A1 and A2A receptors on glutamate and dopamine release in the shell of the nucleus accumbens. Effects of chronic caffeine exposure. *Journal of neurochemistry* **88**(5)**:** 1151-1158.

Rada P, Avena NM, Hoebel BG (2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **134**(3)**:** 737-744.

Rader R, McCauley L, Callen EC (2009). Current strategies in the diagnosis and treatment of childhood attention-deficit/hyperactivity disorder. *Am Fam Physician* **79**(8)**:** 657-665.

Raiker JS, Rapport MD, Kofler MJ, Sarver DE (2012). Objectively-measured impulsivity and attention-deficit/hyperactivity disorder (ADHD): testing competing predictions from the working memory and behavioral inhibition models of ADHD. *Journal of abnormal child psychology* **40**(5)**:** 699-713.

Rajadhyaksha A, Husson I, Satpute SS, Kuppenbender KD, Ren JQ, Guerriero RM, *et al.* (2004). L-type Ca2+ channels mediate adaptation of extracellular signal-regulated kinase 1/2 phosphorylation in the ventral tegmental area after chronic amphetamine treatment. *J Neurosci* **24**(34)**:** 7464-7476.

Rajadhyaksha AM, Kosofsky BE (2005). Psychostimulants, L-type calcium channels, kinases, and phosphatases. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **11**(5)**:** 494-502.

Ramasamy R, Dadhich P, Dhingra A, Lipshultz L (2014). Case Report: Testicular failure possibly associated with chronic use of methylphenidate. *F1000Research* **3:** 207.

Rapoport JL, Inoff-Germain G (2002). Responses to methylphenidate in Attention-Deficit/Hyperactivity Disorder and normal children: update 2002. *J Atten Disord* **6 Suppl 1:** S57-60.

Rappley MD (1997). Safety issues in the use of methylphenidate. An American perspective. *Drug safety* **17**(3)**:** 143-148.

Rasch R (1979). Control of blood glucose levels in the streptozotocin diabetic rat using a long-acting heat-treated insulin. *Diabetologia* **16**(3)**:** 185-190.

Rebec GV, White IM, Puotz JK (1997). Responses of neurons in dorsal striatum during amphetamine-induced focused stereotypy. *Psychopharmacology (Berl)* **130**(4)**:** 343-351.

Reinius B, Blunder M, Brett FM, Eriksson A, Patra K, Jonsson J, *et al.* (2015). Conditional targeting of medium spiny neurons in the striatal matrix. *Frontiers in behavioral neuroscience* **9:** 71.

Renes JS, de Ridder MA, Breukhoven PE, Lem AJ, Hokken-Koelega AC (2012). Methylphenidate and the response to growth hormone treatment in short children born small for gestational age. *PloS one* **7**(12)**:** e53164.

Ricaurte GA, Mechan AO, Yuan J, Hatzidimitriou G, Xie T, Mayne AH, *et al.* (2005). Amphetamine treatment similar to that used in the treatment of adult attention-deficit/hyperactivity disorder damages dopaminergic nerve endings in the striatum of adult nonhuman primates. *J Pharmacol Exp Ther* **315**(1)**:** 91-98.

Richardson M, Moore DA, Gwernan-Jones R, Thompson-Coon J, Ukoumunne O, Rogers M, *et al.* (2015). Non-pharmacological interventions for attention-deficit/hyperactivity disorder (ADHD) delivered in school settings: systematic reviews of quantitative and qualitative research. *Health technology assessment* **19**(45)**:** 1-470.

Riddle EL, Hanson GR, Fleckenstein AE (2007). Therapeutic doses of amphetamine and methylphenidate selectively redistribute the vesicular monoamine transporter-2. *Eur J Pharmacol* **571**(1)**:** 25-28.

Rimondini R, Ferre S, Gimenez-Llort L, Ogren SO, Fuxe K (1998). Differential effects of selective adenosine A1 and A2A receptor agonists on dopamine receptor agonist-induced behavioural responses in rats. *Eur J Pharmacol* **347**(2-3)**:** 153-158.

Robertson SD, Matthies HJ, Galli A (2009). A closer look at amphetamine-induced reverse transport and trafficking of the dopamine and norepinephrine transporters. *Molecular neurobiology* **39**(2)**:** 73-80.

Robinson TE, Camp DM (1987). Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal locomotion. *Pharmacol Biochem Behav* **26**(4)**:** 821-827.

Robison LM, Skaer TL, Sclar DA, Galin RS (2002). Is attention deficit hyperactivity disorder increasing among girls in the US? Trends in diagnosis and the prescribing of stimulants. *CNS Drugs* **16**(2)**:** 129-137.

Rodgers RJ (1979). Effects of nicotine, mecamylamine, and hexamethonium on shock-induced fighting, pain reactivity, and locomotor behaviour in rats. *Psychopharmacology (Berl)* **66**(1)**:** 93-98.

Rodrigues J, Botelho C, Cadinha S, Castel-Branco MG (2008). Desensitization to methylphenidate--the relevance of continued drug intake for a successful outcome. *Allergologia et immunopathologia* **36**(5)**:** 303-305.

Roitman MF, Patterson TA, Sakai RR, Bernstein IL, Figlewicz DP (1999). Sodium depletion and aldosterone decrease dopamine transporter activity in nucleus accumbens but not striatum. *The American journal of physiology* **276**(5 Pt 2)**:** R1339-1345.

Roland AV, Moenter SM (2011). Prenatal androgenization of female mice programs an increase in firing activity of gonadotropin-releasing hormone (GnRH) neurons that is reversed by metformin treatment in adulthood. *Endocrinology* **152**(2)**:** 618-628.

Roman MW (2010). Treatments for childhood ADHD part II: non-pharmacological and novel treatments. *Issues in mental health nursing* **31**(9)**:** 616-618.

Root DH, Hoffman AF, Good CH, Zhang S, Gigante E, Lupica CR, *et al.* (2015). Norepinephrine activates dopamine D4 receptors in the rat lateral habenula. *J Neurosci* **35**(8)**:** 3460-3469.

Rosa-Neto P, Lou HC, Cumming P, Pryds O, Karrebaek H, Lunding J, *et al.* (2005). Methylphenidate-evoked changes in striatal dopamine correlate with inattention and impulsivity in adolescents with attention deficit hyperactivity disorder. *NeuroImage* **25**(3)**:** 868-876.

Rose GJ, Alluri RK, Vasquez-Opazo GA, Odom SE, Graham JA, Leary CJ (2013). Combining pharmacology and whole-cell patch recording from CNS neurons, in vivo. *Journal of neuroscience methods* **213**(1)**:** 99-104.

Rose SR, Reeves G, Gut R, Germak J (2015). Attention-Deficit/Hyperactivity Disorder Medication Treatment Impact on Response to Growth Hormone Therapy: Results from the ANSWER Program, a Non-Interventional Study. *The Journal of pediatrics* **167**(6)**:** 1389-1396.

Rosenblum K, Dudai Y, Richter-Levin G (1996). Long-term potentiation increases tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit 2B in rat dentate gyrus in vivo. *Proc Natl Acad Sci U S A* **93**(19)**:** 10457-10460.

Rosenkranz JA, Grace AA (1999). Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo. *J Neurosci* **19**(24)**:** 11027-11039.

Ross DC, Fischhoff J, Davenport B (2002). Treatment of ADHD when tolerance to methylphenidate develops. *Psychiatric services* **53**(1)**:** 102.

Ross RG (2006). Psychotic and manic-like symptoms during stimulant treatment of attention deficit hyperactivity disorder. *The American journal of psychiatry* **163**(7)**:** 1149-1152.

Rostas JA, Brent VA, Voss K, Errington ML, Bliss TV, Gurd JW (1996). Enhanced tyrosine phosphorylation of the 2B subunit of the N-methyl-D-aspartate receptor in long-term potentiation. *Proc Natl Acad Sci U S A* **93**(19)**:** 10452-10456.

Rouchet N, Waroux O, Lamy C, Massotte L, Scuvee-Moreau J, Liegeois JF, *et al.* (2008). SK channel blockade promotes burst firing in dorsal raphe serotonergic neurons. *The European journal of neuroscience* **28**(6)**:** 1108-1115.

Routh VH (2010). Glucose sensing neurons in the ventromedial hypothalamus. *Sensors* **10**(10)**:** 9002-9025.

Rowan JD, McCarty MK, Kundey SM, Osburn CD, Renaud SM, Kelley BM, *et al.* (2015). Adolescent exposure to methylphenidate impairs serial pattern learning in the serial multiple choice (SMC) task in adult rats. *Neurotoxicology and teratology* **51:** 21-26.

Rowland LM, Astur RS, Jung RE, Bustillo JR, Lauriello J, Yeo RA (2005). Selective cognitive impairments associated with NMDA receptor blockade in humans. *Neuropsychopharmacology* **30**(3)**:** 633-639.

Rozans M, Dreisbach A, Lertora JJ, Kahn MJ (2002). Palliative uses of methylphenidate in patients with cancer: a review. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **20**(1)**:** 335-339.

Rubia K, Alegria AA, Brinson H (2014). Brain abnormalities in attention-deficit hyperactivity disorder: a review. *Revista de neurologia* **58 Suppl 1:** S3-16.

Rush CR, Higgins ST, Vansickel AR, Stoops WW, Lile JA, Glaser PE (2005). Methylphenidate increases cigarette smoking. *Psychopharmacology* **181**(4)**:** 781-789.

