Effects of chronic modafinil treatment in young rats on social behaviour and dopaminergic neurotransmission

Tesis entregada a
LA UNIVERSIDAD DE VALPARAÍSO
en Cumplimiento Parcial de los requisitos para optar al grado de
Doctor en Ciencias con Mención en Neurociencia

Facultad De Ciencias
Por
Valeska Soledad Cid Jofré
Noviembre, 2020
Dirigida por: Dr. Gonzalo Cruz Neculpán
Codirigida por: Dra. Georgina Renard

FACULTAD DE CIENCIAS

UNIVERSIDAD DE VALPARAÍSO
INFORME DE APROBACION
TESIS DE DOCTORADO

Se informa a la Facultad de Ciencias que la Tesis de Doctorado presentada por:
Valeska Soledad Cid Jofré

Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Doctor en Ciencias con mención en Neurociencia, en el examen de Defensa de Tesis rendido el día 21 del mes de octubre de 2020

Director/a de Tesis:

Dr. Gonzalo Cruz Neculpán ....

Codirector/a de Tesis:

Dra. Georgina Renard ....

Comisión de Evaluación de la Tesis

Dr. Ramón Sotomayor Zárate ....
Dr. Waldo Cerpa ....
Dr. /a. ....

Dedication

I want to dedicate this thesis to my fiancé, Tamara Maldonado for her love and support, especially in the hardest times, when I was near to gave up. You took me out of the hole in which I was trapped. I'm sure that the little family that we started years ago will continue to be full of love, support and kindness. You push me to be a better professional, sister, daughter and human being.

To my family and friends for all the kind words, advices, jokes and beers, especially to Daniela de Giorgis, Jennifer Caroca, Macarena Gárate, Macarena Moreno, Daniela Cáceres, Gabriela Pino, Maximiliano Elgueta, Francisca Tobar, Viviana Valero, Claudia Sanhueza y Carlos Rivacoba.

Acknowledgment

First, I want to thank my advisors Georgina Renard and Gonzalo Cruz for the patience and all the advices and kind critics in the numerous meetings in all these years. I'm sure that this thesis would not be the same without you. Second, I want to thank to Waldo Cerpa for his kind advices and Ramón Sotomayor-Zárate for letting me use the equipment (HPLC) in his laboratory and for all the corrections that aid me to improve this project.

Additionally, I want to thank the DNUV program for the financial support for enable going to SOFARCHI meetings (2018 and 2019) and USA to learn a new technique and work in the final part of the results in the laboratory of Rodrigo España in DUCOM, Philadelphia.

This work was supported by ANID 21171017 doctorate scholarship to VCJ, DA031900 to RAE, FONDECYT Grant 120-1816 to GC, FONDECYT Grant 120–0474 to RSZ, CENFI, Universidad de Valparaíso, Grant DIUV-CI January 2006 to RSZ and GC and DICYT grant 021901R_MED Universidad de Santiago, USACH, to GMR.

Index

Table List

Figure List

Symbols List, abbreviations, or nomenclature

ADHD	attentional deficit hyperactivity disorder
AMPH	amphetamine
COMT	catechol-O-methyltransferase
DA	dopamine
DAT	dopamine transporter
D_1	dopamine receptor type 1
D_2	dopamine receptor type 2
D_2S	dopamine receptor type 2 short isoform
D_2L	dopamine receptor type 2 long isoform
DOPAC	3,4-Dihydroxyphenylacetic acid
FSCV	fast scan cyclic voltammetry
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid receptor type A
$GABA_B$	γ-aminobutyric acid receptor type B

GP	*globus pallidus*
GLU	glutamate
mGluR	metabotropic glutamate receptor
GLT-1	glutamate transporter
MAO	monoamine oxidase
MSNs	medium spiny neurons
MOD	modafinil
MPH	methylphenidate
NAc	nucleus accumbens
NAcc	nucleus accumbens core
NAcSh	nucleus accumbens shell
NE	norepinephrine
NET	norepinephrine transporter
PFC	prefrontal cortex
PND	post natal days
mPFC	medial prefrontal cortex
SN	*substancia nigra*
SPB	social play behaviour
TH	tyroxine hidroxylase

VP	*ventral pallidum*
VTA	ventral tegmental area

Abstract

Modafinil (MOD) is an atypical stimulant used to enhance wakefulness and vigilance. The mechanism of action of MOD includes the blockage of dopamine (DA) and norepinephrine (NE) transporters (DAT and NET, respectively). In humans, it has been demonstrated that MOD binds to DAT in nucleus accumbens (NAc), an important nucleus in the reward circuitry. Moreover, studies have been shown a crucial role for glutamate (GLU) and γ-aminobutyric acid (GABA) in the reward circuitry in relation to drug addiction. Also, MOD administration modifies DA and GABA extracellular levels in NAc in naïve animals. Clinical trials are testing MOD for the treatment of attentional deficit hyperactivity disorder (ADHD) in children. In view of a reported over diagnostic of ADHD, evaluating the effects of MOD in healthy young individuals is crucial.

Herein, we evaluated the effects of 14 days of MOD treatment in behavioural (social play behaviour and locomotor activity) and neurochemical measurements (content levels of DA, DOPAC, glutamate and GABA in NAc and VTA), moreover, we analyzed maximal DA uptake, extracellular DA levels and DA release in NAc and D_2 expression in PFC and NAc after 14 days of treatment in young healthy rats.

MOD altered and decreased the replies to play solicitations, additionally reduced the expression of D_2 in PFC and the response of DAergic neurons in NAc after K^+ 70 mM depolarizing stimulus. The lower responsiveness of DAergic terminals could be explained by the trend for high extracellular GABA basal levels and less inhibition of the glutamatergic projections in PFC due

to lower expression of D_2. To date, our results are the first evidence showing effects on social play behaviour and DA system within reward circuitry after 14 days of MOD treatment in young healthy individuals. Therefore, more data is needed to unravel the effects of MOD in the mesocorticolimbic circuitry in young individuals, specifically in key brain nuclei as NAc, VTA and PFC.

Resumen

El modafinilo (MOD) es un estimulante atípico usado para aumentar la vigilia y el desvelo. El mecanismo de acción del MOD es el bloqueo de los transportadores de dopamina (DA) y norepinefrina (NE) (DAT y NET, respectivamente).

En humanos, se ha demostrado que MOD se une a DAT en el núcleo accumbens (NAc), un núcleo importante en el circuito de la recompensa. Además, estudios han demostrado el rol crucial de los neurotransmisores glutamato (GLU) y ácido γ-aminobutírico (GABA) en el circuito de la recompensa en relación con la drogadicción. Adicionalmente, la administración de MOD modifica los niveles extracelulares de DA y GABA en NAc en animales adultos sin tratamiento previo.

Pruebas clínicas están evaluando el uso de MOD para el tratamiento del desorden de déficit atencional con hiperactividad (ADHD) en niños y niñas. En vista de los reportes del sobre diagnóstico de ADHD, evaluar los efectos de MOD en individuos sanos y jóvenes es crucial.

Aquí, hemos evaluado los efectos de 14 días de tratamiento con MOD en medidas conductuales (juego social y actividad locomotora) y neuroquímicas (niveles de contenido de DA, DOPAC, glutamato y GABA en NAc y VTA), además, hemos analizado la recaptura máxima de DA, niveles extracelulares de DA y liberación de DA en NAc, expresión de D_2 en PFC y NAc luego de 14 días de tratamiento en ratas jóvenes y sanas.

MOD disminuyó y alteró las respuestas a solicitudes de juego, adicionalmente, redujo la expresión de D_2 en PFC y la respuesta en neuronas dopaminérgicas en NAc luego de un estímulo despolarizante.

La baja respuesta de los terminales dopaminérgicos puede ser explicada por la tendencia a mayores niveles extracelulares basales de GABA y una menor inhibición de proyecciones glutamatérgicas en PFC mediadas por la menor expresión de D_2.

A la fecha, nuestros resultados son los primeros en mostrar efectos en el comportamiento de juego social y sistema dopaminérgico de recompensa, luego de 14 días de tratamiento con MOD en individuos sanos y jóvenes. Por lo tanto, se necesitan más datos para revelar los efectos de MOD en el circuito mesocorticolimbico de individuos jóvenes, en áreas cerebrales claves como son los núcleos NAc, VTA y PFC.

Graphical abstract

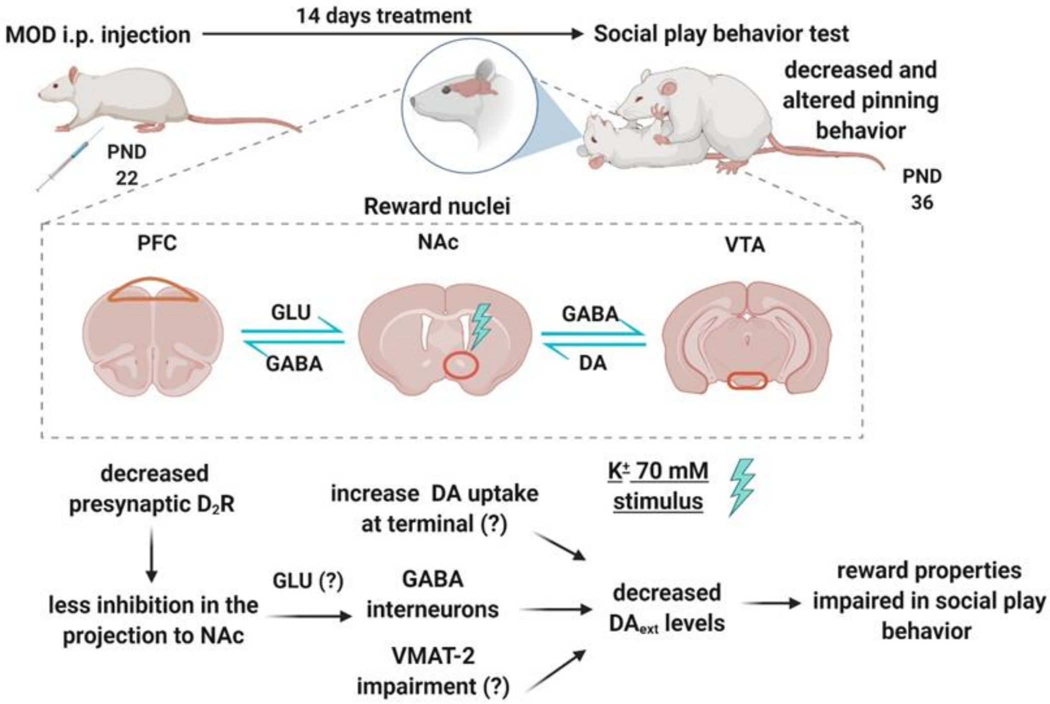


Figure 1. Graphical abstract. Chronic MOD treatment during adolescence altered social behaviour and neurochemistry of DA homeostasis in nucleus accumbens (NAc). MOD treatment during adolescence leads fewer responses to play solicitation in treated rats. The rewarding value of social play is apparently decreased during MOD treatment due to lower DA release levels in NAc after K^+ induced depolarization. Illustration created with Biorender.com

Introduction

Modafinil (MOD) is a wake promoting drug considered an atypical central nervous system stimulant which is commonly prescribed for narcolepsy and idiopathic hypersomnia (Kumar, 2008; US Modafinil in Narcolepsy Multicentre Study Group, 2000). MOD was first synthetized in the late seventies in France (Billiard & Broughton, 2018). FDA express concerns about MOD use under 17 years of age due to unknown effects (FDA, 2015), therefore, to date MOD is recommended only for adults and after physician prescription.

MOD and other drugs, like methylphenidate (MPH) or amphetamines (AMPH) are commonly misused by young people as a neuroenhancer (Dance, 2016; Teter, Falone, Cranford, Boyd & McCabe, 2010). Especially, those drugs are being used by college and high school students with the aiming of increase academic performance (Teter, McCabe, LaGrange, Cranford, & Boyd, 2006; Singh, Bard & Jackson, 2014). The background of this off-label use, is that MPH and AMPH have been used for long time for children and adolescent with ADHD helping them to focus, study and having a better performance in high school. However, the evidence of efficacy in academic performance reached by healthy individuals taking these drugs is arguable (Baranski Pigeau, Dinich, & Jacobs, 2004; Bisagno, González & Urbano, 2016; Gonzalez, Jayanthi, Gomez, Torres, Sosa, Bernardi,... & Bisagno, 2018; Li, Yang, Zhou, Liu, Wei, Xin, ... & Becker, 2020; Minzenberg & Carter, 2008). Therefore, the use of these psychostimulants as neuroenhancers in young healthy individuals remain elusive.

It is important to mention that MPH, AMPH and cocaine are drugs situated in the second higher category of addiction potential (Schedule II) according by DEA (United States Drug Enforcement Administration) but MOD is in the Schedule IV. Therefore, MOD could be safer than MPH and AMPH in the development of drug addiction. In this context, research groups are investigating whether MOD could be prescribed for children and adolescents with ADHD and early work has shown auspicious results (Biederman, Swanson, Wigal, Kratochvil, Boellner, Earl, ... & Greenhill, 2005; Kahbazhi, Ghoreishi, Rahiminejad, Mohammadi, Kamalipour & Akhondzadeh, 2009; Rugino & Samsock, 2003). However, there are concerns about psychostimulants use in children and adolescents with ADHD. Reports show an increase in worldwide ADHD diagnosis in the last years (Polanczyk, De Lima, Horta, Biederman & Rohde, 2007; Thomas, Sanders, Doust, Beller & Glasziou, 2015), and also increase in consumption of MPH and other drugs with off-label use in health young individuals (Teter et al., 2006; Singh & Jackson, 2014). Importantly, high ADHD misdiagnosis rates make difficult to accurately assess for the deficit in young populations (Polanczyk, Willcutt, Salum, Kieling & Rohde, 2014). Having those worries in mind, MOD appears as a good alternative, due to present a low liability to develop addiction than other psychostimulants (Myrick, Malcolm, Taylor & LaROWE, 2004; Mignon, Nishino, Guilleminault & Dement, 1994; Paterson, Fedolak, Olivier, Hanania, Ghavami & Caldarone, 2010; but see Wuo-Sila, Fukushiro, Borçoi, Fernandes, Procópio-Souza, Hollais, ... & Saito, 2011). However, there is a lack of research studying the long-term effects of MOD in healthy individuals especially in children and adolescents.

MOD acts on mesocorticolimbic dopaminergic system, targeting dopamine transporter (DAT) (Federici, Latagliata, Rizzo, Ledonne, Gu, Romigi,... & Mercuri, 2013; Volkow, Fowler, Logan, Alexoff, Zhu, Telang,… & Apelskog-Torres, 2009b) like MPH, AMPH and cocaine but showing a lower affinity for DAT than those drugs (Mereu, Bonci, Newman & Tanda, 2013). The mechanism of action of MOD is blocking DAT but also norepinephrine transporter (NET), with a lower affinity (Mereu et al., 2013). The immediate outcome of inhibiting transporters is blocking the ability to uptake neurotransmitters, leading to an increase of extracellular concentration of the monoamine. The mesocorticolimbic circuitry is known as the reward system. Drugs of abuse, natural rewards and motivated behaviours such as feeding, social interactions like pair bonding, parental care, play and sexual intercourse, activate this circuitry, resulting in the increase of dopamine (DA) levels in the nucleus accumbens (NAc) (reviewed at Volkow & Morales, 2015). One of the main dopaminergic nuclei of the reward system is the ventral tegmental area (VTA) that projects dopaminergic fibres to the NAc, prefrontal cortex (PFC) and other limbic areas. VTA has a crucial role in the control of motivated behaviours. DA transmission from VTA to NAc and other limbic structures participates in the processes behind motivated behaviour and reinforcement and is a core structure for drug addiction (Koob & Volkow, 2016; Volkow, Koob & McLellan, 2016). Besides, NAc projects GABAergic neurons to VTA in returns and PFC projects glutamatergic neurons to VTA. Additionally, PFC projects glutamatergic neurons to NAc and GABAergic medium spiny neurons (MSNs) in NAc have DA receptors (reviewed at Russo & Nestler, 2013).

Oral MOD in humans binds to DAT, thus increasing extracellular DA levels in putamen, caudate and NAc (Volkow et al., 2009b). Oral MOD in adult Sprague Dawley rats also showed increasing levels of DA in both PFC and striatum only with the two higher doses (300 and 600 mg/kg). Moreover, microdialysis studies have shown increases in extracellular DA levels in NAc after MOD subcutaneous administration in naïve adult rats (Ferraro, Sergio, O’Connor, Tiziana, Francis & Kjell, 1996). In mice, 6 days of MOD i.p. injections increase binding of DAT agonist [^{3}H] mazindol and decreases dopamine type 2 receptor (D_2) binding in NAc and PFC (Nguyen, Tian, You, Lee & Jang, 2011). Evidence in mice lacking D_2 demonstrated that this D_2 is essential for arousal MOD effects (Qu, Huang, Xu, Matsumoto & Urade, 2008) and MOD induced a reduction in D_2 activity and result in a higher activation of midbrain DA neurons (Korotkova, Klyuch, Ponomarenko, Lin, Haas & Sergeeva, 2007). This data show that MOD acts on DAT and DA receptors to enhance release and function of mesocorticolimbic circuitry.

However, not only DA is important in the reward circuitry. Glutamate (GLU) and GABA are also essential to the regulation of this circuitry. Activation of VTA GLU neurons that project to NAc can reinforce behaviour and stimulate DA co-release in NAc (Zell, Steinkellner, Hollon, Warlow, Souter, Faget... & Hnasko, 2020). Regarding psychostimulants, cocaine exposure increases extracellular GLU levels in VTA (Kalivas, McFarland, Bowers, Szumlinski, Xi & Baker, 2003), however, the opposite was observed in NAc of cocaine addicted individuals (Engeli, Zoelch, Hock, Nordt, Hulka, Kirschner,… & Seifritz, 2020). Within the NAc, there is a highly specialization and relationship between different types of interneurons, neurons and receptors that set a more complex frame for psychostimulants effects in this nucleus. Briefly, GABAergic MSNs express GLU, GABA and DA receptors and receives glutamatergic inputs from VTA and PFC and DAergic innervation from VTA (Haber, 2003). The DA terminals from VTA express D_2 autoreceptors controlling DA release in NAc (as negative feedback). Communication between NAc and VTA is mediated by GABAergic projections from NAc to VTA (Nicola, Surmeier & Malenka, 2000; Zahm, 2000). It is important to mention that dopamine type 1 receptor (D_1) or D_2 are not equally expressed in all MSNs, rather they are selectively expressed (Le Moine & Bloch, 1995a, b). Therefore, the release of DA in NAc is under GABAergic inhibitory tone from NAc GABA projections to VTA and GABAergic interneurons within NAc. Regarding to GLU and GABA, acute subcutaneous MOD administration in adult rats diminished GABA release in brain areas such as NAc, *substantia nigra* (SN) and *globus pallidus* (GP) and increases striatal glutamate release (Ferraro et al., 1996, 1997, 1998). Those results suggest that part of the effects of MOD could be due to a modulation of GABA and/or GLU neurotransmission. It is not completely clear how MOD could lead to lower levels of extracellular GABA levels in NAc, and the chronic use of MOD, especially in young individuals remain to be understand. Considering this, MOD directly impacts DA system within mesocorticolimbic circuitry and indirectly can impact both glutamatergic and GABAergic systems in the reward circuitry.

Social interactions are motivated and rewarding behaviour confirmed by behavioural tests like conditioned place preference (CPP) (Thiel, Okun & Neisewander, 2008). Moreover, there is evidence revealing the importance of reward circuitry on social interactions in both animal models and humans (Insel & Fernald, 2004). For example, social interactions increase DA release in NAc in both adult and juvenile rats (Robinson et al., 2011a, b). In addition, studies had highlighted the role of the reward circuitry on social behaviours, especially in the NAc, where pharmacological manipulations showed the role of D_2 in the formation of pair bonding and partner preference both in males and females (Liu, Aragona, Young, Dietz, Kabbaj, Mazei-Robison,... & Wang, 2010; Wang, Yu, Cascio, Liu, Gingrich, & Insel, 1999).

Within different types of social behaviours, there is one highly performed in youth, called social play behaviour (SPB). Playing is a type of motivated rewarding behaviour, inducing CPP in rats (Calcagnetti & Schechter, 1992; Trezza, Damsteegt & Vanderschuren, 2009). Moreover, play is a behaviour observed in a wide range of animals, especially in mammals (Burghardt, 2005). Regardless of being an early developed and young type of social behaviour, social play in rats has a structure, with two main components: 1) a pounce is occurring when one rat vigorously pounces at the nape of the other rat with either its snout or front paws, 2) a pin occurs when one rat rolls complete or partially onto its back with the other rat on top. Pins often, but not always, occur in response to a nape contact intent (Panksepp, Siviy, & Normansell, 1984; Pellis, Field, Smith & Pellis, 1997) therefore, pinning display allows the continuity of social play interactions. In rats, SPB is observed days before weaning and reached the peak near sexual maturation, i.e. PND 35-40. After sexual maturity SPB decreases in frequency and structure and other types of social interactions increases (Blanco-Gandia, Mateos-García, García-Pardo, Montagud-Romero, Rodríguez-Arias, Miñarro, & Aguilar 2015; Vanderschuren, Achterberg, & Trezza, 2016; Vanderschuren & Trezza, 2013). Play has an important role in social, cognitive and brain development, especially in highly social animals. Play therapy have been showed to decreased hyperactivity behaviour in rats after neonatal frontal lesions (Panksepp, Burgdorf, Turner & Gordon, 2003). In humans, ADHD has been related with poor skills or lack of playfulness in children (Alessandri, 1992) and play has been started to be recommended for kids with poor social skills related to neuropsychiatric conditions (Leuzinger-Bohleber, 2010).

Play is sensitive to several factors, including motivational state, isolation periods and housing conditions. For example, in young Sprague Dawley rats, different housing conditions produce high or low levels of playing (Varlinskaya & Spear, 2008) and the total SPB of a young rat dependent on the play level of the playing partner (Varlinskaya, Spear & Spear, 1999).

Play behaviour is also sensitive to drugs, indeed, acute AMPH and MPH treatment reduces play in rats (Achterberg, van Kerkhof, Damsteegt, Trezza & Vanderschuren, 2015; Field & Pellis, 1994). Chronic MPH treatment reduces SPB which is evidenced by a decrease in crossover of one young rat to another (Thor & Holloway, 1983) and decrease in social investigation time (Thor & Holloway, 1986). Acute MPH treatment also decreases play (Vanderschuren, Trezza, Griffioen-Roose, Schiepers, Van Leeuwen, De Vries & Schoffelmeer, 2008), however, contrary results have been shown when the treatment is longer (Bolaños, Barrot, Berton, Wallace-Black & Nestler, 2003). Acute AMPH and cocaine administration after 3.5 hrs of isolation reduces both pouncing and pinning frequencies without affecting social exploration time (in the case of AMPH), although, with different mechanisms involvement (Achterberg, Trezza, Siviy, Schrama, Schoffelmeer & Vanderschuren, 2014). In young animals, D_2 have been related to regulation of playfulness. When D_2 antagonist quinpirole was used in young rats before social play test, both solicitations and responses decreased (Siviy et al., 1996). Finally, when naturally less playful rats are compared with other strains it was shown that less playful rats exhibit lower levels of DA release in dorsal and ventral striatum (Siviy, Crawford, Akopian & Walsh, 2011; Siviy, 2020).

The D_2 is expressed post- and pre-synaptically in whole striatum, specifically in GABAergic MSNs neurons and DA projections from VTA, respectively (Xia, Driscoll, Wilbrecht, Margolis, Fields & Hjelmstad, 2011). It is important to mention that D_2 present two isoforms, long (D_2L) and short (D_2S) that are mainly expressed in post and pre-synaptic neurons, respectively (Khan et al., 1998a, 1998b). The D_2S have been related to regulate via negative feedback DA release from terminals (Cass & Gerhardt, 1994; Ford, Gantz, Phillips & Williams, 2010), although, interaction with DAT have been proposed to regulate extracellular levels of DA (Ferris, España, Locke, Konstantopoulos, Rose, Chen & Jones, 2014; Ford, 2014) and this interaction is affected by psychostimulants (Siciliano, Calipari, Ferris & Jones, 2015; Williams & Galli, 2006). Ergo, extracellular DA levels could reveal the homeostasis between release and uptake, being the two outcomes regulated by D_2 autoreceptor and DAT, respectively. The effects of psychostimulants on DA receptors and transporters are well known (Schmitt & Reith, 2010), however their exact molecular mechanisms are not completely elucidated, especially with MOD. Although, there is evidence regarding to cocaine, where it has been shown that produces a lower spontaneous activity of DA neurons in VTA (Ackerman & White, 1992) or desensitisation of D_2 in VTA leading to higher DA release in NAc (Striplin & Kalivas, 1992). Also, cocaine leads to lower levels of striatal D_2 (Nader, Morgan, Gage, Nader, Calhoun, Buchheimer, … & Mach, 2006) even after 1 and 4 months of withdrawal (Volkow, Fowler, Wang, Hitzemann, Logan, Schlyer, … & Wolf, 1993). Moreover, others have shown that the changes in DA circuitry are not directly related to D_2

autoreceptor levels, rather being in signalling pathways within de VTA and NAc (Lu, Grimm, Shaham & Hope, 2003) including up regulation on cAMP.

The neurobiological underpinnings of SPB implicate several brain areas and neurotransmitters. PFC has received attention considering the site of action of psychostimulants used as pharmacological treatment of ADHD. When MPH is infused directly to anterior cingulate or infralimbic cortex play is reduced, however social exploration is increased (Achterberg et al., 2015). In this context, D_1 and D_2 DA receptor antagonists, injected in NAc, impair social play behaviour (Manduca, Servadio, Damsteegt, Campolongo, Vanderschuren & Trezza, 2016). Neonatal 6-OHDA lesions of the striatum produces that rats that receives play soliciting attempts run away more (Pellis, Castañeda, McKenna, Tran-Nguyen, & Whishaw, 1993a) and neonatal frontal lesions produces low pinning frequencies without pouncing alterations in adolescent rats (Panksepp et al., 1994) and similar results are observed after mPFC lesions (Bell, McCaffrey, Forgie, Kolb & Pellis, 2009). However, it is important to mention that those type of lesions are made shortly after birth and are permanent, ergo, compensatory effects cannot be ruled out. Additionally, there is an increase in neuronal activity (measured by c-Fos expression) in both dorsal and ventral striatum and in NAc after social play (van Kerkhof et al., 2012, 2013, 2014) indicating rewarding effects of social play at neurochemical level. Interestingly, only in females that played, neuronal activity was detected in VTA and the number of cells was directly correlated with play and pinning duration (Northcutt & Nguyen, 2014).

Considering that the effects of the early use of MOD on social behaviour and DA homeostasis in the reward circuitry, especially in young and healthy individuals are not elucidated, in this study, we evaluate SPB and its association with changes on the dopaminergic pathway from VTA to NAc after a chronic MOD treatment during preadolescence.

Research question

Does chronic administration of MOD alter social play behaviour through changes in DA neurotransmission in young healthy rats?

Hypothesis

Chronic MOD treatment in healthy juvenile rats impairs social behaviour through an alteration in dopaminergic transmission in the VTA-NAc reward circuitry.

General aim

Evaluate the effects of chronic MOD treatment on social behaviour and dopaminergic neurotransmission in NAc and VTA in healthy juvenile rats.

Specific aims

1. To evaluate the effects of chronic MOD treatment during preadolescence on social play behaviour and locomotor activity in healthy juvenile rats.

2. To evaluate the effects of chronic MOD treatment during preadolescence on dopamine and DOPAC content in NAc and VTA, D_2 expression in NAc and dopamine extracellular levels in NAc in healthy juvenile rats.

Complementary aims

1. To evaluate the effect of chronic MOD on tissue content and extracellular glutamate and GABA levels in NAc of healthy juvenile rats.

2. To evaluate the effect of chronic MOD administration during preadolescence on locomotor activity induced by MOD.

Methods and materials

Animals

Male rats were purchased from the vivarium of the Faculty of Biology at the Pontificia Universidad Católica de Chile (UC CINBIOT Animal Facility funded by PIA CONICYT ECM-07) and arrived our animal facility at Universidad de Santiago de Chile at PND 21. All animals (n= 84) were housed in groups of three, four or five (depending on weight) in polysulphonate cages. They were maintained with food and water *ad libitum*, a 12h:12h light-dark cycle (lights on at 7.00 h), with controlled room temperature and humidity. All animal procedures were performed under approval of the ethical committee for animal care of Universidad de Santiago de Chile (N°615/2017).

Experimental design

All rats were randomly assigned into two groups: vehicle (control) or modafinil (MOD). The treatment consisted in one daily intraperitoneal (i.p.) injection that started at PND 22 and finished 14 days after at PND 35. Both vehicle and MOD-injected rats were isolated for 24 hours immediately after the last injection of vehicle or MOD (see table 1). All the behavioural tests were performed one night after the last injection (PND 36). Rats remained isolated in-home cages with access to food and water ad libitum until the social behaviour tests. All the experiments were conducted during the dark phase, between 19.00 and 23.00 hours.

PND 21	PND 22–PND 35	PND 35–PND 36	PND 36	PND 36	PND 36
Facility arrival	MOD i.p. treatment or vehicle	Isolation for behavioural test group	Behavioural tests, microdialysis or FSCV	euthanasia	Sample collection

Table 1. Methodology synthesis and general group configuration.

Drug preparation and administration

MOD was donated by Laboratorio Saval S.A. and was prepared as shown by García and colleagues (2013). Briefly, every rat received a vehicle or MOD i.p. injection (75 mg/kg; prepared in saline with tween 80 at 16:1 volume:volume, respectively). Administration was performed between 5 and 15 minutes before the dark phase started.

Behavioural tests

a) Basal locomotor activity: After acclimation, basal locomotor activity was recorded for 10 minutes in the experimental cage, just before initiating the interaction test (social play test). Several parameters such as mean and maximum speed, distance traveled, mobile time, immobile time and number of rearing were analysed using ANY-MazeTM software (Stoelting Co., Illinois, USA).

<u>b) Social exploration:</u> The test of 10 minutes was carried out immediately after the injection in a new cage with bedding. Social exploration time was assessed via analysis of each video. Each of the approaches initiated and performed by experimental rats (vehicle or modafinil) was considered. Social behaviour included were sniffing, grooming, playing and chasing.