Rush CR, Stoops WW, Sevak RJ, Hays LR (2010). Cocaine choice in humans during D-amphetamine maintenance. *Journal of clinical psychopharmacology* **30**(2)**:** 152-159.

Rush RA, Geffen LB (1980). Dopamine beta-hydroxylase in health and disease. *Critical reviews in clinical laboratory sciences* **12**(3)**:** 241-277.

Russell VA (2011). Overview of animal models of attention deficit hyperactivity disorder (ADHD). *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* **Chapter 9:** Unit9 35.

Russell VA, Sagvolden T, Johansen EB (2005). Animal models of attention-deficit hyperactivity disorder. *Behav Brain Funct* **1:** 9.

Russo RE, Velluti JC (1992). Inhibitory effects of excitatory amino acids on pyramidal cells of the in vitro turtle medial cortex. *Experimental brain research* **92**(1)**:** 85-93.

Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci* **33**(6)**:** 267-276.

Safer D, Allen R, Barr E (1972). Depression of growth in hyperactive children on stimulant drugs. *The New England journal of medicine* **287**(5)**:** 217-220.

Safer DJ, Allen RP, Barr E (1975). Growth rebound after termination of stimulant drugs. *The Journal of pediatrics* **86**(1)**:** 113-116.

Sagvolden T (2000). Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neurosci Biobehav Rev* **24**(1)**:** 31-39.

Sagvolden T, Johansen EB, Woien G, Walaas SI, Storm-Mathisen J, Bergersen LH, *et al.* (2009). The spontaneously hypertensive rat model of ADHD--the importance of selecting the appropriate reference strain. *Neuropharmacology* **57**(7-8)**:** 619-626.

Sagvolden T, Russell VA, Aase H, Johansen EB, Farshbaf M (2005). Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry* **57**(11)**:** 1239-1247.

Saigusa T, Aono Y, Uchida T, Takada K, Verheij MM, Koshikawa N, *et al.* (2012). The alpha(1)-, but not alpha(2)-, adrenoceptor in the nucleus accumbens plays an inhibitory role upon the accumbal noradrenaline and dopamine efflux of freely moving rats. *Eur J Pharmacol* **688**(1-3)**:** 35-41.

Salek RL, Claussen CM, Perez A, Dafny N (2012). Acute and chronic methylphenidate alters prefrontal cortex neuronal activity recorded from freely behaving rats. *Eur J Pharmacol* **679**(1-3)**:** 60-67.

Sallinen J, Haapalinna A, Viitamaa T, Kobilka BK, Scheinin M (1998). D-amphetamine and L-5-hydroxytryptophan-induced behaviours in mice with genetically-altered expression of the alpha2C-adrenergic receptor subtype. *Neuroscience* **86**(3)**:** 959-965.

Sams-Dodd F (1998). Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. *Neuropsychopharmacology* **19**(1)**:** 18-25.

Samuels ER, Szabadi E (2008). Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. *Current neuropharmacology* **6**(3)**:** 235-253.

Sandhu JK, Pandey S, Ribecco-Lutkiewicz M, Monette R, Borowy-Borowski H, Walker PR, *et al.* (2003). Molecular mechanisms of glutamate neurotoxicity in mixed cultures of NT2-derived neurons and astrocytes: protective effects of coenzyme Q10. *Journal of neuroscience research* **72**(6)**:** 691-703.

Sandstrom MI, Rebec GV (2003). Characterization of striatal activity in conscious rats: contribution of NMDA and AMPA/kainate receptors to both spontaneous and glutamate-driven firing. *Synapse* **47**(2)**:** 91-100.

Sara SJ, Herve-Minvielle A (1995). Inhibitory influence of frontal cortex on locus coeruleus neurons. *Proc Natl Acad Sci U S A* **92**(13)**:** 6032-6036.

Sarantis K, Matsokis N, Angelatou F (2009). Synergistic interactions of dopamine D1 and glutamate NMDA receptors in rat hippocampus and prefrontal cortex: involvement of ERK1/2 signaling. *Neuroscience* **163**(4)**:** 1135-1145.

Sargent PB (1993). The diversity of neuronal nicotinic acetylcholine receptors. *Annual review of neuroscience* **16:** 403-443.

Sasaki K, Omotuyi OI, Ueda M, Shinohara K, Ueda H (2015). NMDA receptor agonists reverse impaired psychomotor and cognitive functions associated with hippocampal Hbegf-deficiency in mice. *Molecular brain* **8**(1)**:** 83.

Sato S, Hatta M, Nagumo JI (1974). Response characteristics of a neuron model to a periodic input. *Kybernetik* **16**(1)**:** 1-8.

Sauer JM, Ponsler GD, Mattiuz EL, Long AJ, Witcher JW, Thomasson HR, *et al.* (2003). Disposition and metabolic fate of atomoxetine hydrochloride: the role of CYP2D6 in human disposition and metabolism. *Drug Metab Dispos* **31**(1)**:** 98-107.

Schachar R, Tannock R, Marriott M, Logan G (1995). Deficient inhibitory control in attention deficit hyperactivity disorder. *Journal of abnormal child psychology* **23**(4)**:** 411-437.

Schaefer TL, Davenport MH, Erickson CA (2015). Emerging pharmacologic treatment options for fragile X syndrome. *The application of clinical genetics* **8:** 75-93.

Schank JR, Ventura R, Puglisi-Allegra S, Alcaro A, Cole CD, Liles LC, *et al.* (2006). Dopamine beta-hydroxylase knockout mice have alterations in dopamine signaling and are hypersensitive to cocaine. *Neuropsychopharmacology* **31**(10)**:** 2221-2230.

Schelleman H, Bilker WB, Kimmel SE, Daniel GW, Newcomb C, Guevara JP, *et al.* (2012). Methylphenidate and risk of serious cardiovascular events in adults. *The American journal of psychiatry* **169**(2)**:** 178-185.

Scherer D, Hassel D, Bloehs R, Zitron E, von Lowenstern K, Seyler C, *et al.* (2009). Selective noradrenaline reuptake inhibitor atomoxetine directly blocks hERG currents. *British journal of pharmacology* **156**(2)**:** 226-236.

Schilstrom B, Yaka R, Argilli E, Suvarna N, Schumann J, Chen BT, *et al.* (2006). Cocaine enhances NMDA receptor-mediated currents in ventral tegmental area cells via dopamine D5 receptor-dependent redistribution of NMDA receptors. *J Neurosci* **26**(33)**:** 8549-8558.

Schippers MC, Schetters D, De Vries TJ, Pattij T (2016). Differential effects of the pharmacological stressor yohimbine on impulsive decision making and response inhibition. *Psychopharmacology (Berl)* **233**(14)**:** 2775-2785.

Schmeichel BE, Berridge CW (2013). Neurocircuitry underlying the preferential sensitivity of prefrontal catecholamines to low-dose psychostimulants. *Neuropsychopharmacology* **38**(6)**:** 1078-1084.

Schmitz F, Pierozan P, Rodrigues AF, Biasibetti H, Coelho DM, Mussulini BH, *et al.* (2015). Chronic Treatment with a Clinically Relevant Dose of Methylphenidate Increases Glutamate Levels in Cerebrospinal Fluid and Impairs Glutamatergic Homeostasis in Prefrontal Cortex of Juvenile Rats. *Molecular neurobiology*.

Schneider M, Koch M (2005). Behavioral and morphological alterations following neonatal excitotoxic lesions of the medial prefrontal cortex in rats. *Experimental neurology* **195**(1)**:** 185-198.

Schober AL, Mongin AA (2015). Intracellular levels of glutamate in swollen astrocytes are preserved via neurotransmitter reuptake and de novo synthesis: implications for hyponatremia. *Journal of neurochemistry* **135**(1)**:** 176-185.

Schoemaker JH, Jansen WT, Schipper J, Szegedi A (2014). The selective glycine uptake inhibitor org 25935 as an adjunctive treatment to atypical antipsychotics in predominant persistent negative symptoms of schizophrenia: results from the GIANT trial. *Journal of clinical psychopharmacology* **34**(2)**:** 190-198.

Schousboe A, Waagepetersen HS (2007). GABA: homeostatic and pharmacological aspects. *Progress in brain research* **160:** 9-19.

Schubert D, Piasecki D (2001). Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *J Neurosci* **21**(19)**:** 7455-7462.

Schuch V, Utsumi DA, Costa TV, Kulikowski LD, Muszkat M (2015). Attention Deficit Hyperactivity Disorder in the Light of the Epigenetic Paradigm. *Frontiers in psychiatry* **6:** 126.

Schur RR, Draisma LW, Wijnen JP, Boks MP, Koevoets MG, Joels M, *et al.* (2016). Brain GABA levels across psychiatric disorders: A systematic literature review and meta-analysis of H-MRS studies. *Hum Brain Mapp*.

Schwabe U, Ukena D, Lohse MJ (1985). Xanthine derivatives as antagonists at A1 and A2 adenosine receptors. *Naunyn-Schmiedeberg's archives of pharmacology* **330**(3)**:** 212-221.

Schwartz S, Correll CU (2014). Efficacy and safety of atomoxetine in children and adolescents with attention-deficit/hyperactivity disorder: results from a comprehensive meta-analysis and metaregression. *Journal of the American Academy of Child and Adolescent Psychiatry* **53**(2)**:** 174-187.

Schweimer JV, Ungless MA (2010). Phasic responses in dorsal raphe serotonin neurons to noxious stimuli. *Neuroscience* **171**(4)**:** 1209-1215.

Seamans JK, Yang CR (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Progress in neurobiology* **74**(1)**:** 1-58.

Seeman P, Guan HC, Hirbec H (2009). Dopamine D2High receptors stimulated by phencyclidines, lysergic acid diethylamide, salvinorin A, and modafinil. *Synapse* **63**(8)**:** 698-704.

Sellers EM (1978). Addictive drugs: disposition, tolerance, and dependence interrelationships. *Drug metabolism reviews* **8**(1)**:** 5-11.

Sembower MA, Ertischek MD, Buchholtz C, Dasgupta N, Schnoll SH (2013). Surveillance of diversion and nonmedical use of extended-release prescription amphetamine and oral methylphenidate in the United States. *Journal of addictive diseases* **32**(1)**:** 26-38.

Semenova S, Stolerman IP, Markou A (2007). Chronic nicotine administration improves attention while nicotine withdrawal induces performance deficits in the 5-choice serial reaction time task in rats. *Pharmacol Biochem Behav* **87**(3)**:** 360-368.

Semrud-Clikeman M, Pliszka SR, Bledsoe J, Lancaster J (2012). Volumetric MRI Differences in Treatment Naive and Chronically Treated Adolescents With ADHD-Combined Type. *J Atten Disord* **18**(6)**:** 511-520.

Senecky Y, Lobel D, Diamond GW, Weitz R, Inbar D (2002). Isolated orofacial dyskinesia: a methylphenidate-induced movement disorder. *Pediatric neurology* **27**(3)**:** 224-226.

Sengupta P (2013). The Laboratory Rat: Relating Its Age With Human's. *International journal of preventive medicine* **4**(6)**:** 624-630.

Seo D, Patrick CJ, Kennealy PJ (2008). Role of Serotonin and Dopamine System Interactions in the Neurobiology of Impulsive Aggression and its Comorbidity with other Clinical Disorders. *Aggression and violent behavior* **13**(5)**:** 383-395.

Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998). Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* **18(**7)**:** 2697-2708.

Shang CY, Gau SS (2012). Improving visual memory, attention, and school function with atomoxetine in boys with attention-deficit/hyperactivity disorder. *Journal of child and adolescent psychopharmacology* **22**(5)**:** 353-363.

Sheets MF, Hanck DA (2003). Molecular action of lidocaine on the voltage sensors of sodium channels. *The Journal of general physiology* **121**(2)**:** 163-175.

Shen KZ, Johnson SW (2010). Ca2+ influx through NMDA-gated channels activates ATP-sensitive K+ currents through a nitric oxide-cGMP pathway in subthalamic neurons. *J Neurosci* **30**(5)**:** 1882-1893.

Shen KZ, Johnson SW (2013). Group I mGluRs evoke K-ATP current by intracellular Ca2+ mobilization in rat subthalamus neurons. *J Pharmacol Exp Ther* **345**(1)**:** 139-150.

Shen KZ, Yakhnitsa V, Munhall AC, Johnson SW (2014). AMP kinase regulates K-ATP currents evoked by NMDA receptor stimulation in rat subthalamic nucleus neurons. *Neuroscience* **274:** 138-152.

Shen RY, Choong KC (2006). Different adaptations in ventral tegmental area dopamine neurons in control and ethanol exposed rats after methylphenidate treatment. *Biol Psychiatry* **59**(7)**:** 635-642.

Shi WX, Pun CL, Smith PL, Bunney BS (2000a). Endogenous DA-mediated feedback inhibition of DA neurons: involvement of both D(1)- and D(2)-like receptors. *Synapse* **35**(2)**:** 111-119.

Shi WX, Pun CL, Zhang XX, Jones MD, Bunney BS (2000b). Dual effects of D-amphetamine on dopamine neurons mediated by dopamine and nondopamine receptors. *J Neurosci* **20**(9)**:** 3504-3511.

Shi WX, Pun CL, Zhou Y (2004). Psychostimulants induce low-frequency oscillations in the firing activity of dopamine neurons. *Neuropsychopharmacology* **29**(12)**:** 2160-2167.

Shimamura AP (2000). The role of the prefrontal cortex in dynamic filtering. *Psychobiology* **28**(2)**:** 207-218.

Shimamura AP, Janowsky JS, Squire LR (1990). Memory for the temporal order of events in patients with frontal lobe lesions and amnesic patients. *Neuropsychologia* **28**(8)**:** 803-813.

Shippenberg TS, Thompson AC (2001). Overview of microdialysis. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* **Chapter 7:** Unit7 1.

Shlens J, Field GD, Gauthier JL, Greschner M, Sher A, Litke AM, *et al.* (2009). The structure of large-scale synchronized firing in primate retina. *J Neurosci* **29**(15)**:** 5022-5031.

Shorer Z, Becker B, Jacobi-Polishook T, Oddsson L, Melzer I (2012). Postural control among children with and without attention deficit hyperactivity disorder in single and dual conditions. *European journal of pediatrics* **171**(7)**:** 1087-1094.

Shpilenya LS, Muzychenko AP, Gasbarrini G, Addolorato G (2002). Metadoxine in acute alcohol intoxication: a double-blind, randomized, placebo-controlled study. *Alcoholism, clinical and experimental research* **26**(3)**:** 340-346.

Shriner R, Gold M (2014). Food addiction: an evolving nonlinear science. *Nutrients* **6**(11)**:** 5370-5391.

Siddiqui SV, Chatterjee U, Kumar D, Siddiqui A, Goyal N (2008). Neuropsychology of prefrontal cortex. *Indian journal of psychiatry* **50**(3)**:** 202-208.

Silva AP, Prado SO, Scardovelli TA, Boschi SR, Campos LC, Frere AF (2015). Measurement of the effect of physical exercise on the concentration of individuals with ADHD. *PloS one* **10**(3)**:** e0122119.

Silver IA, Erecinska M (1994). Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *J Neurosci* **14**(8)**:** 5068-5076.

Simchon-Tenenbaum Y, Weizman A, Rehavi M (2015). Alterations in brain neurotrophic and glial factors following early age chronic methylphenidate and cocaine administration. *Behavioural brain research* **282:** 125-132.

Simchon Y, Weizman A, Rehavi M (2010). The effect of chronic methylphenidate administration on presynaptic dopaminergic parameters in a rat model for ADHD. *Eur Neuropsychopharmacol* **20**(10)**:** 714-720.

Simmler LD, Rickli A, Hoener MC, Liechti ME (2014). Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. *Neuropharmacology* **79:** 152-160.

Simoes PF, Silva AP, Pereira FC, Marques E, Milhazes N, Borges F, *et al.* (2008). Methamphetamine changes NMDA and AMPA glutamate receptor subunit levels in the rat striatum and frontal cortex. *Ann N Y Acad Sci* **1139:** 232-241.

Simon H, Le Moal M, Stinus L, Calas A (1979). Anatomical relationships between the ventral mesencephalic tegmentum--a 10 region and the locus coeruleus as demonstrated by anterograde and retrograde tracing techniques. *J Neural Transm* **44**(1-2)**:** 77-86.

Simon SM, Llinas RR (1985). Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. *Biophysical journal* **48**(3)**:** 485-498.

Simpson D, Plosker GL (2004). Atomoxetine: a review of its use in adults with attention deficit hyperactivity disorder. *Drugs* **64**(2)**:** 205-222.

Sirois PA, Montepiedra G, Kapetanovic S, Williams PL, Pearson DA, Malee K, *et al.* (2009). Impact of medications prescribed for treatment of attention-deficit hyperactivity disorder on physical growth in children and adolescents with HIV. *Journal of developmental and behavioral pediatrics : JDBP* **30**(5)**:** 403-412.

Snyder SH, Katims JJ, Annau Z, Bruns RF, Daly JW (1981). Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci U S A* **78**(5)**:** 3260-3264.

Solhi H, Jamilian HR, Kazemifar AM, Javaheri J, Rasti Barzaki A (2014). Methylphenidate vs. resperidone in treatment of methamphetamine dependence: A clinical trial. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society* **22**(3)**:** 191-194.

Solinas M, Ferre S, You ZB, Karcz-Kubicha M, Popoli P, Goldberg SR (2002). Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J Neurosci* **22**(15)**:** 6321-6324.

Somkuwar SS, Jordan CJ, Kantak KM, Dwoskin LP (2013). Adolescent atomoxetine treatment in a rodent model of ADHD: effects on cocaine self-administration and dopamine transporters in frontostriatal regions. *Neuropsychopharmacology* **38**(13)**:** 2588-2597.

Somoza EC, Winhusen TM, Bridge TP, Rotrosen JP, Vanderburg DG, Harrer JM, *et al.* (2004). An open-label pilot study of methylphenidate in the treatment of cocaine dependent patients with adult attention deficit/hyperactivity disorder. *Journal of addictive diseases* **23**(1)**:** 77-92.

Sontag TA, Tucha O, Walitza S, Lange KW (2010). Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. *Attention deficit and hyperactivity disorders* **2**(1)**:** 1-20.

Sonuga-Barke EJ, Brandeis D, Cortese S, Daley D, Ferrin M, Holtmann M, *et al.* (2013). Nonpharmacological interventions for ADHD: systematic review and meta-analyses of

randomized controlled trials of dietary and psychological treatments. *The American journal of psychiatry* **170**(3)**:** 275-289.