<u>c) Social play behaviour</u>: The test was carried out in a new cage with bedding, in the same room where the rats were acclimated and immediately after the injection. After 10' minutes in the cage, another unfamiliar rat of the same sex and similar weight (stimuli rat) was introduced by the experimenter. The social play test had a duration of 10 minutes and was recorded with a video camera. We considered a minimum of 6 pouncing events for stimuli rats as acceptable, therefore we only included in the analysis of social play behaviour dyads where stimuli rats display a minimum of 6 pouncing events and excluded pairs that do not meet this criterion. The criterion was chosen after the analyses of normal social play behavior in different papers (Veenema and Neumann, 2009; Bredewold et al., 2014). The basic components of social play behaviour according to Trezza and colleagues (2010) were measured: 1) **pinning**, meaning the positive responses to play solicitation; when the rat performed partial or complete dorsal contact with the surface of the home cage exposing abdominal area in responses to a pouncing event (nape attack or play solicitation) and 2) **pouncing**, which is the behaviour for play solicitation; when the rat puts on top of the other rat and rub the muzzle on or near to the neck of the other rat. Both behaviour peak two weeks after weaning and decline after sexual maturation being changed to other forms of social behaviour, like chasing, following, sniffing and grooming. We performed social tests at PND 36. Frequency of pinning and pouncing was determined by video analysis by two blinded experimenters.

d) Locomotor activity induced by MOD: another group of rats were used. After acclimation and immediately after the injection, locomotor activity was recorded during 60 minutes in the experimental cage at specific different treatment days: 1, 7 and 14. Several measurements as mean and maximum speed, distance travel, mobile time, immobile time and number of rearing were analysed using ANY-MazeTM software (Stoelting Co., Illinois, USA).

Tissue extraction

Immediately after the social play test, rats were decapitated with a guillotine for small animals (model 51330, Stoelting Co., USA) and brains were removed. PFC (Bregma +2.80 to +1.80 mm approximately), NAc (Bregma +2.28 to +1.28 mm approximately) and VTA (Bregma -6.48 to – 7.48 mm approximately) were micro-dissected at 4°C and weighed on an analytical balance. The tissue was collected in 400 µL of 0.2 M perchloric acid (PCA) and then homogenized. The resultant homogenates were centrifuged for 30 min at 12,000 g at 4ºC and then the supernatant was filtrated (PTFE syringe Filter; 0.22 µm pore size, Qing Feng OEM). The filtrates were stored at -80ºC until further analysis for DA, DOPAC, GLU and GABA.

In Vivo Microdialysis in NAc

At PND 36, *in vivo* microdialysis experiments were performed using a previously described protocol (Sotomayor et al., 2005). Briefly, the animals were deeply anesthetized with urethane (1.5 g/kg i.p.) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, MA). Body temperature of the animals was maintained at 37°C with an electric blanket controlled by a thermostat.

A concentric brain microdialysis probe (Microdialysis Probe, Harvard Bioscience; CMA-11, 6,000 Daltons cut off, 2 mm membrane length) was implanted in the NAc using the following coordinates according to the atlas of Paxinos and Watson (2007) NAc: (+1.5 mm rostral to the bregma, 1.5 mm lateral to the midline, and -7.2 mm below dura mater). For juvenile ones it was calibrated the coordinates according to bregma lambda distance: bregma-lambda distance/9 * x-coordinate (X=AP; ML; DL). The microdialysis probe was perfused with artificial cerebro spinal fluid (aCSF: NaCl 147 mM; KCl 2.7 mM; $CaCl_2$ 1.2 mM and $MgCl_2$ 0.85 mM; adjusted to pH 7.4) at a flowrate of 2 μL/min using an infusion pump (model 210 RWD, RWD Life Science Co, Ltd, China). After a stabilization period of 90 min, three perfusion samples of 15 min each were collected in tubes containing 4 μL of 0.2 M PCA. At 45 min, the aCSF solution was changed for 15 min to 70 mM K^+. Between 60 and 105 min, aCSF was again perfused through the microdialysis probe. Collected perfusion samples were stored at -80ºC until analysis. At the end of each experiment, animals were decapitated, and brains were quickly removed and stored in 4% paraformaldehyde. Brain sections of 50 μm were stained with cresyl violet and examined microscopically to test the placement of the probe.

Dopamine and DOPAC analysis

We followed the protocol used in Velásquez et al., 2019. Briefly, an aliquot (10 μL) was injected into the HPLC system. DA and DOPAC content of each experimental condition were quantified by the electrochemical detection using appropriate standards. 10 µL of each sample were injected to the HPLC-ED system with the following equipment: An isocratic pump, (model

PU-2080 Plus, Jasco Co. Ltd., Tokyo, Japan), a C18 column (model Kromasil 100-3.5-C18, AkzoNobel, Bohus, Sweden) and an electrochemical detector (set at 650 mV, 0.5 nA; model LC-4C, BAS, West Lafayette, IN, United States). The mobile phase, containing 0.1 M NaH_2PO_4, 1.0 mM 1- octane sulfonic acid, 1.0 mM EDTA and 8.0% (volume/volume) CH_3CN (pH 3.4) was pumped at a flow rate of 125 µL/min. DA extracellular levels were assessed by comparing the respective peak area and elution time of the sample with a reference standard and the quantification was performed using a calibration curve for each neurotransmitter (Program ChromPass, Jasco Co. Ltd., Tokyo, Japan).

Glutamate and GABA analysis

Glutamate and GABA content in NAc and VTA were determined under the different experimental conditions. An aliquot (20 µL) of the filtrate was injected HPLC-fluorometric determination of GABA and GLU was performed as described previously (Gárate-Pérez et al 2019). Briefly, 20 µL of the sample were mixed with 4 µL of borate buffer (pH 10.8), and then the mixture was derivatized by adding 4 µL of fluorogenic reagent (20 mg of orthophthaldehyde and 10 µL of β-mercapto-ethanol in 5 mL of ethanol). 90 s after derivatization, samples were injected into a HPLC system with the following configuration: isocratic pump (model PU-4180, Jasco Co. Ltd., Tokyo, Japan), a C-18 reverse phase column (Kromasil 3-4.6, Sweden), and a fluorescence detector (model FP-4020, Jasco Co. Ltd., Tokyo, Japan). The mobile phase containing 0.1 M NaH_2PO_4 and 24.0% (volume/volume) CH_3CN (pH adjusted to 5.7) was pumped at a flowrate of 0.8 mL/min. The retention time for glutamate was 1.8 min and for GABA was 11 min while the detection limit for both neurotransmitters was 5 fmol/µL. Dialysate samples were analysed by comparing the peak area and elution time with reference standards (ChromNAV 2.0, Jasco Co. Ltd., Tokyo, Japan).

Western blot (WB) and dopamine receptor type 2

Rats were euthanized using isoflurane and decapitated immediately after the social play behaviour test. Using a brain matrix of 1 mm with micro punches of 1.0- and 1.5-mm diameter, NAc, VTA and PFC were extracted; tissues were homogenized with RIPA buffer with proteases inhibitor cocktail and were frozen until analysis.

The protocol for the western blotting was a modified version of Ambrosetti et al. (2016) and Lopez-Perez et al (2005). Briefly, total protein extracts were prepared by homogenization every tissue obtained from vehicle and MOD-treated animals in a sodium dodecyl sulphate (SDS) buffer containing: 20% glycerol, 4.0% SDS, 10% mercapto-ethanol, 0.1% bromophenol blue in 125 mM Tris– HCl with pH 6.8. Protein concentrations were determined using Bradford protein quantification assay, using 595 nm of absorbance. Samples were denaturized (5 min, 95 ºC) in the buffer previously described, with 30 μg of protein deposited into each lane of a 10% polyacrylamide gel. Electrophoresis was carried out at 80 V 15 minutes and 100 V for 2 hours, and the proteins in the gel subsequently electroblotted onto nitrocellulose membranes using 350 mA for 2.5 hours. The membranes were incubated for 1 h at room temperature with a blocking solution containing: 5% BSA in TBS-T.

They were incubated 1 hour at room temperature with the rabbit anti-dopamine D_2 receptor antibody (AB5084P, Merck Millipore, USA) diluted at 1:1000 in TBS-T. Following incubation with the secondary antibody, namely a rabbit polyclonal anti-GAPDH antibody

(G9545, Sigma-Aldrich Co, LLC) (1:10,000, 1 h incubation). The antibody complexes were detected using a Goat Anti-Rabbit IgG Fc (HRP; ab97200). For detection we used EZ-ECL Kit Enhanced Chemiluminescence Detection Kit (Biological Industries, Israel). Chemiluminescence was captured using C-digit Blot Scanner (LI-COR Bioscience, USA). Results were analysed by measuring the pixel intensities of bands using the semi-quantification tool of the Image J (National Institutes of Health, USA). All Western blots were performed in triplicate for each sample. The coefficient of variation was 6.2% for PFC and 13.7% for NAc among samples.

Ex vivo **fast scan cyclic voltammetry in NAc core**

At PND 36, *ex vivo* FSCV studies were conducted for more detailed examination of changes in DA release and uptake following MOD treatment. Following MOD or vehicle treatment, rats were sacrificed and coronal brain sections (400 µm) containing the NAc core (NAcc) were collected and placed in oxygenated aCSF. A bipolar stimulating electrode was lowered onto the NAcc where it delivered single-pulse stimulations (400 µA, 4 ms). A carbon fiber electrode, placed in the NAcc, recorded DA release and uptake. Stimulations were repeated every 3 min until stable recordings were achieved (<10% variation across three consecutive recordings). For data acquisition, the electrode potential was linearly scanned as a triangular waveform from −0.4 to 1.2 V and back to −0.4 V vs Ag/AgCl during an 8 ms period. Cyclic voltammograms were recorded at the carbon fiber electrode every 100 ms using a scan rate of 400 V/s by means of a voltammeter/amperometer (Chem-Clamp, Dagan Corporation, MN).

The magnitude of electrically evoked DA release and transporter-mediated uptake kinetics, including maximal uptake rate (V_{max}) was monitored. During electrical stimulation, the ascending phase of the DA overflow curve represents both release and uptake while the descending portion of the overflow curves represents primarily uptake. DA overflow curves were fitted to a Michaelis–Menten-based kinetic model (Wu et al., 2001) using Demon Voltammetry and Analysis Software (Yogarson et al., 2011) written in Labview (National Instruments, Austin, TX) to obtain independent measures for DA release and uptake. Changes in release and uptake were obtained by setting baseline Km values to 0.16 μM and establishing a baseline V_{max} individually for each subject. Extracellular concentrations of DA were assessed by comparing the current at the peak oxidation potential for DA in consecutive voltammograms with electrode calibrations of known concentrations of DA (3 μM). Stimulated DA release was calculated as the average of the last three samples of baseline acquisition.

Statistical Analysis

Pinning, pouncing, rearing frequencies, locomotor activity, social exploration time, DA, DOPAC, GLU, GABA content level and D_2R relative expression, are expressed as mean ± SEM. All data were submitted to a D'Agostino-Pearson test to assess normality. Then, data were analysed using unpaired two tailed Student's t-test (pouncing frequency; social exploration time; basal locomotor activity, V_{max} and DA release) or Mann Whitney test both with Welch's correction (pinning frequencies; DA, DOPAC, GLU and GABA tissue content). One-way ANOVA and Two-way ANOVA (weight gain; locomotor activity induced, DA, GLU, GABA release % basal and mean extracellular levels) test with multiple comparison were used with Sidak post-test correction. Pearson correlation test was used (pinning display and play solicitations received). Significance was set at $p<0.05$.

Results

1. Body weight

a) Chronic modafinil treatment did not affect body weight gain

Rats were weighted every day at the same hour (between 17.30 – 17.45) since they arrived at our animal facilities (PND 21). MOD or vehicle treatment started the day after (PND22) and lasted 14 days in total (PND 22 – PND 35). MOD treatment did not change body weight gain during the experiment. The repeated measurements Two-Way ANOVA revealed a significance main effect of time F(13780) = 2604; $p<0.001$; but not treatment F(1,60) = 0.34 $p=0.5595$; neither the interaction F(13,780) = 0.1094; $p>0.99$ (Figure 2).

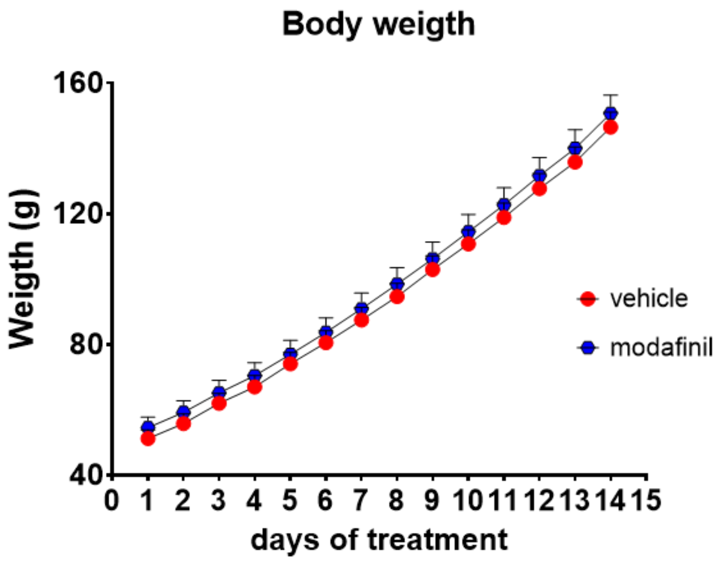


Figure 2. Weight gain from PND21 (day 1) until the day of euthanasia (day 15). The figure shows the body weight from the day of weaning at PND21 until the day of euthanasia in vehicle (red dots) and MOD (blue dots) injected rats. Vehicle or MOD were injected daily from PND22 (day 2 in the figure) to PND35. N = 31 for both vehicle and modafinil treated rats. All data are represented as mean ± SEM.

2. Social play behaviour

a) Modafinil impaired responses to play solicitations (pinning events)

The number of pouncing events (play solicitations) performed by MOD rats was not different from the vehicle group (t=0,3889 df=17,94; p= 0.7019; Figure 3 A). Interestingly, total number of pining events decreased in MOD group compared with vehicles (t=3,729 df=9,717; p= 0.004; Figure 2 B). Correlation analysis (Figure 3 C and 3 D) showed a positive and significant correlation between solicitations performed by stimuli rats and pinning behaviour displayed only in the vehicle group (r=0.857; p= 0.003; Figure 3 C).

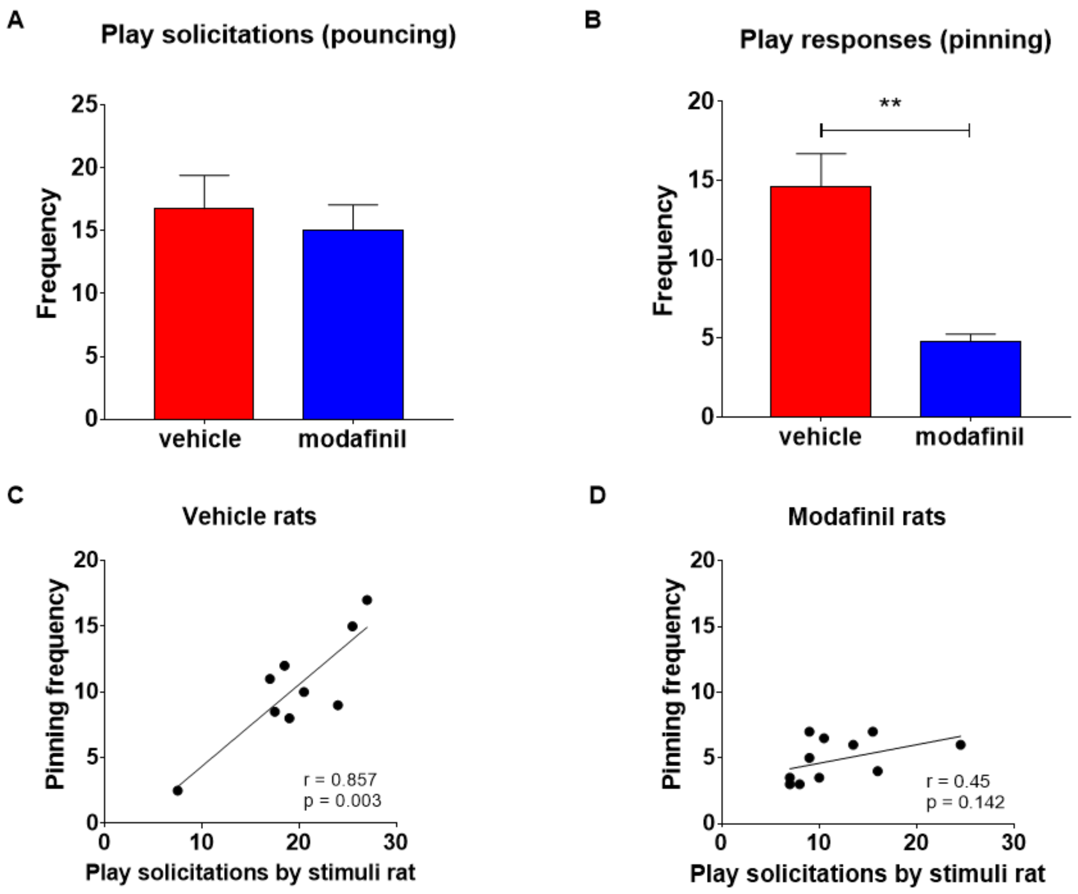


Figure 3. Social play behaviour after 14 days of MOD treatment. (A) Play solicitations (Pouncing) frequency in 10 minutes play test; p= 0.7019 (Unpaired t test w/Welch correction was used) **(B)** Play responses (pinning) frequency in 10 minutes test; p= 0.004; All data are represented as mean ± SEM (Unpaired Mann-Whitney w/Welch correction). **(C)** Positive correlation between play solicitations received and responses display for the vehicle group (r = 0.857; p= 0.003) **(D)** No correlation between play solicitation received and pinning display in the MOD group (r= 0.45; p= 0.142). Vehicle (n=9) Modafinil (n=12). Pearson correlation test was used.

b) MOD did not modify social exploration time

No change in total social exploration time was observed after chronic MOD treatment (t=0,3799 df=21,53; p= 0.707; Figure 4).

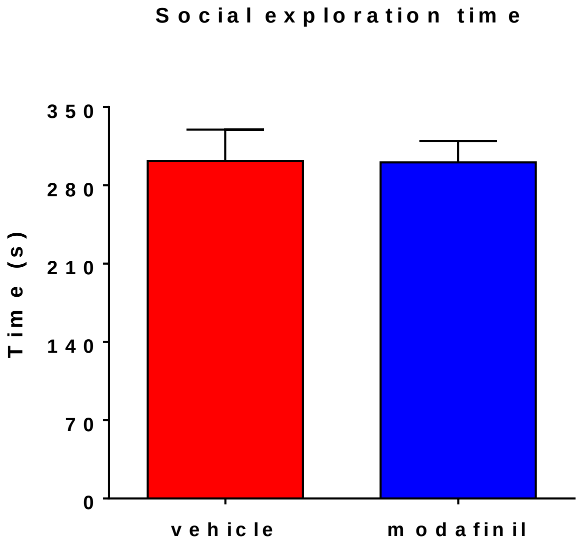


Figure 4. Social exploration time (s) performed by experimental rats. Every approach made by the experimental rat including sniffing, following, grooming or touching the stimuli rat was considered. Vehicle group (n=12), MOD group (n=12); p= 0.707; Data are represented as mean ± SEM (Unpaired t-test w/Welch correction).

3. Basal locomotor activity

a) MOD did not affect basal horizontal and vertical locomotor activity

Total distance travelled (t=0.8317 df=13.21; p=0.4203, Figure 5 A), total immobile time (t=1.308 df=13.71; p=0.2125, Figure 5 B) mean speed (t=0.8606 df=13.14; p=0.4049, data non showed) and maximum speed (t=0.4376 df=9.753; p= 0.6712, data non showed) were not affected by chronic MOD treatment.

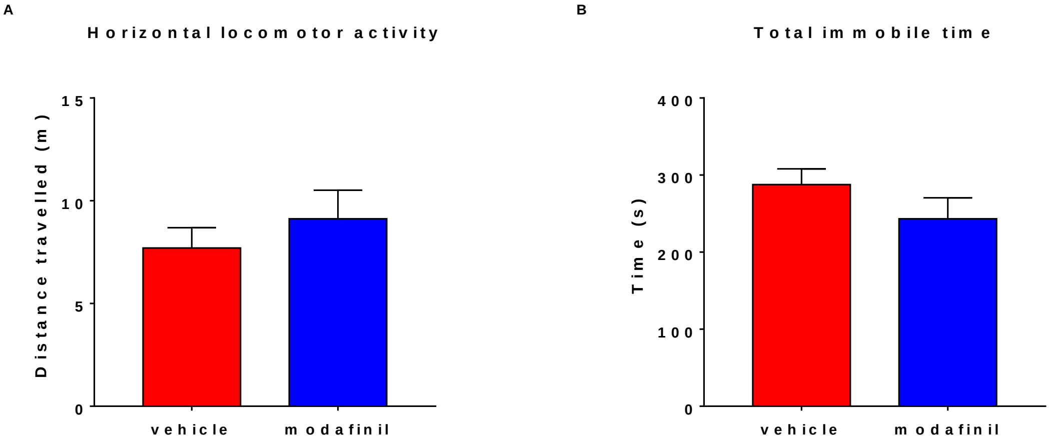


Figure 5. Basal horizontal locomotor activity performed by experimental rats after MOD treatment. **(A)** Total distance travelled (m) by experimental rats in the basal locomotor activity test. Vehicle (n=10), MOD (n=8); p= 0.4203; **(B)** Total immobile time (s) by experimental rats in the basal locomotor activity test. Data are represented as mean ± SEM (Unpaired t-test w/Welch correction).

In the case of basal vertical locomotor activities like total number of rearing (t=0.6884 df=27.86; p=0.4969; Figure 6), and relative time of rearing (see annexes) was measured. Chronic MOD treatment did not affect any of the parameters above mentioned.

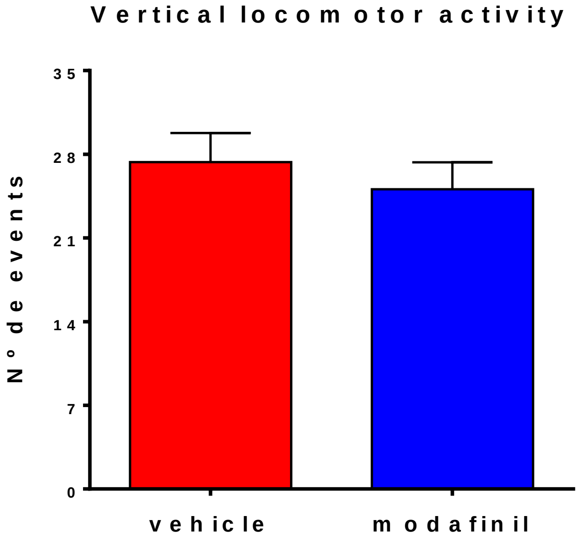


Figure 6. Basal vertical locomotor activity performed by experimental rats after MOD treatment. Vehicle (n=17), MOD (n=13); p=0.4969; Data is expressed as mean ± SEM (Unpaired t-test w/Welch correction).

4. Horizontal locomotor activity induced by MOD

Locomotor activity was assessed in a 60 minutes test immediately after the injection at days 1, 7 and 14. Two-Way ANOVA showed that total distance travelled and mean speed were increased by MOD injection in each of testing: day 1 (Figure 7 A and B), day 7 (Figure 8 A and B) and day 14 (Figure 9 A and B). As it is noted, the acute increase and decrease over time in locomotor activity by MOD is also showed as accumulative distance travelled along the entire treatment regime (Figure 10). For additionally locomotor measurements, see annexes.

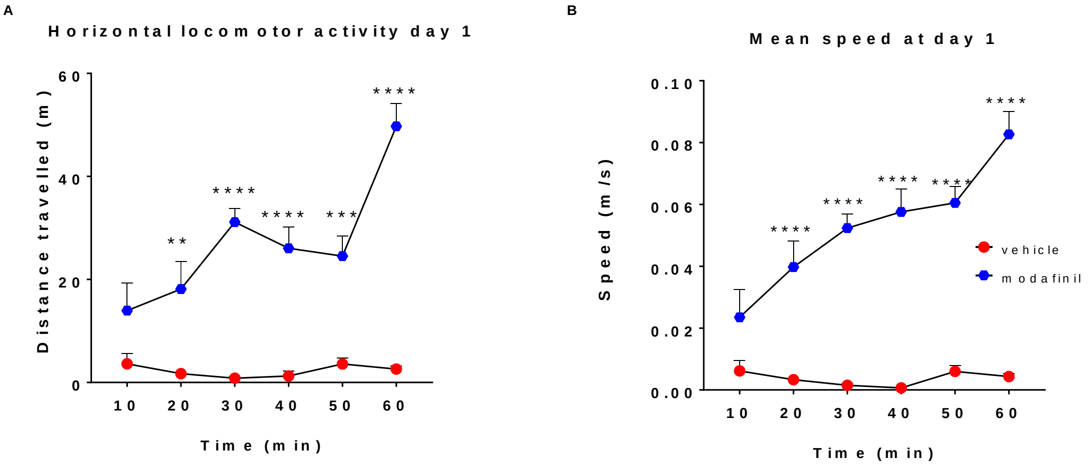


Figure 7. Effects of MOD injection at day 1 of treatment on locomotor activity in 60 minutes test. (A) Distance travelled (m) after first injection. Vehicle (red dots) and MOD (blue dots) groups (n=6). Interaction (F (5, 55) = 8.149), p<0.0001; Time factor (F (5, 55) = 7.618), p<0.0001; treatment factor (F (1, 55) = 185.3), p<0.0001; **p=0.001; ***p=0.0003; ****p<0.0001. **(B)** Mean speed reached by rats after first injection. Interaction (F (5, 55) = 7.78), p<0.0001; Time factor (F (5, 55) = 7.128), p<0.0001; treatment factor (F (1, 55) = 265.2), p<0.0001; ****p<0.0001. All data are represented as mean ± SEM. (Two-Way ANOVA w/ Sidak's multiple comparison test).

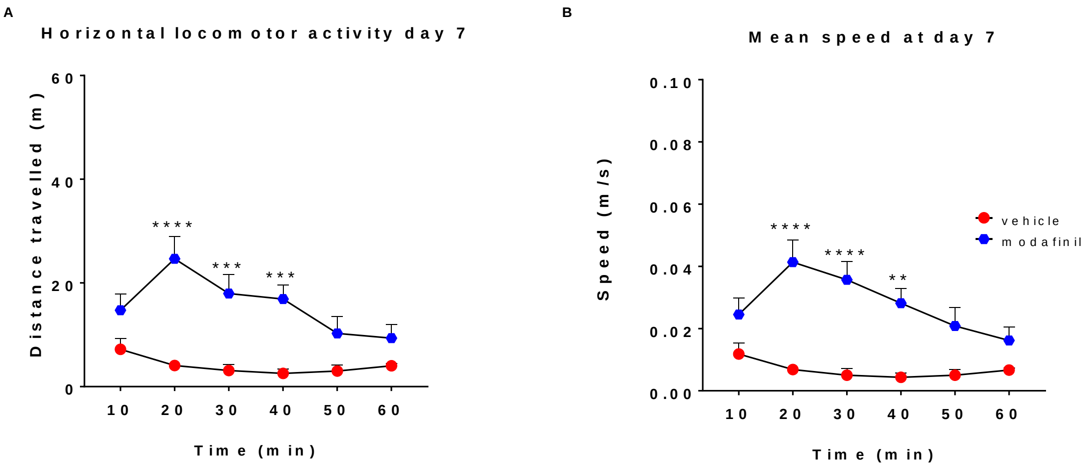


Figure 8. Effects of MOD injection at day 7 of treatment on locomotor activity in 60 minutes. **(A)** Distance travelled (m) after seventh injection. Vehicle (red dots) and MOD (blue dots) groups (n=6); Interaction (F (5, 60) = 2.755), p=0.026; Time factor (F (5, 60) = 2.707), p=0.028; treatment factor (F (1, 60) = 64.63), p<0.0001; ***p<0.001; ****p<0.0001; **(B)** Mean speed reached by rats after the seventh injection. Interaction (F (5, 60) = 2.875), p=0.021; Time factor (F (5, 60) = 2.49), p=0.040; treatment factor (F (1, 60) = 75.49), p<0.0001; **p=0.0011; ****p<0.0001. All data are represented as mean ± SEM. (Two-Way ANOVA w/ Sidak's multiple comparison test).

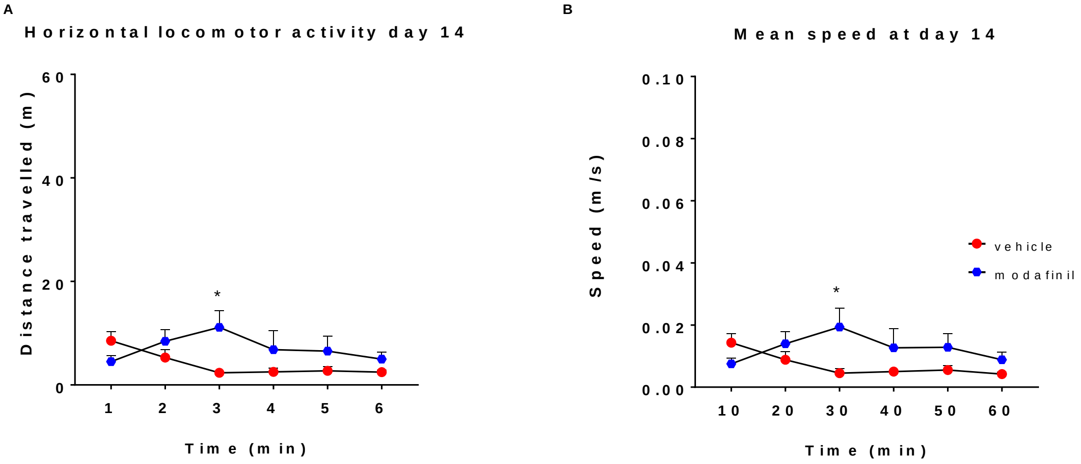


Figure 9. Effects of MOD injection at day 14 of treatment on locomotor activity in 60 minutes test. **(A)** Distance travelled (m) after the fourteenth injection. Vehicle (red dots) and MOD (blue dots) groups (n=6). Interaction (F (5, 59) = 2.064), p=0.0828; Time factor (F (5, 59) =0.8683), p=0.5079; treatment factor (F (1, 59) = 6.897), p=0.011; *p=0.0188; **(B)** Mean speed reached by rats after day 14 of injections. Interaction (F (5, 59) = 2.079), p=0.0807; Time factor (F (5, 59) = 0.6856), p=0.6362; treatment factor (F (1, 59) = 7.512), p=0.0081; *p=0.0197. All data are represented as mean ± SEM. (Two-Way ANOVA w/ Sidak's multiple comparison test).