Sostek AJ, Buchsbaum MS, Rapoport JL (1980). Effects of amphetamine on vigilance performance in normal and hyperactive children. *Journal of abnormal child psychology* **8**(4)**:** 491-500.

Spencer TJ, Brown A, Seidman LJ, Valera EM, Makris N, Lomedico A, *et al.* (2013). Effect of psychostimulants on brain structure and function in ADHD: a qualitative literature review of magnetic resonance imaging-based neuroimaging studies. *The Journal of clinical psychiatry* **74**(9)**:** 902-917.

Spencer TJ, Faraone SV, Biederman J, Lerner M, Cooper KM, Zimmerman B, *et al.* (2006). Does prolonged therapy with a long-acting stimulant suppress growth in children with ADHD? *Journal of the American Academy of Child and Adolescent Psychiatry* **45**(5)**:** 527-537.

Spielewoy C, Biala G, Roubert C, Hamon M, Betancur C, Giros B (2001). Hypolocomotor effects of acute and daily d-amphetamine in mice lacking the dopamine transporter. *Psychopharmacology (Berl)* **159**(1)**:** 2-9.

Spiller HA, Hays HL, Aleguas A, Jr. (2013). Overdose of drugs for attention-deficit hyperactivity disorder: clinical presentation, mechanisms of toxicity, and management. *CNS Drugs* **27**(7)**:** 531-543.

Sproson EJ, Chantrey J, Hollis C, Marsden CA, Fonel KC (2001). Effect of repeated methylphenidate administration on presynaptic dopamine and behaviour in young adult rats. *J Psychopharmacol* **15**(2)**:** 67-75.

Stalnaker TA, Berg B, Aujla N, Schoenbaum G (2016). Cholinergic Interneurons Use Orbitofrontal Input to Track Beliefs about Current State. *J Neurosci* **36**(23)**:** 6242-6257.

Stanley EF (1997). The calcium channel and the organization of the presynaptic transmitter release face. *Trends Neurosci* **20**(9)**:** 404-409.

Stefanini GF, Addolorato G, Caputo F, Bernardi M, Gasbarrini G (1999). Treatment of alcoholic fatty liver: is the metabolic effect of metadoxine the only reason for improved liver function? *Journal of hepatology* **30**(4)**:** 739-740.

Steffensen SC, Taylor SR, Horton ML, Barber EN, Lyle LT, Stobbs SH, *et al.* (2008). Cocaine disinhibits dopamine neurons in the ventral tegmental area via use-dependent blockade of GABA neuron voltage-sensitive sodium channels. *The European journal of neuroscience* **28**(10)**:** 2028-2040.

Stein DJ, Hollander E, Liebowitz MR (1993). Neurobiology of impulsivity and the impulse control disorders. *The Journal of neuropsychiatry and clinical neurosciences* **5**(1)**:** 9-17.

Steinau S (2013). Diagnostic Criteria in Attention Deficit Hyperactivity Disorder - Changes in DSM 5. *Frontiers in psychiatry* **4:** 49.

Steinkellner T, Freissmuth M, Sitte HH, Montgomery T (2011). The ugly side of amphetamines: short- and long-term toxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), methamphetamine and D-amphetamine. *Biological chemistry* **392**(1-2)**:** 103-115.

Steketee JD (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev* **41**(2-3)**:** 203-228.

Stephan KM, Thaut MH, Wunderlich G, Schicks W, Tian B, Tellmann L, *et al.* (2002). Conscious and subconscious sensorimotor synchronization--prefrontal cortex and the influence of awareness. *NeuroImage* **15**(2)**:** 345-352.

Stevens MC, Haney-Caron E (2012). Comparison of brain volume abnormalities between ADHD and conduct disorder in adolescence. *J Psychiatry Neurosci* **37**(6)**:** 389-398.

Stice E, Burger KS, Yokum S (2013). Relative ability of fat and sugar tastes to activate reward, gustatory, and somatosensory regions. *The American journal of clinical nutrition* **98**(6)**:** 1377-1384.

Stoner GR, Skirboll LR, Werkman S, Hommer DW (1988). Preferential effects of caffeine on limbic and cortical dopamine systems. *Biol Psychiatry* **23**(8)**:** 761-768.

Stratton P, Cheung A, Wiles J, Kiyatkin E, Sah P, Windels F (2012). Action potential waveform variability limits multi-unit separation in freely behaving rats. *PloS one* **7**(6)**:** e38482.

Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P (2004). Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* **29**(11)**:** 2007-2017.

Stumm G, Schlegel J, Schafer T, Wurz C, Mennel HD, Krieg JC, *et al.* (1999). Amphetamines induce apoptosis and regulation of bcl-x splice variants in neocortical neurons. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **13**(9)**:** 1065-1072.

Sturgess JE, Ting AKRA, Podbielski D, Sellings LH, Chen JF, van der Kooy D (2010). Adenosine A1 and A2A receptors are not upstream of caffeine's dopamine D2 receptor-dependent aversive effects and dopamine-independent rewarding effects. *The European journal of neuroscience* **32**(1)**:** 143-154.

Sullivan RM, Brake WG (2003). What the rodent prefrontal cortex can teach us about attention-deficit/hyperactivity disorder: the critical role of early developmental events on prefrontal function. *Behavioural brain research* **146**(1-2)**:** 43-55.

Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J Neurosci* **15**(5 Pt 2)**:** 4102-4108.

Sulzer D, Maidment NT, Rayport S (1993). Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. *Journal of neurochemistry* **60**(2)**:** 527-535.

Sun X, Zhao Y, Wolf ME (2005). Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci* **25**(32)**:** 7342-7351.

Sur C, Kinney GG (2007). Glycine transporter 1 inhibitors and modulation of NMDA receptor-mediated excitatory neurotransmission. *Current drug targets* **8**(5)**:** 643-649.

Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci* **30**(5)**:** 228-235.

Svanborg P, Thernlund G, Gustafsson PA, Hagglof B, Schacht A, Kadesjo B (2009). Atomoxetine improves patient and family coping in attention deficit/hyperactivity disorder: a randomized, double-blind, placebo-controlled study in Swedish children and adolescents. *Eur Child Adolesc Psychiatry* **18**(12)**:** 725-735.

Svensson TH, Tung CS (1989). Local cooling of pre-frontal cortex induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area in vivo. *Acta physiologica Scandinavica* **136**(1)**:** 135-136.

Swanson J, Baler RD, Volkow ND (2011). Understanding the effects of stimulant medications on cognition in individuals with attention-deficit hyperactivity disorder: a decade of progress. *Neuropsychopharmacology* **36**(1)**:** 207-226.

Swanson J, Gupta S, Guinta D, Flynn D, Agler D, Lerner M, *et al.* (1999). Acute tolerance to methylphenidate in the treatment of attention deficit hyperactivity disorder in children. *Clinical pharmacology and therapeutics* **66**(3)**:** 295-305.

Swanson JM (2005). Long-acting stimulants: development and dosing. *The Canadian child and adolescent psychiatry review = La revue canadienne de psychiatrie de l'enfant et de l'adolescent* **14**(Supplement 1)**:** 4-9.

Swanson JM, Elliott GR, Greenhill LL, Wigal T, Arnold LE, Vitiello B, *et al.* (2007). Effects of stimulant medication on growth rates across 3 years in the MTA follow-up. *Journal of the American Academy of Child and Adolescent Psychiatry* **46**(8)**:** 1015-1027.

Swanson JM, Volkow ND (2003). Serum and brain concentrations of methylphenidate: implications for use and abuse. *Neurosci Biobehav Rev* **27**(7)**:** 615-621.

Swanson JM, Wigal SB, Wigal T, Sonuga-Barke E, Greenhill LL, Biederman J, *et al.* (2004). A comparison of once-daily extended-release methylphenidate formulations in children with attention-deficit/hyperactivity disorder in the laboratory school (the Comacs Study). *Pediatrics* **113**(3 Pt 1)**:** e206-216.

Swanson LW (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain research bulletin* **9**(1-6)**:** 321-353.

Swanson LW, Hartman BK (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. *The Journal of comparative neurology* **163**(4)**:** 467-505.

Symmes A, Winters KC, Fahnhorst T, Botzet A, Lee S, August G, *et al.* (2015). The Association Between Attention-Deficit Hyperactivity Disorder and Nicotine Use Among Adolescents and Young Adults. *Journal of child & adolescent substance abuse* **24**(1)**:** 37-45.

Takamatsu Y, Hagino Y, Sato A, Takahashi T, Nagasawa SY, Kubo Y, *et al.* (2015). Improvement of learning and increase in dopamine level in the frontal cortex by methylphenidate in mice lacking dopamine transporter. *Current molecular medicine* **15**(3)**:** 245-252.

Tang M, Jiang P, Li H, Liu Y, Cai H, Dang R, *et al.* (2015). Fish oil supplementation alleviates depressant-like behaviors and modulates lipid profiles in rats exposed to chronic unpredictable mild stress. *BMC complementary and alternative medicine* **15:** 239.

Taylor BF, Ramirez HE, Battles AH, Andrutis KA, Neubert JK (2016). Analgesic Activity of Tramadol and Buprenorphine after Voluntary Ingestion by Rats (Rattus norvegicus). *Journal of the American Association for Laboratory Animal Science : JAALAS* **55**(1)**:** 74-82.