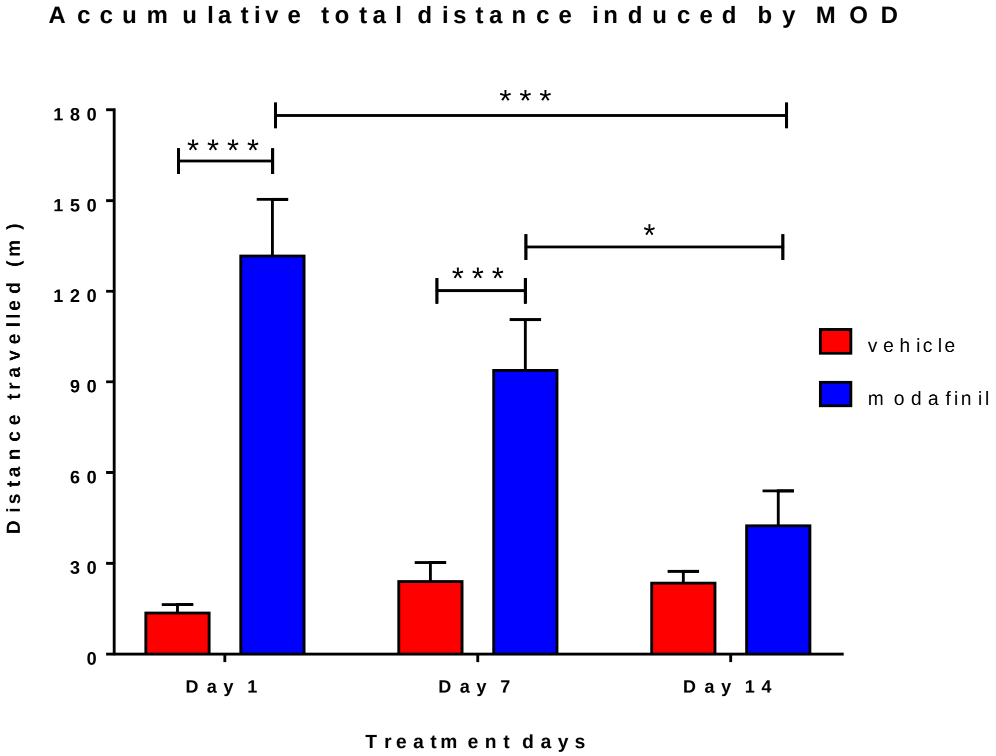


Figure 10. Locomotor activity induced by MOD expressed in accumulative distance travelled (m) on days 1, 7 and 14 of the treatment in vehicle and modafinil rats. Each bar represents the mean of metres travelled per group in the entire MOD treatment. Vehicle and MOD groups (n=6); ****p<0.0001 (day 1 vehicle vs MOD); ***p= 0.0007 (day 7 vehicle vs MOD); ***p= 0.0002 (day 1 vs day 14 MOD group); *p=0.0025 (day 7 vs day 14 MOD group). Interaction (F (2, 20) = 7.91); p=0.0029; time (F (2, 20) = 5.219); p=0.0150; treatment (F (1, 10) = 67.68); p<0,0001; data are represented as mean ± SEM. (Two-Way ANOVA w/Sidak's multiple comparison test).

5. Dopamine and DOPAC tissue content

a) Modafinil reduces DOPAC but not DA content in VTA

MOD treatment tend to reduced DOPAC content (Sum of ranks in column A, B =23, 32; Mann-Whitney U = 4; p= 0.1833; figure 11 A) and the same tendency is observed with DA levels (Sum of ranks in column A, B =23, 32; Mann-Whitney U = 4; p= 0.1833; figure 11 B). Additionally, to estimate the DA metabolism was measured with DOPAC/DA ratio. DA metabolism was lower in the modafinil group compared to the vehicle group but not statistically significant (Sum of ranks in column A, B = 14, 41; Mann-Whitney U = 8; p= 0.667; figure 11 C).

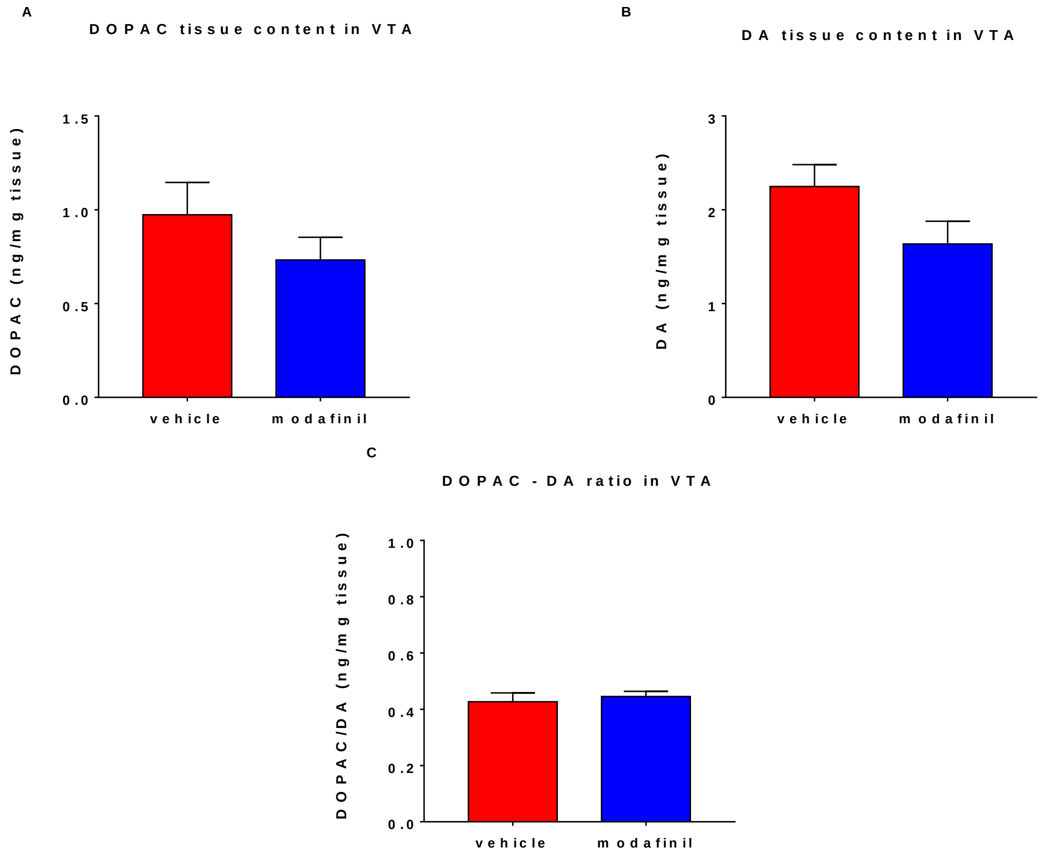


Figure 11. Tissue content of dopamine, DOPAC and DOPAC/DA ratio in ventral tegmental area (VTA) after 14 days of MOD treatment. vehicle and MOD (n=7). **(A)** Dopamine tissue content in VTA. **(B)** DOPAC tissue content in VTA. **(C)** DOPAC/DA ratio. This ratio was used

as a DA metabolism measure. All data are represented as mean ± SEM (Unpaired Mann-Whitney w/Welch correction test).

b) MOD treatment does not affect DA or DOPAC tissue content in NAc

DA and DOPAC tissue content were not affected by MOD treatment; DA (Sum of ranks in column A, B =14, 41; Mann-Whitney U = 8; p= 0.6667) and DOPAC (Sum of ranks in column A, B =13, 42; Mann-Whitney U = 7; p=0.5167). As expected, DOPAC/DA ratio was not different between treatment conditions (Sum of ranks in column A, B= 12, 43; Mann-Whitney U = 6; p=0.3833). Figures 12 A, 12 B and 12 C.

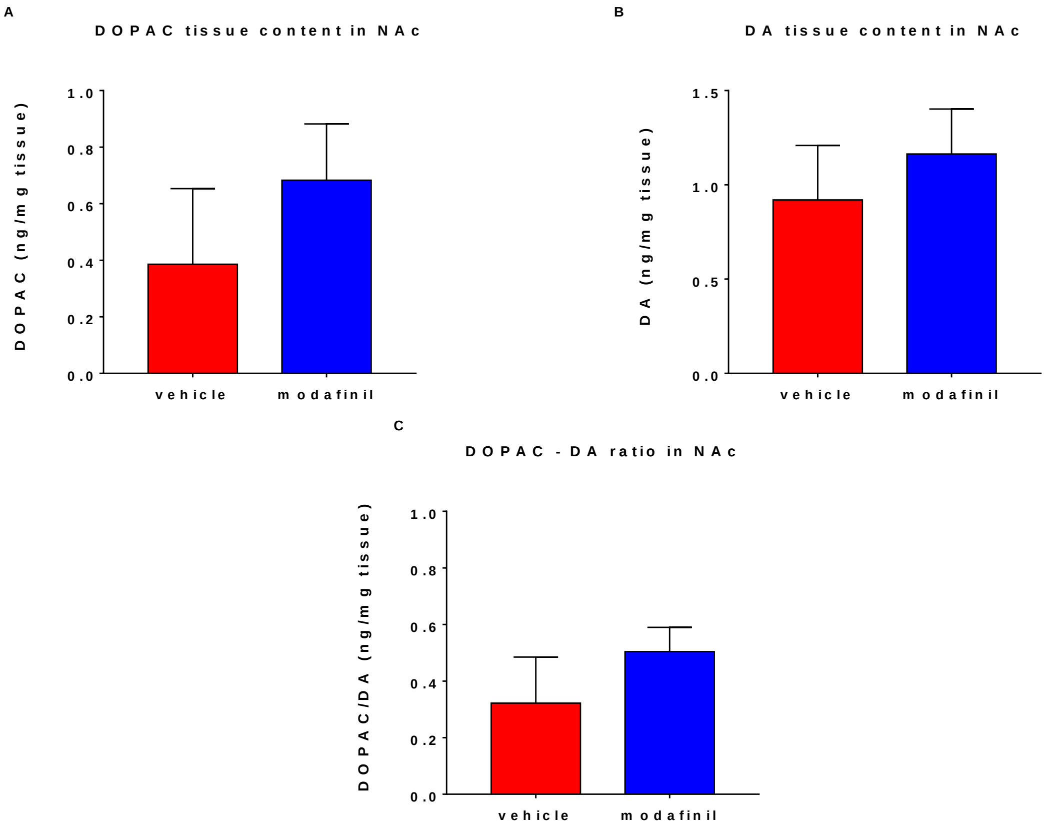


Figure 12. Tissue content of dopamine, dopamine metabolite DOPAC and DOPAC/DA ratio in nucleus accumbens (NAc) after 14 days of MOD treatment in young healthy rats. Vehicle and MOD groups (n=7). **(A)** Dopamine content levels in nucleus accumbens; p= 0.6667; **(B)** DOPAC content levels in nucleus accumbens; p=0.5167; **(C)** DOPAC/DA ratio. This ratio was used as a DA metabolism measure, p= 0.3833, all data is represented as mean ± SEM (Unpaired Mann-Whitney w/Welch correction test).

7. Modafinil reduces D_2 expression in PFC but not in NAc

We found a notorious reduction in PFC D_2 expression in MOD treated group compared to vehicle group (Sum of ranks in column A, B = 52, 26; Mann-Whitney U = 5; p= 0.0411; figure 13 A). When analysed the D_2 expression in NAc no difference was observed between treatments (Sum of ranks in column A, B = 70, 50; Mann-Whitney U = 22; p= 0.5358; figure 13 B).

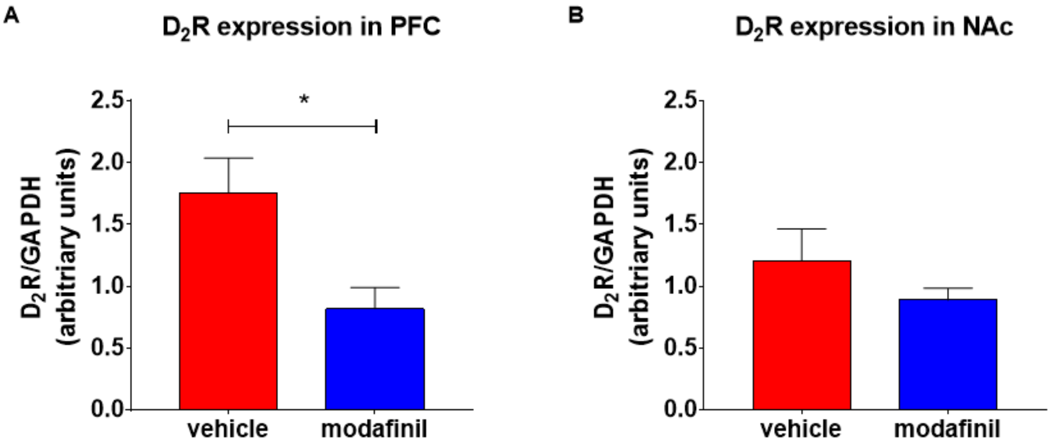


Figure 13. Dopamine type 2 receptor (D2) expression in prefrontal cortex (PFC) and nucleus accumbens (NAc). (A) D2 expression in PFC. Vehicle and modafinil groups (n=6), *p= 0.0411; **(B)** D_2 expression in NAc. Vehicle (n=8) and modafinil (n=7) groups, p= 0.5358. Data are expressed as mean ± SEM of arbitrary units of D_2 immunoreactivity normalized to GAPDH immunoreactivity (Unpaired Mann-Whitney test w/Welch's correction).

8. Neurotransmitters release in NAc by *in vivo* microdialysis and *ex vivo* fast scan cyclic voltammetry

a) MOD treatment impairs DA release in NAc

Reverse dialysis shows that 70 mM K+ infused in NAc increases DA extracellular levels in both vehicle and in MOD rats (Figure 14 A). However, the magnitude of the response of NAc DA to 70 mM K^{+} was significantly lower in MOD rats compared with vehicle in minutes 60 and 75 (minute 60 (t = 3.231), df = 68; p= 0.0151; minute 75 (t = 2.912; df = 68; p= 0.0382) (Fig. 14 A and B). Examples of probe localization see annexes.

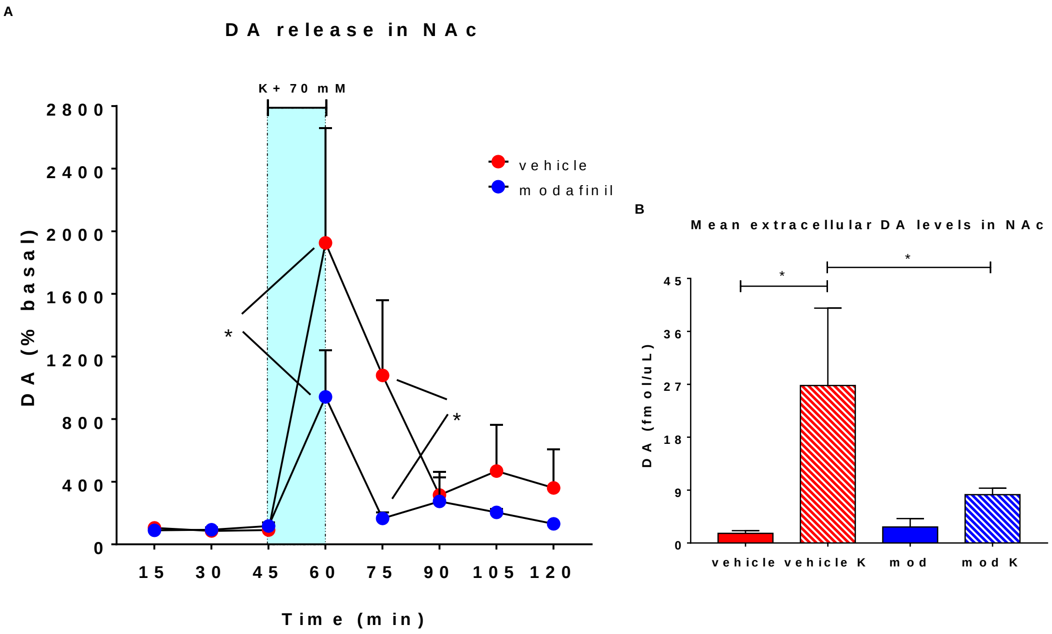


Figure 14. Effect of chronic MOD treatment on extracellular and release of dopamine (DA) levels in nucleus accumbens (NAc) after K+70 mM stimulation by *in vivo* microdialysis. (A) Forty-five minutes after beginning the collection of samples, an aCSF containing 70 mM K^{+} was

perfused through the dialysis probe during 15 min. DA is expressed as percentage of baseline. Basal DA levels (fmol/µL) for vehicle group was 1.376 ± 0.269 and for MOD group was 3.192 ± 0.795. Vehicle (n=4) and MOD (n=7); *p<0.05; **(B)** Extracellular DA levels in nucleus accumbens is expressed as mean of DA levels ± SEM. *p<0.05 (Two- Way ANOVA Sidak's multiple comparison test and One-way ANOVA Sidak's multiple comparison test).

9. MOD did not modify baseline DA release and uptake in NAcc

DA release and maximal DA uptake (V_{max}) were analysed at baselines using *ex vivo* fast scan cyclic voltammetry (FSCV) in NAcc 24 hrs after the last MOD or vehicle injection (Figure 15 A B, C); Vehicle: n= 8; MOD: n= 6. Baseline evoked DA release and maximal uptake rate were higher in MOD-treated rats, although this difference was not statistically significant (t(12)=0.905, p=0.192; t(12)=1.438, p=0.088, respectively).

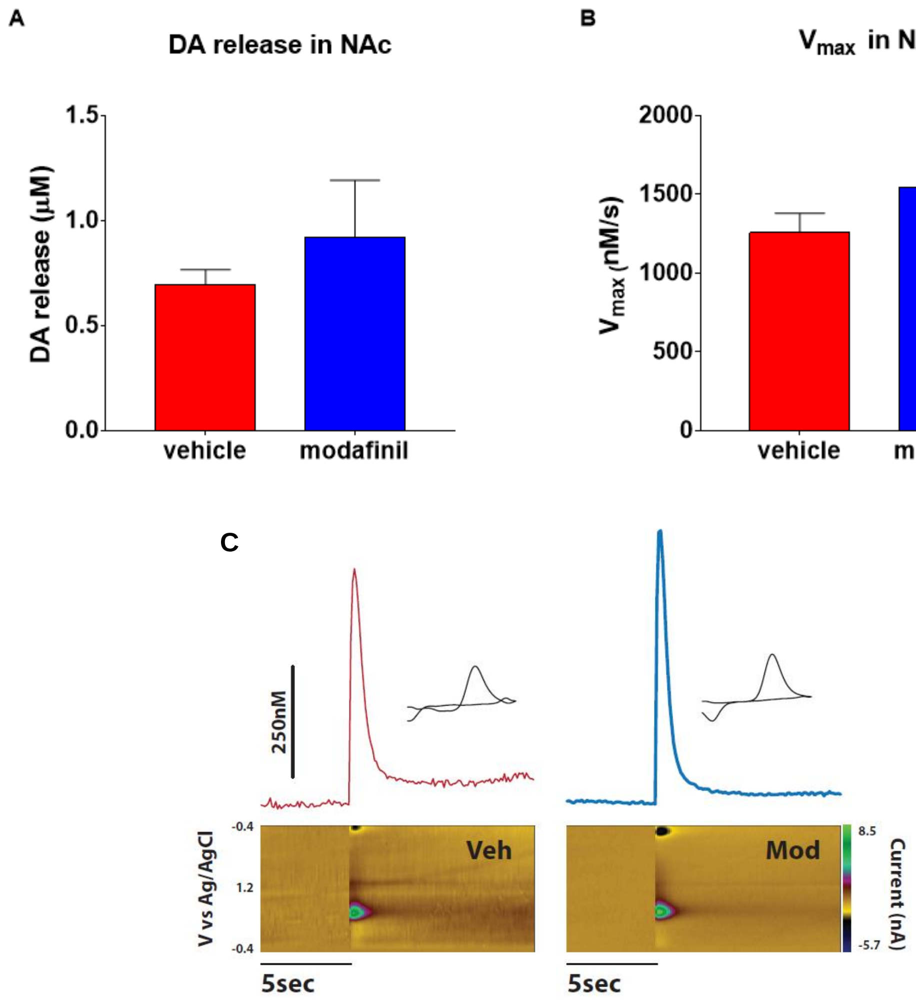


Figure 15. Effect of chronic MOD treatment on DA release and maximal DA uptake in nucleus accumbens core (NAcc) by *ex vivo* fast scan cyclic voltammetry (FSCV). (A) Average evoked DA release. **(B)** Average uptake rate (V_{max}). **(C)** Example traces (above) and colour plots (below) of evoked DA release for chronic vehicle and MOD-treated rats. Vehicle (red) n= 8; MOD (blue) n= 6.

6. Glutamate and GABA content in NAc and VTA were not affected by treatment

As mentioned, we included a complementary aim, we incorporated glutamate and GABA measures. GLU and GABA tissue content were not affected by MOD treatment either in VTA nor NAc; GLU in VTA (Sum of ranks in column A, B = 44, 47; Mann-Whitney U = 19; p= 0.8357; figure 16 A), GABA in VTA (Sum of ranks in column A, B = 52, 39; Mann-Whitney U =11; p= 0.1807; Figure 16 B), GLU in NAc (Sum of ranks in column A, B = 31.5, 46.5; Mann-Whitney U = 16,5; p= 0.9091; Figure 17 A) and GABA in NAc (Sum of ranks in column A, B =28, 50; Mann-Whitney U =13; p=0.5303; Figure 17 B).

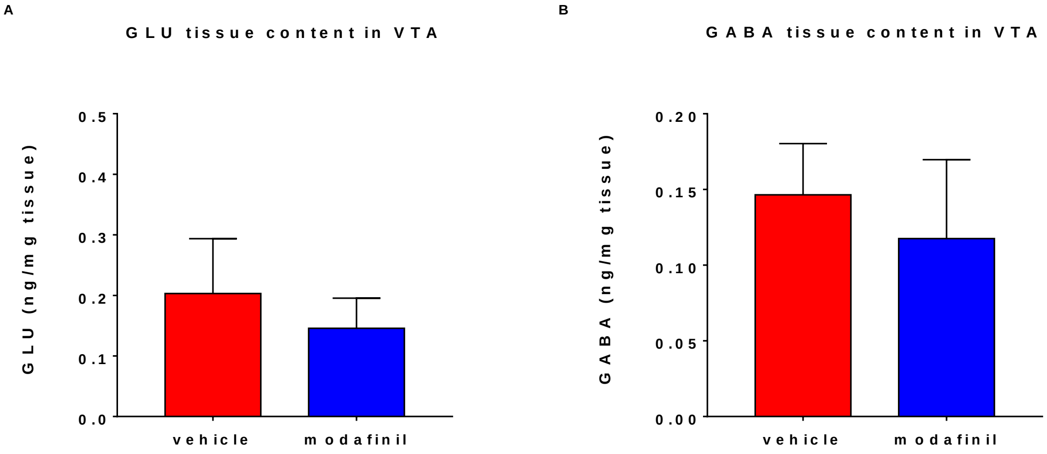


Figure 16. Glutamate (GLU) and GABA tissue content in ventral tegmental area (VTA) after MOD treatment of 14 days in young healthy rats. **(A)** GLU content in Ventral tegmental area; p=0.8357; **(B)** GABA content in ventral tegmental area; p=0.1807. Vehicle and MOD groups (n=7); All data are represented as mean ± SEM (Unpaired Mann-Whitney w/Welch's correction test).

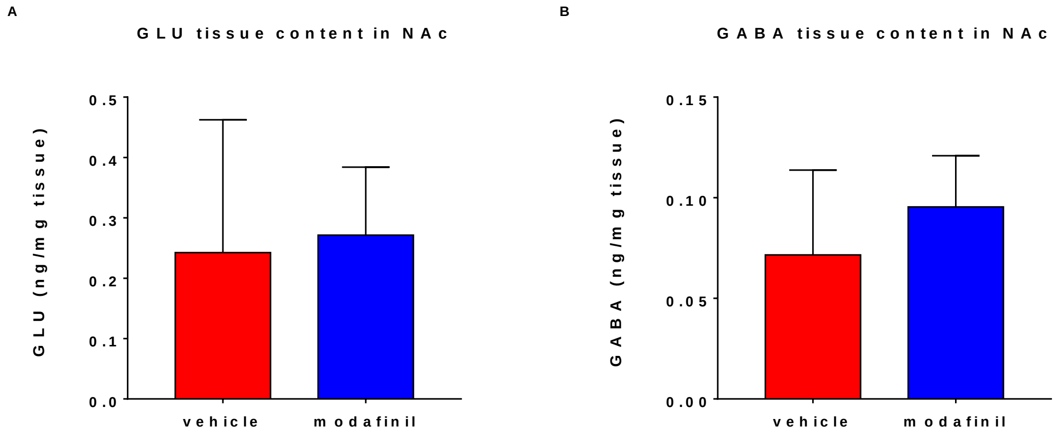


Figure 17. GLU and GABA tissue content in nucleus accumbens (NAc) after MOD treatment of 14 days in young healthy rats. (A) GLU content in nucleus accumbens after modafinil treatment; vehicle (n=8) MOD (=7); p=0.9091; **(B)** GABA levels in nucleus accumbens after 14 days treatment. Vehicle (n=8) and modafinil (n=7); p= 0.5303; All data are represented as mean ± SEM (Unpaired Mann-Whitney w/Welch's correction test).

b) MOD treatment did not change glutamate or GABA release in nucleus accumbens

Reverse dialysis of 70 mM K^+ in NAc failed to change extracellular GLU or GABA levels in vehicle and MOD rats (Figure 18 A and B and Figure 19 A and B). However, as the figure 19 B showed, the mean of basal levels of GABA are higher in the treated group (t = 3.688; df = 18; p= 0.01).

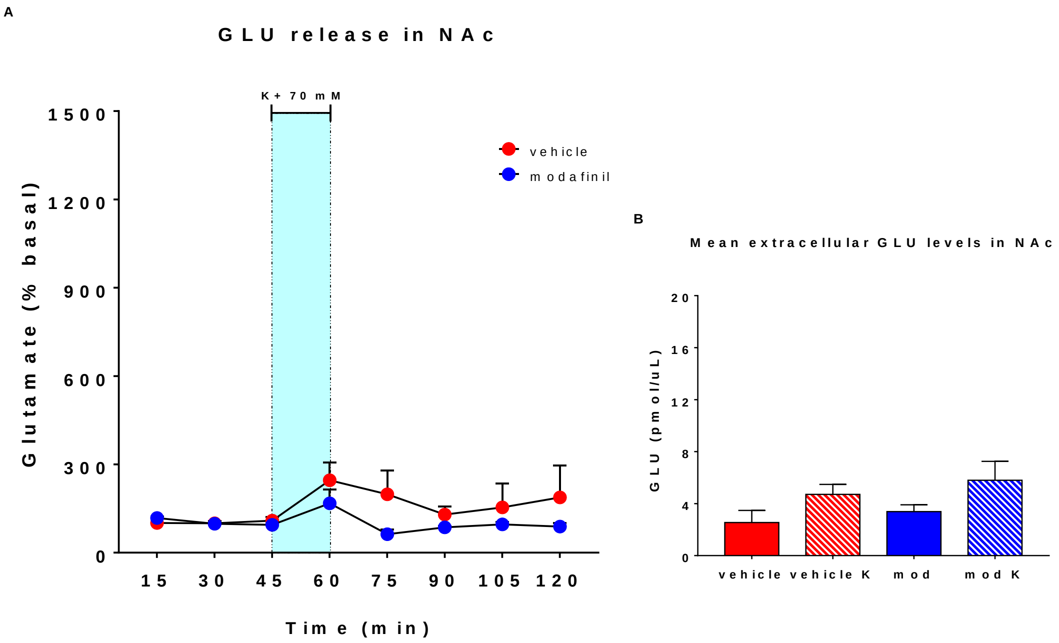


Figure 18. Effect of chronic MOD treatment on extracellular and release of glutamate levels in nucleus accumbens (NAc) after K$^+$ 70 mM stimulation by *in vivo* microdialysis. (A) Forty-five minutes after beginning the collection of samples, an aCSF containing 70 mM K$^+$ was perfused through the dialysis probe during 15 min. GLU is expressed as percentage of baseline. Basal GLU levels (pmol/µL) for vehicle was 4.544 ± 0.789; vehicle K$^+$ 4.803 ± 0.749; MOD basal 4.925 ± 0.739 and modafinil K$^+$ 6.276 ± 1.118; Vehicle (n=5) and MOD (n=5); **(B)** GLU release levels in NAc expressed as mean of glutamate levels ± SEM; Two- Way ANOVA Sidak's multiple comparison test and one-way ANOVA with Sidak's multiple comparison test.