Taylor JL, Rajbhandari AK, Berridge KC, Aldridge JW (2010). Dopamine receptor modulation of repetitive grooming actions in the rat: potential relevance for Tourette syndrome. *Brain research* **1322:** 92-101.

Taylor SB, Lewis CR, Olive MF (2013). The neurocircuitry of illicit psychostimulant addiction: acute and chronic effects in humans. *Substance abuse and rehabilitation* **4:** 29-43.

Tehovnik EJ, Sommer MA (1997). Effective spread and timecourse of neural inactivation caused by lidocaine injection in monkey cerebral cortex. *Journal of neuroscience methods* **74**(1)**:** 17-26.

Teng L, Crooks PA, Dwoskin LP (1998). Lobeline displaces [3H]dihydrotetrabenazine binding and releases [3H]dopamine from rat striatal synaptic vesicles: comparison with d-amphetamine. *Journal of neurochemistry* **71**(1)**:** 258-265.

Tepper JM, Sun BC, Martin LP, Creese I (1997). Functional roles of dopamine D2 and D3 autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo. *J Neurosci* **17**(7)**:** 2519-2530.

ter Laak MA, Temmink AH, Koeken A, van 't Veer NE, van Hattum PR, Cobbaert CM (2010). Recognition of impaired atomoxetine metabolism because of low CYP2D6 activity. *Pediatric neurology* **43**(3)**:** 159-162.

Tercyak KP, Lerman C, Audrain J (2002). Association of attention-deficit/hyperactivity disorder symptoms with levels of cigarette smoking in a community sample of adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry* **41**(7)**:** 799-805.

Terkawi AS, Sharma S, Durieux ME, Thammishetti S, Brenin D, Tiouririne M (2015). Perioperative lidocaine infusion reduces the incidence of post-mastectomy chronic pain: a double-blind, placebo-controlled randomized trial. *Pain physician* **18**(2)**:** E139-146.

Terstappen GC, Pellacani A, Aldegheri L, Graziani F, Carignani C, Pula G, *et al.* (2003). The antidepressant fluoxetine blocks the human small conductance calcium-activated potassium channels SK1, SK2 and SK3. *Neurosci Lett* **346**(1-2)**:** 85-88.

Teter CJ, McCabe SE, LaGrange K, Cranford JA, Boyd CJ (2006). Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. *Pharmacotherapy* **26**(10)**:** 1501-1510.

Thanos PK, Michaelides M, Benveniste H, Wang GJ, Volkow ND (2007). Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. *Pharmacol Biochem Behav* **87**(4)**:** 426-433.

Thapar A, Cooper M, Jefferies R, Stergiakouli E (2012). What causes attention deficit hyperactivity disorder? *Arch Dis Child* **97**(3)**:** 260-265.

Thiele SL, Warre R, Nash JE (2012). Development of a unilaterally-lesioned 6-OHDA mouse model of Parkinson's disease. *Journal of visualized experiments : JoVE*(60).

Thomas D, Gut B, Wendt-Nordahl G, Kiehn J (2002). The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. *J Pharmacol Exp Ther* **300**(2)**:** 543-548.

Thomsen M, Han DD, Gu HH, Caine SB (2009). Lack of cocaine self-administration in mice expressing a cocaine-insensitive dopamine transporter. *J Pharmacol Exp Ther* **331**(1)**:** 204-211.

Tianzhu Z, Shihai Y, Juan D (2014). Antidepressant-like effects of cordycepin in a mice model of chronic unpredictable mild stress. *Evidence-based complementary and alternative medicine : eCAM* **2014:** 438506.

Tiihonen J, Kuoppasalmi K, Fohr J, Tuomola P, Kuikanmaki O, Vorma H, *et al.* (2007). A comparison of aripiprazole, methylphenidate, and placebo for amphetamine dependence. *The American journal of psychiatry* **164**(1)**:** 160-162.

Tirosh O, Sen CK, Roy S, Packer L (2000). Cellular and mitochondrial changes in glutamate-induced HT4 neuronal cell death. *Neuroscience* **97**(3)**:** 531-541.

Tomasi D, Volkow ND (2012). Abnormal functional connectivity in children with attention-deficit/hyperactivity disorder. *Biol Psychiatry* **71**(5)**:** 443-450.

Torvinen M, Torri C, Tombesi A, Marcellino D, Watson S, Lluis C, *et al.* (2005). Trafficking of adenosine A2A and dopamine D2 receptors. *Journal of molecular neuroscience : MN* **25**(2)**:** 191-200.

Tran-Van-Minh A, Caze RD, Abrahamsson T, Cathala L, Gutkin BS, DiGregorio DA (2015). Contribution of sublinear and supralinear dendritic integration to neuronal computations. *Frontiers in cellular neuroscience* **9:** 67.

Trepanier CH, Jackson MF, MacDonald JF (2012). Regulation of NMDA receptors by the tyrosine kinase Fyn. *The FEBS journal* **279**(1)**:** 12-19.

Tsacopoulos M, Magistretti PJ (1996). Metabolic coupling between glia and neurons. *J Neurosci* **16**(3)**:** 877-885.

Tseng KY, O'Donnell P (2004). Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J Neurosci* **24**(22)**:** 5131-5139.

Tseng KY, O'Donnell P (2007). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cereb Cortex* **17**(5)**:** 1235-1240.

Turic D, Langley K, Mills S, Stephens M, Lawson D, Govan C, *et al.* (2004). Follow-up of genetic linkage findings on chromosome 16p13: evidence of association of N-methyl-D aspartate glutamate receptor 2A gene polymorphism with ADHD. *Mol Psychiatry* **9**(2)**:** 169-173.

Turner DA, Adamson DC (2011a). Neuronal-astrocyte metabolic interactions: understanding the transition into abnormal astrocytoma metabolism. *Journal of neuropathology and experimental neurology* **70**(3)**:** 167-176.

Turner PV, Brabb T, Pekow C, Vasbinder MA (2011b). Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science : JAALAS* **50**(5)**:** 600-613.

Turner PV, Vaughn E, Sunohara-Neilson J, Ovari J, Leri F (2012). Oral gavage in rats: animal welfare evaluation. *Journal of the American Association for Laboratory Animal Science : JAALAS* **51**(1)**:** 25-30.

Tzavara ET, Bymaster FP, Overshiner CD, Davis RJ, Perry KW, Wolff M, *et al.* (2006). Procholinergic and memory enhancing properties of the selective norepinephrine uptake inhibitor atomoxetine. *Mol Psychiatry* **11**(2)**:** 187-195.

Udvardi PT, Fohr KJ, Henes C, Liebau S, Dreyhaupt J, Boeckers TM, *et al.* (2013). Atomoxetine affects transcription/translation of the NMDA receptor and the norepinephrine transporter in the rat brain--an in vivo study. *Drug Des Devel Ther* **7:** 1433-1446.

Uehara T, Sumiyoshi T, Matsuoka T, Itoh H, Kurachi M (2007). Effect of prefrontal cortex inactivation on behavioral and neurochemical abnormalities in rats with excitotoxic lesions of the entorhinal cortex. *Synapse* **61**(6)**:** 391-400.

Umemiya M, Raymond LA (1997). Dopaminergic modulation of excitatory postsynaptic currents in rat neostriatal neurons. *Journal of neurophysiology* **78**(3)**:** 1248-1255.

Ungless MA, Grace AA (2012). Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. *Trends Neurosci* **35**(7)**:** 422-430.

Ungless MA, Magill PJ, Bolam JP (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science* **303**(5666)**:** 2040-2042.

Upadhyaya HP, Desaiah D, Schuh KJ, Bymaster FP, Kallman MJ, Clarke DO, *et al.* (2013). A review of the abuse potential assessment of atomoxetine: a nonstimulant medication for attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl)* **226**(2)**:** 189-200.

Uranova NA, Klintzova AJ, Istomin VV, Haselhorst U, Schenk H (1989). The effects of amphetamine on synaptic plasticity in rat's medial prefrontal cortex. *J Hirnforsch* **30**(1)**:** 45-50.

Urban KR, Gao WJ (2013a). Methylphenidate and the juvenile brain: enhancement of attention at the expense of cortical plasticity? *Medical hypotheses* **81**(6)**:** 988-994.

Urban KR, Gao WJ (2014). Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain. *Frontiers in systems neuroscience* **8:** 38.

Urban KR, Li YC, Gao WJ (2013b). Treatment with a clinically-relevant dose of methylphenidate alters NMDA receptor composition and synaptic plasticity in the juvenile rat prefrontal cortex. *Neurobiology of learning and memory* **101:** 65-74.

Urban KR, Waterhouse BD, Gao WJ (2012). Distinct age-dependent effects of methylphenidate on developing and adult prefrontal neurons. *Biol Psychiatry* **72**(10)**:** 880-888.

Urcelay GP, Dalley JW (2012). Linking ADHD, impulsivity, and drug abuse: a neuropsychological perspective. *Current topics in behavioral neurosciences* **9:** 173-197.

Vaarmann A, Kovac S, Holmstrom KM, Gandhi S, Abramov AY (2013). Dopamine protects neurons against glutamate-induced excitotoxicity. *Cell death & disease* **4:** e455.

Valenti O, Cifelli P, Gill KM, Grace AA (2011). Antipsychotic drugs rapidly induce dopamine neuron depolarization block in a developmental rat model of schizophrenia. *J Neurosci* **31**(34)**:** 12330-12338.