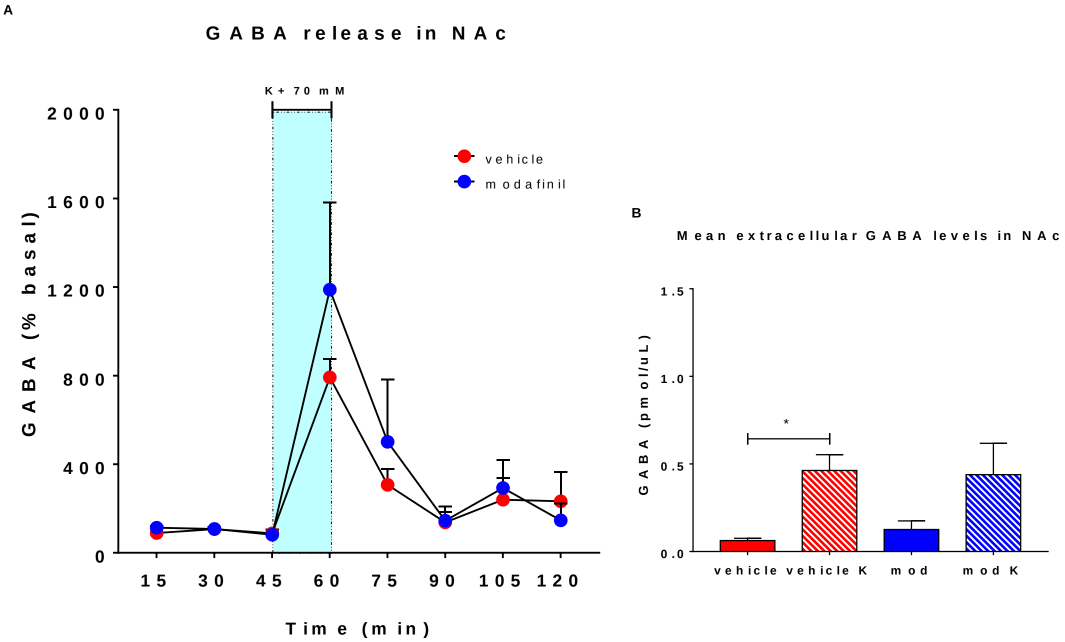


Figure 19. Effect of chronic MOD treatment on extracellular and release of GABA levels in nucleus accumbens (NAc) after K$^+$ 70 mM stimulation by in vivo microdialysis. **(A)** Forty-five minutes after beginning the collection of samples, an aCSF containing 70 mM K$^+$ was perfused through the dialysis probe during 15 min. GABA is expressed as percentage of baseline. Basal GABA levels (pmol/µL) for vehicle was 0.063 ± 0.006; vehicle K$^+$ 0.476 ± 0.115; MOD basal 0.898 ± 0.413 and MOD K$^+$ 1.206 ± 0.498; Vehicle (n=7) and MOD (n=5); **(B)** GABA release levels in NAc expressed mean of GABA levels ± SEM. *p<0.05; Two-way ANOVA Sidak's multiple comparison test and one-way ANOVA with Sidak's multiple comparison test.

Discussion

MOD is a wake promoting drug and an atypical psychostimulant prescribed for narcolepsy (Kallweit & Bassetti, 2017) and used to enhance attention and vigilance in adults (Dance, 2016). MOD acts on DA transmission by blocking DAT and enhancing extracellular DA levels (Volkow, 2009). GABAergic neurons have crucial roles in the reward circuitry and has been demonstrated that MOD modifies extracellular DA and GABA levels in NAc. Clinical trials are investigating MOD treatment for ADHD (Amiri et al., 2008; Arnold et al., 2014; Goez et al., 2012). On the other hand, ADHD is overdiagnosed (Polanczyk et al., 2014) and there are a few researches studying the long terms effects of MOD in healthy individuals, especially in children and adolescents.

The aim of this work was to evaluate the effects of chronic MOD treatment during preadolescence on social play behaviour, locomotor activity, DA, DOPAC, GABA and GLU in NAc and VTA. Chronic treatment with MOD in healthy young rats impaired social play behaviour, reduced the response of dopaminergic neurons in NAc after depolarization and reduced the expression of D_2 in PFC.

MOD did not change bodyweight gain in adolescent rats compared to controls. Considering the dose, route of administration and the duration of treatment we did not expected changes in body weight, or the rate of weight gained. Early studies reported weight loss after acute oral MOD administration (20 mg/kg) (Nicolaidis & De Saint Hilaire, 1993) but with higher doses

(80 mg/kg) they observed the opposite effect. Also, children treated with MOD have a lower appetite but not weight loss during 6 weeks of treatment (Kahbazhi et al., 2009). Having these mixed results, the effect of MOD on bodyweight remains unclear.

Social interactions are a key feature of social animals, being “social play” one of the earliest manifestations of those behaviours. Social play behaviour is highly rewarding, and rats begin displaying it some days before weaning. The maximum display of social play behaviour is by 2 weeks after weaning and then changes in structure and declines in frequency and changes to other types of social interactions, after sexual maturation (review at Blanco-Gandia et al., 2015). This behaviour is fundamental to normal cognitive and social development thus impacting quality of life.

The engagement and/or the amount of interaction between two young rats in social play behaviour could be altered according to developmental conditions. In our study, a chronic MOD treatment impaired social play behaviour. Rats treated with MOD failed in responding to play solicitations performed by stimuli rats (same sex, similar weight, untreated and unfamiliar rats), but they asked for play as much as the vehicle group. This result is intriguing because it shows that the main behavioural components of social play behaviour (pouncing and pinning) may differ in their rewarding effects. In fact, the interaction between two rats rather than just the initiative of the soliciting animal is the event that produces the rewarding value of social play behaviour (Calcagnetti et al., 1992). For example, reducing pinning behaviour with scopolamine (a cholinergic receptor antagonist) reduces the rewarding value of social play behaviour in the other rat, measured with CPP paradigm (Calcagnetti et al., 1992; Pellis et al, 1995). Therefore, eventually, the partners performing the solicitations will decrease this behaviour due to lower rewards received (positive plays responses).

Correlation analyses in this thesis are clear in demonstrating that vehicle rats respond proportionally to play solicitations (positive and significant correlation) while MOD treated rats do not (no correlation). This could mean that MOD treated rats respond inadequately and imply that the interaction with MOD rats is less attractive to stimuli rats and this interaction is also less rewarding of for MOD treated rats (Pellis and McKenna, 1995). It is important to mention that pinning is one of the most important juvenile social behavior (Panksepp 1981; Panksepp & Beatty, 1980; Panksepp et al., 1984) and the structure of social play behavior affects adult social behaviors and dominance hierarchy (Nijhof, Vinkers, van Geelen, Duijff, Achterberg, Van Der Net, ... & van der Brug, 2018; Siviy, 2016). In male rats, the subject that elicited more pinning are the one that become dominant after sexual maturation (Pellis & Pellis, 1991). Dominance hierarchy directly impacts important adult behaviours such as: mating behavior and female access (Carr et al., 1982), aggression (Albert, Walsh, Gorzalka, Siemens & Louie, 1986) and sociability (Pellis et al., 1993). Our results suggest that MOD treatment in young male rats produce long-term effects in social interaction in adulthood.

Importantly, in the present study, MOD administration for 14 days impaired social play behaviour without altering social exploration, thus indicating a selective and specific impact of the treatment on a young and highly rewarding behaviour as social play. Similar consequences have been observed in young rats neonatally treated with cocaine where the treatment impaired learning when the reward was access to a play partner but there were no deficits in learning when the reward was food (Willford, Segar, Hansen-Trench & Barron, 1999).

In our knowledge, this is the first evidence demonstrating that chronic MOD administration impairs social behaviour in juvenile rats, specifically social play behaviour which has been proposed as the highest in reward in this age. However, other psychostimulants as MPH, cocaine and AMPH have demonstrated to impair social play behaviour, but when administrated in acute fashion (Manduca et al., 2016; Vanderschuren et al., 2008). On the other hand, social play behaviour was not affected after a chronic i.p. MPH treatment (Bolaños et al., 2003). Taking together, it is essential to highlight that the discrepancy in results might be related to length of the treatment. Accordingly, the length of psychostimulant exposure is crucial for determining behavioural and molecular outputs in brain reward circuitry. For example, molecular outcomes such as receptor expression, second messenger signalling, transporter function, trafficking, among others, after cocaine exposure differ between administration protocols and/or length of treatment (reviewed by Anderson & Pierce, 2005).

Considering that acute administration of psychostimulants such as MPH, AMPH and cocaine decreases social play behaviour, it could be possible that this impairment is a consequence of increased locomotor activity, thus we analyzed the possible effects on locomotor activity after acute and chronic MOD administration in young rats. MOD provokes an increase in locomotor activity after an acute administration, however, this effect is almost completely lost after 14 days of daily MOD treatment. On the contrary, Paterson and colleagues show that activity was augmented with MOD after 5 days of administration (Paterson et al., 2010). Similarly, rats receiving a daily dose of psychostimulants like MPH, AMPH or cocaine showed an increase in locomotor activity after several days of exposure (Adriani, Leo, Greco, Rea, di Porzio, Laviola & Perrone-Capano, 2006; Ahumada, Bahamondes, Cerda, Silva, Cruz, Moya, Sotomayor-Zárate & Renard, 2017; Freese et al, 2012; Yang, Swann, & Dafny, 2007).

DAT efficiency to clearance DA in NAc have been indirectly related to cocaine exposure and locomotor activity (Sabeti, Gerhardt & Zahniser, 2002, 2003). Though, the mechanisms underlying these differences with MOD are not elucidated. Conventional DAT inhibitors such as cocaine and MPH binds to outward-facing DAT, this property leads to lower DA uptake rate in striatum and affects directly the subjective effects or reward properties of the drug in animals

(Kohut, Hiranita, Hong, Ebbs, Tronci, Green, ... & Katz, 2014; reviewed by Zanhiser & Sorkin, 2004). The same relationship has been established with DAT occupancy degree in humans (Volkow et al., 1997). The core effects of typical psychostimulants are augmentation of locomotor activity and reinforcement made them prone to abuse. This increase in locomotion is mediated by increases of extracellular DA in brain areas engaged in locomotor activity and reward, such as striatum and NAc (Delfs, Schreiber & Kelley, 1990; Kalivas et al., 1993; Stewart and Vezina, 1989). Moreover, electrolytic lesion in NAc reverted effects on locomotor activity in sensitized animals after chronic cocaine exposure (Todtenkopf et al., 2002). Higher levels of DA in NAc in response to MPH were associated with locomotor hyperactivity in rats exposed to prenatal alcohol (Muñoz-Villegas et a., 2016). Thus, NAc have a crucial role in both locomotor effect and reinforce properties of psychostimulants.

MOD is an atypical DAT inhibitor and produces an increase in extracellular DA concentration in a lesser extent than cocaine and is less effective blocking DAT than cocaine (Loland et al., 2012; Schmitt et al., 2011, 2013). Atypical DAT inhibitors block DA uptake, however, they do not share behavioural cocaine-like effect *in vivo* related to abuse liability and locomotor activity stimulation (reviewed by Reith et al., 2015; Tanda et al., 2009). Therefore, using MOD in young age might be safer than other psychostimulants for treatment of ADHD, since we did not observe an increase locomotor activity over time.

Taking into account our behavioral results and since MOD blocks DAT on the DAergic terminals in NAc and we observed an impairment in a highly reward behaviour (social play), we aimed to investigate whether the reward circuitry (mesolimbic and mesocortical) is altered after MOD treatment. We measure DA content and its main metabolite DOPAC in VTA and NAc and DA extracellular levels after a depolarizing stimulus. No changes were observed in DA and

DOPAC levels in NAc and VTA after 14 days of treatment but a decrease in K^+ -induced DA extracellular levels was observed.

Considering that we did not observe changes in content tissue levels of DA in NAc and the responsiveness in DAergic terminals are altered after 14 days of treatment, the unique inhibitor profile of MOD might explain the decrease in locomotion activity augmentation over time. There are few evidences related to MOD tolerance after chronic use and the evidence suggest contrary results (Kim, 2012; Krishnan & Chary, 2015; Mitler et al., 2000). According to our data of locomotor activity induced by MOD and neurochemical results, we cannot discard a possible tolerance effect after 14 days of MOD i.p. injections in healthy young rats.

The reason behind the fewer play responses and locomotor activity after 14 days of treatment with MOD could be a disruption in the VTA-NAc circuit leading a lower rewarding effect of social play in young animals. NAc DA release induced by a depolarizing stimulus was lower compared to vehicle, while basal extracellular DA levels were the same between both groups. Lower levels of DA release in NAc could be related to the vesicular transporter of monoamines 2 (VMAT-2), modifying the capacity of responsiveness to a stimulation in NAc. Interestingly, one study reported that low doses of MPH are capable to alters VMAT-2, redistributing vesicles in monoaminergic striatal neurons in rat (Riddle et al., 2007). In this line, chronic AMPH treatment also decrease DA release induced by a depolarizing stimulus (Renard et al., 2014). Mice previously exposed to cocaine exhibit higher locomotor response and DA release levels after cocaine challenge, but similar NAc basal DA levels compared to vehicle (Zapata et al., 2003) and locomotor hyperactivity were associated with higher levels of DA in NAc in response to MPH (Muñoz-Villegas et a., 2016). Decrease DA response observed in NAc after chronic MOD treatment, reflects the lack of increase in locomotor activity at the end of the protocol suggest a normalization over time), Also, DAT functionality is modified following psychostimulant administration depending on their mechanism of action and protocol length: upregulated or unaltered after acute cocaine and MPH exposure (Daws et al., 2002; Mortensen & Amara, 2003; Sandoval et al., 2001), downregulated or upregulated following acute AMPH treatment (Boudanova et al., 2008; L'Aurelle et al., 2005) or higher after MOD 6 days administration (Nguyen et al., 2011). In post-mortem cryoprotected human and baboon tissue, Mash and colleagues (2002) demonstrated that both DA uptake and DAT density were increased in cocaine users compared to control subjects in striatum. Furthermore, in humans using *in vivo* single-photon emission computed tomography (SPECT) for measurement of DAT densities, cocaine consumption leads to up regulation compared with drug-free individuals depending of withdrawal period, showing a tendency for stabilisation over time (Malison et al. 1995, 1998). Extracellular DA levels are altered via increase uptake rate mediated by DAT rather than D_2 release inhibition (Ferris et al., 2014). Thus, chronic MOD treatment might increase DAT levels or functionality in NAc dopaminergic terminals. An altered DAT functionality could explain lower extracellular DA levels due to higher levels of DA uptake.

To evaluate this possibility, we performed Fast Scan Cyclic Voltammetry (FSCV) in our 14 days MOD exposure protocol in young male rats. NAcc FSCV was performed the next day after the last MOD or vehicle injection. We observed at baselines that MOD treated rats shows a trend to higher DA release and increased levels of maximal DA uptake (V_{max}). This result might explain, at least in part, the lower extracellular DA levels observed in microdialysis after depolarizing stimulus.

On the other hand, D_2 autoreceptors in DA terminals modulate the release and the synthesis of DA in NAc or striatum (Anzalone et al., 2012). DA uptake can be modulated by D_2 autoreceptors in NAc and mPFC; in particular, *in vivo* experiments have exposed that when local

NAc application of raclopride (D_2 antagonist) is performed, the DA uptake is slower (Cass et al., 1994) showing that DAT and D_2 autoreceptors interact with each other and both regulate extracellular DA levels in NAc.

D_2 receptor has two isoforms, the short (D_2S) and the long (D_2L) and the former is mainly presynaptic in striatum acting as an autoreceptor in the DAergic terminal. The molecular weight is 50 KDa and 60 kDa for of D_2S and D_2L, respectively (Khan et al., 1998a, 1998b). Therefore, our blots reflect the D_2S isoform in both nuclei. In NAc we did not find differences between groups in the expression of D_2 after 14 days of MOD treatment. It is possible that the decrease response of DAergic terminals to a depolarizing stimulation might be due mainly to altered DAT or VMAT-2 function in NAc.

Interestingly, our data show lower levels of D_2 in PFC due to MOD chronic treatment. PFC and NAc have both types of receptors (D_1 and D_2) and receive DA projections from VTA as well. PFC has been related with executive functions including working memory, future planning, rule learning, goal directed behaviour, reward value association and decision making (Fuster, 2001; Kennerley & Watson, 2011; Rushworth, Noonan, Boorman, Walton & Behrens, 2011). Nevertheless, PFC have also a crucial role in social behaviour, such as social recognition and social approach in rodents (Ko, 2017; Lee et al., 2016). When excitation/inhibition balance in PFC was disturbed, social dysfunction is observed (Yizhar, Fenno, Prigge, Schneider, Davidson, O'shea,... & Stehfest, 2011). PFC have been implicated in the normal development of social cognition in both mice and humans (Bicks et al., 2015). Moreover, PFC lesions showed the importance of PFC in decision making regarding to reward value (Rudebeck et al., 2008). Considering the reward value associated to social play and the role of PFC in social behaviour and decision making, lower D_2 expression in PFC suggest an altered relationship between reward and decision to play in MOD treated rats.