Valenti O, Grace AA (2009). Entorhinal cortex inhibits medial prefrontal cortex and modulates the activity states of electrophysiologically characterized pyramidal neurons in vivo. *Cereb Cortex* **19**(3)**:** 658-674.

Valera EM, Faraone SV, Murray KE, Seidman LJ (2007). Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry* **61**(12)**:** 1361-1369.

Valvassori SS, Frey BN, Martins MR, Reus GZ, Schimidtz F, Inacio CG, *et al.* (2007). Sensitization and cross-sensitization after chronic treatment with methylphenidate in adolescent Wistar rats. *Behavioural pharmacology* **18**(3)**:** 205-212.

van der Marel K, Klomp A, Meerhoff GF, Schipper P, Lucassen PJ, Homberg JR, *et al.* (2014). Long-term oral methylphenidate treatment in adolescent and adult rats: differential effects on brain morphology and function. *Neuropsychopharmacology* **39**(2)**:** 263-273.

van Gaalen MM, van Koten R, Schoffelmeer AN, Vanderschuren LJ (2006). Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biol Psychiatry* **60**(1)**:** 66-73.

Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP (2012). hERG K(+) channels: structure, function, and clinical significance. *Physiological reviews* **92**(3)**:** 1393-1478.

Vandermaelen CP, Aghajanian GK (1983). Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain research* **289**(1-2)**:** 109-119.

Vansickel AR, Stoops WW, Glaser PE, Poole MM, Rush CR (2011). Methylphenidate increases cigarette smoking in participants with ADHD. *Psychopharmacology* **218**(2)**:** 381-390.

Vaughan RA, Foster JD (2013). Mechanisms of dopamine transporter regulation in normal and disease states. *Trends in pharmacological sciences* **34**(9)**:** 489-496.

Vazirani RP, Fioramonti X, Routh VH (2013). Membrane potential dye imaging of ventromedial hypothalamus neurons from adult mice to study glucose sensing. *Journal of visualized experiments : JoVE*(81).

Vedantham V, Cannon SC (1999). The position of the fast-inactivation gate during lidocaine block of voltage-gated Na+ channels. *The Journal of general physiology* **113**(1)**:** 7-16.

Velcea G, Winsberg BG (2004). Atomoxetine and nonresponders to stimulants. *The American journal of psychiatry* **161**(9)**:** 1718-1719.

Vendruscolo LF, Izidio GS, Takahashi RN (2009). Drug reinforcement in a rat model of attention deficit/hyperactivity disorder--the Spontaneously Hypertensive Rat (SHR). *Current drug abuse reviews* **2**(2)**:** 177-183.

Vetter VL, Elia J, Erickson C, Berger S, Blum N, Uzark K, *et al.* (2008). Cardiovascular monitoring of children and adolescents with heart disease receiving medications for attention deficit/hyperactivity disorder [corrected]: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young Congenital Cardiac Defects Committee and the Council on Cardiovascular Nursing. *Circulation* **117**(18)**:** 2407-2423.

Viggiano D, Vallone D, Sadile A (2004). Dysfunctions in dopamine systems and ADHD: evidence from animals and modeling. *Neural plasticity* **11**(1-2)**:** 97-114.

Vitiello B (2008). Understanding the risk of using medications for attention deficit hyperactivity disorder with respect to physical growth and cardiovascular function. *Child and adolescent psychiatric clinics of North America* **17**(2)**:** 459-474, xi.

Vles JS, Feron FJ, Hendriksen JG, Jolles J, van Kroonenburgh MJ, Weber WE (2003). Methylphenidate down-regulates the dopamine receptor and transporter system in children with attention deficit hyperkinetic disorder (ADHD). *Neuropediatrics* **34**(2)**:** 77-80.

Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley JS, *et al.* (1995). Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. *Archives of general psychiatry* **52**(6)**:** 456-463.

Volkow ND, Wang GJ, Fowler JS, Fischman M, Foltin R, Abumrad NN, *et al.* (1999a). Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. *Life sciences* **65**(1)**:** PL7-12.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Ding YS, Logan J, *et al.* (1996a). Relationship between psychostimulant-induced "high" and dopamine transporter occupancy. *Proc Natl Acad Sci U S A* **93**(19)**:** 10388-10392.

Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Angrist B, Gatley SJ, *et al.* (1999b). Association of methylphenidate-induced craving with changes in right striato-orbitofrontal metabolism in cocaine abusers: implications in addiction. *The American journal of psychiatry* **156**(1)**:** 19-26.

Volkow ND, Wang GJ, Gatley SJ, Fowler JS, Ding YS, Logan J, *et al.* (1996b). Temporal relationships between the pharmacokinetics of methylphenidate in the human brain and its behavioral and cardiovascular effects. *Psychopharmacology (Berl)* **123**(1)**:** 26-33.

Volkow ND, Wang GJ, Newcorn J, Fowler JS, Telang F, Solanto MV, *et al.* (2007a). Brain dopamine transporter levels in treatment and drug naive adults with ADHD. *NeuroImage* **34**(3)**:** 1182-1190.

Volkow ND, Wang GJ, Newcorn J, Telang F, Solanto MV, Fowler JS, *et al.* (2007b). Depressed dopamine activity in caudate and preliminary evidence of limbic involvement in adults with attention-deficit/hyperactivity disorder. *Archives of general psychiatry* **64**(8)**:** 932-940.

Volkow ND, Wang GJ, Tomasi D, Kollins SH, Wigal TL, Newcorn JH, *et al.* (2012). Methylphenidate-elicited dopamine increases in ventral striatum are associated with long-term symptom improvement in adults with attention deficit hyperactivity disorder. *J Neurosci* **32**(3)**:** 841-849.

Volz TJ, Farnsworth SJ, Rowley SD, Hanson GR, Fleckenstein AE (2008). Methylphenidate-induced increases in vesicular dopamine sequestration and dopamine release in the striatum: the role of muscarinic and dopamine D2 receptors. *J Pharmacol Exp Ther* **327**(1)**:** 161-167.

Walderhaug E, Herman AI, Magnusson A, Morgan MJ, Landro NI (2010). The short (S) allele of the serotonin transporter polymorphism and acute tryptophan depletion both increase impulsivity in men. *Neurosci Lett* **473**(3)**:** 208-211.

Walker DJ, Mason O, Clemow DB, Day KA (2015). Atomoxetine treatment in adults with attention-deficit/hyperactivity disorder. *Postgraduate medicine* **127**(7)**:** 686-701.

Wallace LJ (2012). Effects of amphetamine on subcellular distribution of dopamine and DOPAC. *Synapse* **66**(7)**:** 592-607.

Wallace LJ, Connell LE (2008). Mechanisms by which amphetamine redistributes dopamine out of vesicles: a computational study. *Synapse* **62**(5)**:** 370-378.

Wang GJ, Volkow ND, Wigal T, Kollins SH, Newcorn JH, Telang F, *et al.* (2013). Long-term stimulant treatment affects brain dopamine transporter level in patients with attention deficit hyperactive disorder. *PloS one* **8**(5)**:** e63023.

Wang GX, Ma YH, Wang SF, Ren GF, Guo H (2012). Association of dopaminergic/GABAergic genes with attention deficit hyperactivity disorder in children. *Molecular medicine reports* **6**(5)**:** 1093-1098.

Wang H, Stradtman GG, 3rd, Wang XJ, Gao WJ (2008). A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proc Natl Acad Sci U S A* **105**(43)**:** 16791-16796.

Wang HX, Mittleman MA, Leineweber C, Orth-Gomer K (2006). Depressive symptoms, social isolation, and progression of coronary artery atherosclerosis: the Stockholm Female Coronary Angiography Study. *Psychotherapy and psychosomatics* **75**(2)**:** 96-102.

Wang J, Carnicella S, Phamluong K, Jeanblanc J, Ronesi JA, Chaudhri N, *et al.* (2007a). Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. *J Neurosci* **27**(13)**:** 3593-3602.

Wang J, Lanfranco MF, Gibb SL, Yowell QV, Carnicella S, Ron D (2010a). Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. *J Neurosci* **30**(30)**:** 10187-10198.

Wang J, O'Donnell P (2001). D(1) dopamine receptors potentiate nmda-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. *Cereb Cortex* **11**(5)**:** 452-462.

Wang RY, Aghajanian GK (1977). Recording of single unit activity during electrical stimulation and microiontophoresis: a method of minimizing stimulus artifacts. *Electroencephalography and clinical neurophysiology* **43**(3)**:** 434-437.

Wang W, Pizzonia JH, Richerson GB (1998). Chemosensitivity of rat medullary raphe neurones in primary tissue culture. *The Journal of physiology* **511 (Pt 2):** 433-450.

Wang Y, Krishnan HR, Ghezzi A, Yin JC, Atkinson NS (2007b). Drug-induced epigenetic changes produce drug tolerance. *PLoS biology* **5**(10)**:** e265.

Wang Y, Liu J, Gui ZH, Ali U, Fan LL, Hou C, *et al.* (2011). alpha2-Adrenoceptor regulates the spontaneous and the GABA/glutamate modulated firing activity of the rat medial prefrontal cortex pyramidal neurons. *Neuroscience* **182:** 193-202.

Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M (2010b). [Estimation of the normal range of blood glucose in rats]. *Wei sheng yan jiu = Journal of hygiene research* **39**(2)**:** 133-137, 142.

Wang ZJ, Yu B, Zhang XQ, Sheng ZF, Li SJ, Huang YL, *et al.* (2014). Correlations between depression behaviors and sleep parameters after repeated corticosterone injections in rats. *Acta pharmacologica Sinica* **35**(7)**:** 879-888.

Warburton DM (1992). Nicotine as a cognitive enhancer. *Progress in neuro-psychopharmacology & biological psychiatry* **16**(2)**:** 181-191.

Warton FL, Howells FM, Russell VA (2009). Increased glutamate-stimulated release of dopamine in substantia nigra of a rat model for attention-deficit/hyperactivity disorder--lack of effect of methylphenidate. *Metabolic brain disease* **24**(4)**:** 599-613.

Wayment HK, Schenk JO, Sorg BA (2001). Characterization of extracellular dopamine clearance in the medial prefrontal cortex: role of monoamine uptake and monoamine oxidase inhibition. *J Neurosci* **21**(1)**:** 35-44.

Wee S, Woolverton WL (2004). Evaluation of the reinforcing effects of atomoxetine in monkeys: comparison to methylphenidate and desipramine. *Drug Alcohol Depend* **75**(3)**:** 271-276.

Weinshenker D, Ferrucci M, Busceti CL, Biagioni F, Lazzeri G, Liles LC, *et al.* (2008). Genetic or pharmacological blockade of noradrenaline synthesis enhances the neurochemical, behavioral, and neurotoxic effects of methamphetamine. *Journal of neurochemistry* **105**(2)**:** 471-483.

Whalen CK, Henker B, Dotemoto S (1981). Teacher response to the methylphenidate (ritalin) versus placebo status of hyperactive boys in the classroom. *Child development* **52**(3)**:** 1005-1014.

Whalen CK, Jamner LD, Henker B, Delfino RJ, Lozano JM (2002). The ADHD spectrum and everyday life: experience sampling of adolescent moods, activities, smoking, and drinking. *Child development* **73**(1)**:** 209-227.

Wheeler DD, Edwards AM, Chapman BM, Ondo JG (1993). A model of the sodium dependence of dopamine uptake in rat striatal synaptosomes. *Neurochem Res* **18**(8)**:** 927-936.

Wheeler TL, Eppolito AK, Smith LN, Huff TB, Smith RF (2007). A novel method for oral stimulant administration in the neonate rat and similar species. *Journal of neuroscience methods* **159**(2)**:** 282-285.

White FJ (1996). Synaptic regulation of mesocorticolimbic dopamine neurons. *Annual review of neuroscience* **19:** 405-436.

White FJ, Wang RY (1984). A10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. *Life sciences* **34**(12)**:** 1161-1170.

White FJ, Wang RY (1986). Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. *J Neurosci* **6**(1)**:** 274-280.

Wickham RJ, Solecki WB, Nunes EJ, Addy NA (2015). Distinct effects of ventral tegmental area NMDA and acetylcholine receptor blockade on conditioned reinforcement produced by food-associated cues. *Neuroscience* **301:** 384-394.

Wietecha L, Williams D, Shaywitz S, Shaywitz B, Hooper SR, Wigal SB, *et al.* (2013a). Atomoxetine improved attention in children and adolescents with attention-deficit/hyperactivity disorder and dyslexia in a 16 week, acute, randomized, double-blind trial. *Journal of child and adolescent psychopharmacology* **23**(9)**:** 605-613.

Wietecha LA, Ruff DD, Allen AJ, Greenhill LL, Newcorn JH (2013b). Atomoxetine tolerability in pediatric and adult patients receiving different dosing strategies. *The Journal of clinical psychiatry* **74**(12)**:** 1217-1223.

Wigal SB (2009). Efficacy and safety limitations of attention-deficit hyperactivity disorder pharmacotherapy in children and adults. *CNS Drugs* **23 Suppl 1:** 21-31.

Wignall ND, de Wit H (2011). Effects of nicotine on attention and inhibitory control in healthy nonsmokers. *Exp Clin Psychopharmacol* **19**(3)**:** 183-191.

Wilens TE (2008). Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder. *Journal of clinical psychopharmacology* **28**(3 Suppl 2)**:** S46-53.

Wilens TE (2004). Impact of ADHD and its treatment on substance abuse in adults. *The Journal of clinical psychiatry* **65 Suppl 3:** 38-45.

Wilens TE, Biederman J, Faraone SV, Martelon M, Westerberg D, Spencer TJ (2009). Presenting ADHD symptoms, subtypes, and comorbid disorders in clinically referred adults with ADHD. *The Journal of clinical psychiatry* **70**(11)**:** 1557-1562.

Wilens TE, Faraone SV, Biederman J, Gunawardene S (2003). Does stimulant therapy of attention-deficit/hyperactivity disorder beget later substance abuse? A meta-analytic review of the literature. *Pediatrics* **111**(1)**:** 179-185.

Wilens TE, Spencer TJ (2010). Understanding attention-deficit/hyperactivity disorder from childhood to adulthood. *Postgraduate medicine* **122**(5)**:** 97-109.

Willcutt EG (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* **9**(3)**:** 490-499.

Williams JT, Henderson G, North RA (1985). Characterization of alpha 2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. *Neuroscience* **14**(1)**:** 95-101.

Willner P (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* **134**(4)**:** 319-329.

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* **93**(3)**:** 358-364.

Wilson CJ, Chang HT, Kitai ST (1990). Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. *J Neurosci* **10**(2)**:** 508-519.

Winsberg B, Matinsky S, Kupietz S, Richardson E (1987). Is there dose-dependent tolerance associated with chronic methylphenidate therapy in hyperactive children: oral dose and plasma considerations. *Psychopharmacology bulletin* **23**(1)**:** 107-110.

Winstanley CA, Dalley JW, Theobald DE, Robbins TW (2003). Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology (Berl)* **170**(3)**:** 320-331.

Winstanley CA, Theobald DE, Cardinal RN, Robbins TW (2004). Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J Neurosci* **24**(20)**:** 4718-4722.

Winterstein AG, Gerhard T, Shuster J, Saidi A (2009). Cardiac safety of methylphenidate versus amphetamine salts in the treatment of ADHD. *Pediatrics* **124**(1)**:** e75-80.

Wolf ME (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in neurobiology* **54**(6)**:** 679-720.

Wolf ME, Ferrario CR (2010). AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci Biobehav Rev* **35**(2)**:** 185-211.

Wolf ME, White FJ, Nassar R, Brooderson RJ, Khansa MR (1993). Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J Pharmacol Exp Ther* **264**(1)**:** 249-255.

Wolosin SM, Richardson ME, Hennessey JG, Denckla MB, Mostofsky SH (2009). Abnormal cerebral cortex structure in children with ADHD. *Hum Brain Mapp* **30**(1)**:** 175-184.

Wolraich ML, McGuinn L, Doffing M (2007). Treatment of attention deficit hyperactivity disorder in children and adolescents: safety considerations. *Drug safety* **30**(1)**:** 17-26.

Woo HD, Kim DW, Hong YS, Kim YM, Seo JH, Choe BM, *et al.* (2014). Dietary patterns in children with attention deficit/hyperactivity disorder (ADHD). *Nutrients* **6**(4)**:** 1539-1553.

Wood J, Kim Y, Moghaddam B (2012). Disruption of prefrontal cortex large scale neuronal activity by different classes of psychotomimetic drugs. *J Neurosci* **32**(9)**:** 3022-3031.

Woodward JJ, Compton DM, Balster RL, Martin BR (1995). In vitro and in vivo effects of cocaine and selected local anesthetics on the dopamine transporter. *Eur J Pharmacol* **277**(1)**:** 7-13.

Wooters TE, Bardo MT (2011). Methylphenidate and fluphenazine, but not amphetamine, differentially affect impulsive choice in spontaneously hypertensive, Wistar-Kyoto and Sprague-Dawley rats. *Brain research* **1396:** 45-53.

Wooters TE, Bardo MT (2009). Nicotinic receptors differentially modulate the induction and expression of behavioral sensitization to methylphenidate in rats. *Psychopharmacology (Berl)* **204**(3)**:** 551-562.

Worbe Y, Savulich G, Voon V, Fernandez-Egea E, Robbins TW (2014). Serotonin depletion induces 'waiting impulsivity' on the human four-choice serial reaction time task: cross-species translational significance. *Neuropsychopharmacology* **39**(6)**:** 1519-1526.

Wu YN, Shen KZ, Johnson SW (1999). Presynaptic inhibition preferentially reduces in NMDA receptor-mediated component of transmission in rat midbrain dopamine neurons. *British journal of pharmacology* **127**(6)**:** 1422-1430.

Wulbert M, Dries R (1977). The relative efficacy of methylphenidate (ritalin) and behavior-modification techniques in the treatment of a hyperactive child. *Journal of applied behavior analysis* **10**(1)**:** 21-31.

Xiu X, Puskar NL, Shanata JA, Lester HA, Dougherty DA (2009). Nicotine binding to brain receptors requires a strong cation-pi interaction. *Nature* **458**(7237)**:** 534-537.

Xu C, Shen RY (2001). Amphetamine normalizes the electrical activity of dopamine neurons in the ventral tegmental area following prenatal ethanol exposure. *J Pharmacol Exp Ther* **297**(2)**:** 746-752.

Xu TX, Ma Q, Spealman RD, Yao WD (2010). Amphetamine modulation of long-term potentiation in the prefrontal cortex: dose dependency, monoaminergic contributions, and paradoxical rescue in hyperdopaminergic mutant. *Journal of neurochemistry* **115**(6)**:** 1643-1654.

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015). The ins and outs of the striatum: role in drug addiction. *Neuroscience* **301:** 529-541.

Yamada K, Nabeshima T (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *Journal of pharmacological sciences* **91**(4)**:** 267-270.

Yamaguchi T, Sheen W, Morales M (2007). Glutamatergic neurons are present in the rat ventral tegmental area. *The European journal of neuroscience* **25**(1)**:** 106-118.

Yamamoto DJ, Nelson AM, Mandt BH, Larson GA, Rorabaugh JM, Ng CM, *et al.* (2013). Rats classified as low or high cocaine locomotor responders: a unique model involving striatal dopamine transporters that predicts cocaine addiction-like behaviors. *Neurosci Biobehav Rev* **37**(8)**:** 1738-1753.

Yamashita M, Sakakibara Y, Hall FS, Numachi Y, Yoshida S, Kobayashi H, *et al.* (2013). Impaired cliff avoidance reaction in dopamine transporter knockout mice. *Psychopharmacology (Berl)* **227**(4)**:** 741-749.

Yang CR, Chen L (2005). Targeting prefrontal cortical dopamine D1 and N-methyl-D-aspartate receptor interactions in schizophrenia treatment. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **11**(5)**:** 452-470.

Yang FN, Pan JS, Li X (2016). Beta-adrenoreceptor blockade abolishes atomoxetine-induced risk taking. *Physiology & behavior* **153:** 125-132.

Yang P, Swann A, Dafny N (2000). NMDA receptor antagonist disrupts acute and chronic effects of methylphenidate. *Physiology & behavior* **71**(1-2)**:** 133-145.

Yang PB, Atkins KD, Dafny N (2011). Behavioral sensitization and cross-sensitization between methylphenidate amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) in female SD rats. *Eur J Pharmacol* **661**(1-3)**:** 72-85.

Yang PB, Swann AC, Dafny N (2003). Chronic pretreatment with methylphenidate induces cross-sensitization with amphetamine. *Life sciences* **73**(22)**:** 2899-2911.

Yang PB, Swann AC, Dafny N (2007). Methylphenidate treated at the test cage--dose-dependent sensitization or tolerance depend on the behavioral assay used. *Crit Rev Neurobiol* **19**(1)**:** 59-77.

Yanofski J (2011). The Dopamine Dilemma-Part II: Could Stimulants Cause Tolerance, Dependence, and Paradoxical Decompensation? *Innovations in clinical neuroscience* **8**(1)**:** 47-53.

Yee A, Chin SC, Hashim AH, Harbajan Singh MK, Loh HS, Sulaiman AH, *et al.* (2015). Anhedonia in depressed patients on treatment with selective serotonin reuptake inhibitor anti-depressant--A two-centered study in Malaysia. *International journal of psychiatry in clinical practice* **19**(3)**:** 182-187.

Yeh GC, Chen JC, Tsai HC, Wu HH, Lin CY, Hsu PC, *et al.* (2002). Amphetamine inhibits the N-methyl-D-aspartate receptor-mediated responses by directly interacting with the receptor/channel complex. *J Pharmacol Exp Ther* **300**(3)**:** 1008-1016.

Yeh JZ, Tanguy J (1985). Na channel activation gate modulates slow recovery from use-dependent block by local anesthetics in squid giant axons. *Biophysical journal* **47**(5)**:** 685-694.

Yeung SY, Millar JA, Mathie A (1999). Inhibition of neuronal KV potassium currents by the antidepressant drug, fluoxetine. *British journal of pharmacology* **128**(7)**:** 1609-1615.

Yin R, French ED (2000). A comparison of the effects of nicotine on dopamine and non-dopamine neurons in the rat ventral tegmental area: an in vitro electrophysiological study. *Brain research bulletin* **51**(6)**:** 507-514.

Yoshimura Y, Ohmura T, Komatsu Y (2003). Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex. *J Neurosci* **23**(16)**:** 6557-6566.

Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, *et al.* (2004). Nicotine improves sustained attention in mice: evidence for involvement of the alpha7 nicotinic acetylcholine receptor. *Neuropsychopharmacology* **29**(5)**:** 891-900.

Young JW, Light GA, Marston HM, Sharp R, Geyer MA (2009). The 5-choice continuous performance test: evidence for a translational test of vigilance for mice. *PloS one* **4**(1)**:** e4227.

Yung SS, Lai SF, Lam MT, Lee VC, Li RH, Ho PC, *et al.* (2015). A randomized, controlled, double-blinded trial of local lidocaine gel and intrauterine lidocaine infusion for pain relief during saline infusion sonogram. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*.

Zahniser NR, Doolen S (2001). Chronic and acute regulation of Na+/Cl- -dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. *Pharmacology & therapeutics* **92**(1)**:** 21-55.

Zanier-Gomes PH, de Abreu Silva TE, Zanetti GC, Benati ER, Pinheiro NM, Murta BM, *et al.* (2015). Depressive behavior induced by social isolation of predisposed female rats. *Physiology & behavior* **151:** 292-297.

Zehle S, Bock J, Jezierski G, Gruss M, Braun K (2007). Methylphenidate treatment recovers stress-induced elevated dendritic spine densities in the rodent dorsal anterior cingulate cortex. *Developmental neurobiology* **67**(14)**:** 1891-1900.

Zetterling M, Hillered L, Enblad P, Karlsson T, Ronne-Engstrom E (2011). Relation between brain interstitial and systemic glucose concentrations after subarachnoid hemorrhage. *Journal of neurosurgery* **115**(1)**:** 66-74.

Zhang B, Sun L, Yang YM, Huang HP, Zhu FP, Wang L, *et al.* (2011). Action potential bursts enhance transmitter release at a giant central synapse. *The Journal of physiology* **589**(Pt 9)**:** 2213-2227.

Zhang CL, Feng ZJ, Liu Y, Ji XH, Peng JY, Zhang XH, *et al.* (2012a). Methylphenidate enhances NMDA-receptor response in medial prefrontal cortex via sigma-1 receptor: a novel mechanism for methylphenidate action. *PloS one* **7**(12)**:** e51910.

Zhang D, Gao M, Xu D, Shi WX, Gutkin BS, Steffensen SC, *et al.* (2012b). Impact of prefrontal cortex in nicotine-induced excitation of ventral tegmental area dopamine neurons in anesthetized rats. *J Neurosci* **32**(36)**:** 12366-12375.

Zhang H, Du M, Zhuang S (2010a). Impact of long-term treatment of methylphenidate on height and weight of school age children with ADHD. *Neuropediatrics* **41**(2)**:** 55-59.

Zhang JT, Ma SS, Yip SW, Wang LJ, Chen C, Yan CG, *et al.* (2015). Decreased functional connectivity between ventral tegmental area and nucleus accumbens in Internet gaming disorder: evidence from resting state functional magnetic resonance imaging. *Behav Brain Funct* **11**(1)**:** 37.

Zhang TA, Placzek AN, Dani JA (2010b). In vitro identification and electrophysiological characterization of dopamine neurons in the ventral tegmental area. *Neuropharmacology* **59**(6)**:** 431-436.

Zhang W, Klimek V, Farley JT, Zhu MY, Ordway GA (1999). alpha2C adrenoceptors inhibit adenylyl cyclase in mouse striatum: potential activation by dopamine. *J Pharmacol Exp Ther* **289**(3)**:** 1286-1292.

Zhao J, Ramadan E, Cappiello M, Wroblewska B, Bzdega T, Neale JH (2001). NAAG inhibits KCl-induced [(3)H]-GABA release via mGluR3, cAMP, PKA and L-type calcium conductance. *The European journal of neuroscience* **13**(2)**:** 340-346.

Zhou FM, Wilson CJ, Dani JA (2002). Cholinergic interneuron characteristics and nicotinic properties in the striatum. *Journal of neurobiology* **53**(4)**:** 590-605.

Zhou J (2004). Norepinephrine transporter inhibitors and their therapeutic potential. *Drugs of the future* **29**(12)**:** 1235-1244.

Zhou Y, Bunney BS, Shi WX (2006). Differential effects of cocaine on firing rate and pattern of dopamine neurons: role of alpha1 receptors and comparison with L-dopa and apomorphine. *J Pharmacol Exp Ther* **317**(1)**:** 196-201.

Zhuo M (2009). Plasticity of NMDA receptor NR2B subunit in memory and chronic pain. *Molecular brain* **2:** 4.

Ziereis S, Jansen P (2015). Effects of physical activity on executive function and motor performance in children with ADHD. *Research in developmental disabilities* **38:** 181-191.

Zigova T, Pencea V, Wiegand SJ, Luskin MB (1998). Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb. *Molecular and cellular neurosciences* **11**(4)**:** 234-245.

Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V, *et al.* (2009). Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J Pharmacol Exp Ther* **329**(2)**:** 738-746.

Zosel A, Bartelson BB, Bailey E, Lowenstein S, Dart R (2013). Characterization of adolescent prescription drug abuse and misuse using the Researched Abuse Diversion and Addiction-related Surveillance (RADARS((R))) System. *Journal of the American Academy of Child and Adolescent Psychiatry* **52**(2)**:** 196-204 e192.

Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, *et al.* (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc Natl Acad Sci U S A* **106**(18)**:** 7281-7288.