Neonatal lesions in PFC impairs the structure of social play (Bell et al, 2009; Schneider and Koch, 2005), showing alterations in frequency and structure of pinning behaviour in both young and adult rats (Pellis et al., 2006). When muscimol and baclofen ($GABA_A$ and $_B$ receptor agonists) are injected in areas of PFC, young rats reduces pinning and pouncing frequencies and time spent in social behaviour (van Kerkhof, Damsteeg, Trezza, Voorn & Vanderschuren, 2013). Additionally, when MPH is injected, both pinning and pouncing frequency are diminished (Achterberg et al., 2015). Therefore, it might be possible that decrease pinning behaviour observed due to chronic MOD treatment is indirectly related to D_2 PFC expression.

PFC has also been related with ADHD. Interestingly, when PFC neonatal lesions are use in rats, they developed hyperactivity (ADHD-like phenotype) and changed their play behaviour pattern (Panksepp et al., 2003). We measured the time of rearing (a proxy of attention behaviour, see Aspide, Gironi Carnevale, Sergeant & Sadile, 1998) and found differences between groups only in days 1 and 7 of MOD treatment (see annexes). It would be interesting considering future attentional tasks where it is known the participation of PFC and the possible consequences mediated by MOD.

PFC and NAc are connected. A decrease in D_2 expression in PFC can explain the mechanism underlying the trend for higher basal extracellular GABA measured in NAc by *in vivo* microdialysis. PFC D_2 autoreceptors were impaired with MOD treatment, suggesting a lower inhibition on the glutamatergic projections to NAc, resulting in more GLU release in NAc, thus, more extracellular GABA levels are expected.

Regarding to extracellular GLU and GABA levels we observed higher extracellular GABA levels after K^+ 70 mM depolarising stimulus in the vehicle group, indicating again, an impaired response to a depolarising stimulus in the treated group, however we observed a trend for extracellular GABA basal levels to be higher in treated group. Early microdialysis studies in

NAc with acute i.p. administration of MOD provokes decreases in GABA release (Ferraro, Antonelli, O'Connor, Tanganelli, Rambert & Fuxe, 1997) and the same doses of MOD did not elicit changes in DA release levels. However, when MOD is administrated subcutaneously with GABA antagonist the effects is reverted (Ferraro et al., 1996). It is important to mention that in Ferraro and colleagues' studies, adult rats and acute protocol were used, therefore, length of treatment and age of animals might be behind the differences observed in our results. In the case of GLU, the same team reported increase release in striatum only with high doses (300 mg/kg; Ferraro et al., 1998). Noticeable, single doses of metamphetamine did not affect levels of neither glutamate and GABA in SN or striatum, but repeated doses results in impressive high extracellular GABA levels, leading to extracellular glutamate levels similar to single doses condition (Bustamante et al., 2002). As we showed, basal extracellular GABA levels seems to be higher in treated rats and DA release was lower, therefore chronic MOD might increase the inhibitory balance in NAc due to an augmentation of GABAergic communication with VTA. Thus, provoking lower DA release after a depolarizing stimulus and this enhancement of inhibitory balance is not related to glutamate levels.

Finally, to date this is the first study to reveal social play behavior impairment related to changes in D_2 autoreceptor expression in PFC, extracellular GABA and DA levels in NAc after MOD chronic treatment. Indicating the relationships between GABA and DAergic systems within reward circuitry and social behavior in healthy young rats.

General conclusion

Chronic MOD treatment during adolescence leads to fewer responses to play solicitation in treated rats but no changes in other behaviours as social exploration or basal locomotor activity. The rewarding value of social play is apparently decreased during MOD treatment since we found a lower DA release in NAc after K^+ induced depolarization.

These findings were also accompanied with lower expression of D_2 in PFC. This decrease in presynaptic D_2 in PFC could lead to an augmented glutamatergic tone from PFC to NAc, leading to an impaired DAergic responses in NAc mediated by GABA levels. Though, the absence of changes in glutamate and GABA in NAc make difficult to speculate, then more studies are necessary to conclude in this point. Additionally, we observed an increase in locomotor activity induced by acute MOD, however, this outcome disappears after 14 days of treatment. Considering that MOD prefers other DAT conformation than cocaine or other psychostimulants, the decrease of locomotor activity over time could reflect tolerance (see figure 20).

To our knowledge, this is the first study revealing alterations in social behaviour and neurochemistry of DA homeostasis in nucleus accumbens after chronic MOD treatment during adolescence, drawing attention and inviting to rigorously evaluate the utilization of MOD during childhood and adolescence in a ADHD context or in relation to misutilization as a neuroenhancer.

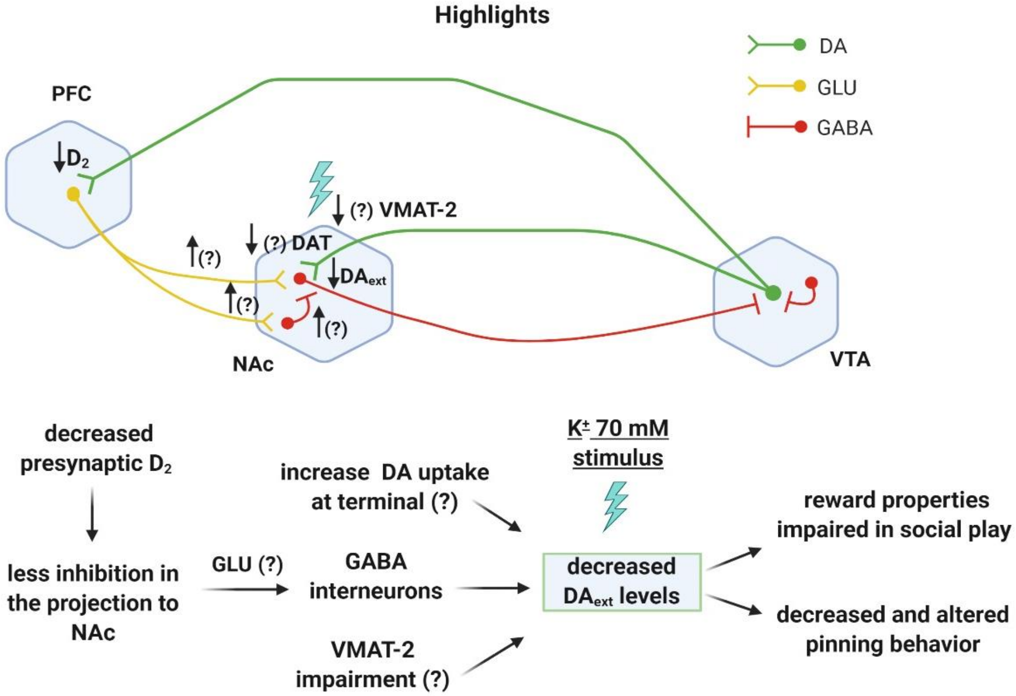


Figure 20. General conclusion. Alterations in social behaviour and neurochemistry of DA homeostasis in nucleus accumbens (NAc) after chronic MOD treatment during adolescence. Chronic MOD treatment during adolescence leads to fewer responses to play solicitation in treated rats but no changes in other behaviours as social exploration or basal locomotor activity. The rewarding value of social play is apparently decreased during MOD treatment since we found a lower DA release in NAc after K^+ induced depolarization. Illustration created with Biorender.com

References

Achterberg, E. M., Trezza, V., Siviy, S. M., Schrama, L., Schoffelmeer, A. N., & Vanderschuren, L. J. (2014). Amphetamine and cocaine suppress social play behaviour in rats through distinct mechanisms. Psychopharmacology, 231(8), 1503-1515.

Achterberg, E. M., van Kerkhof, L. W., Damsteegt, R., Trezza, V., & Vanderschuren, L. J. (2015). Methylphenidate and atomoxetine inhibit social play behaviour through prefrontal and subcortical limbic mechanisms in rats. Journal of Neuroscience, 35(1), 161-169.

Ackerman, J. M., & White, F. J. (1992). Decreased activity of rat A10 dopamine neurons following withdrawal from repeated cocaine. European Journal of Pharmacology, 218(1), 171–173. https://doi.org/10.1016/0014-2999(92)90161-V

Adriani, W., Leo, D., Greco, D., Rea, M., di Porzio, U., Laviola, G.; & Perrone-Capano, C. (2006). Methylphenidate Administration to Adolescent Rats Determines Plastic Changes on Reward-Related Behaviour and Striatal Gene Expression. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 31. 1946-56. 10.1038/sj.npp.1300962

Albert, D. J., Walsh, M. L., Gorzalka, B. B., Siemens, Y., & Louie, H. (1986). Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance. Physiology & behavior, 36(3), 401-407.

Ahumada, C., Bahamondes, C., Cerda, C. A., Silva, R. A., Cruz, G., Moya, P. R., ... & Renard, G. M. (2017). Amphetamine treatment affects the extra-hypothalamic vasopressinergic system in a sex-and nucleus-dependent manner. Journal of Neuroendocrinology, 29(4).

Alessandri, S. M. (1992). Attention, play, and social behaviour in ADHD preschoolers. Journal of abnormal child psychology, 20(3), 289-302.

Amiri, S., Mohammadi, M., Mohammadi, M., Nouroozinejad, G.H., (2006) Modafinil as a treatment for Attention-Deficit/Hyperactivity Disorder in children and adolescents: A double blind, randomized clinical trial. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32, 145–149

Anderson, S. M., & Pierce, R. C. (2005). Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. Pharmacology & therapeutics, 106(3), 389-403.

Anzalone, A., Lizardi-Ortiz, J. E., Ramos, M., De Mei, C., Hopf, F. W., Iaccarino, C., ... & Caron, M. G. (2012). Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. Journal of Neuroscience, 32(26), 9023-9034.

Arnold, L. E., Ganocy, S. J., Mount, K., Youngstrom, E. A., Frazier, T., Fristad, M., Horwitz, S. M., Birmaher, B., Findling, R., Kowatch, R. A., Demeter, C., Axelson, D., Gill, M. K., & Marsh, L. (2014). Three-year latent class trajectories of attention-deficit/hyperactivity disorder (ADHD) symptoms in a clinical sample not selected for ADHD. Journal of the American Academy of Child and Adolescent Psychiatry, 53(7), 745–760. https://doi.org/10.1016/j.jaac.2014.03.007

Aspide, R., Gironi Carnevale, U. A., Sergeant, J. A., & Sadile, A. G. (1998). Non-selective attention and nitric oxide in putative animal models of Attention-Deficit Hyperactivity Disorder. Behavioural Brain Research, 95(1), 123–133. https://doi.org/10.1016/S0166-4328(97)00217-9

Avelar, A. J., Juliano, S. A., & Garris, P. A. (2013). Amphetamine augments vesicular dopamine release in the dorsal and ventral striatum through different mechanisms. Journal of Neurochemistry, 125(3), 373–385. https://doi.org/10.1111/jnc.12197

Baarendse, P. J., Limpens, J. H., & Vanderschuren, L. J. (2014). Disrupted social development enhances the motivation for cocaine in rats. Psychopharmacology, 231(8), 1695-1704.

Baranski, J. V., Pigeau, R., Dinich, P., & Jacobs, I. (2004). Effects of modafinil on cognitive and meta-cognitive performance. Human Psychopharmacology: Clinical and Experimental, 19(5), 323-332.

Bell, H. C., McCaffrey, D. R., Forgie, M. L., Kolb, B., & Pellis, S. M. (2009). The role of the medial prefrontal cortex in the play fighting of rats. Behavioural neuroscience, 123(6), 1158.

Bicks, L. K., Koike, H., Akbarian, S., & Morishita, H. (2015). Prefrontal cortex and social cognition in mouse and man. Frontiers in psychology, 6, 1805.

Biederman, J., Swanson, J. M., Wigal, S. B., Kratochvil, C. J., Boellner, S. W., Earl, C. Q., ... & Greenhill, L. (2005). Efficacy and safety of modafinil film–coated tablets in children and adolescents with attention-deficit/hyperactivity disorder: results of a randomized, double-blind, placebo-controlled, flexible-dose study. Pediatrics, 116(6), e777-e784.

Billiard, M., & Broughton, R. (2018). Modafinil: its discovery, the early European and North American experience in the treatment of narcolepsy and idiopathic hypersomnia, and its subsequent use in other medical conditions. Sleep medicine, 49, 69-72.

Bisagno, V., González, B., & Urbano, F. J. (2016). Cognitive enhancers versus addictive psychostimulants: The good and bad side of dopamine on prefrontal cortical circuits. Pharmacological Research, 109, 108-118.

Blanco-Gandía, M. C., Mateos-García, A., García-Pardo, M. P., Montagud-Romero, S., Rodríguez-Arias, M., Miñarro, J., & Aguilar, M. A. (2015). Effect of drugs of abuse on social behaviour: a review of animal models. Behavioural pharmacology, 26(6), 541-570.

Bredewold, R., Smith, C. J., Dumais, K. M., & Veenema, A. H. (2014). Sex-specific modulation of juvenile social play behaviour by vasopressin and oxytocin depends on social context. Frontiers in behavioural neuroscience, 8, 216.

Bustamante, D., You, Z., Castel, M., Johansson, S., Goiny, M., Terenius, L., Hökfelt, T. and Herrera-Marschitz, M. (2002). Effect of single and repeated methamphetamine treatment on neurotransmitter release in substantia nigra and neostriatum of the rat. Journal of Neurochemistry, 83: 645-654. doi:10.1046/j.1471-4159.2002. 01171.x

Boellner, S. W., Earl, C. Q. & Arora, S. (2006) Modafinil in children and adolescents with attention-deficit/hyperactivity disorder:a preliminary 8-week, open-label study. Current Medical Research and Opinion 22, 2457–2465

Bolaños, C. A., Barrot, M., Berton, O., Wallace-Black, D., & Nestler, E. J. (2003). Methylphenidate treatment during pre- and peri adolescence alters behavioural responses to emotional stimuli at adulthood. Biological Psychiatry, 54(12), 1317–1329. http://doi.org/10.1016/S0006-3223(03)00570-5

Boudanova, E., Navaroli, D. M., & Melikian, H. E. (2008). Amphetamine-induced decreases in dopamine transporter surface expression are protein kinase C-independent. Neuropharmacology, 54(3), 605-612.

Burghardt, G. M. (2005). The genesis of animal play: Testing the limits. Mit Press.

Calcagnetti, D. J., & Schechter, M. D. (1992). Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. Physiology and Behaviour, 51(4), 667–672. https://doi.org/10.1016/0031-9384(92)90101-7

Cass, W. A., & Gerhardt, G. A. (1994). Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. Neuroscience letters, 176(2), 259-263

Dance, A. (2016). Smart drugs: A dose of intelligence. Nature, 531(7592), S2–S3. http://doi.org/10.1038/531S2a

Daws, L. C., Callaghan, P. D., Morón, J. A., Kahlig, K. M., Shippenberg, T. S., Javitch, J. A., & Galli, A. (2002). Cocaine increases dopamine uptake and cell surface expression of dopamine transporters. Biochemical and biophysical research communications, 290(5), 1545-1550.

Delfs, J. M., Schreiber, L., & Kelley, A. E. (1990). Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. Journal of Neuroscience, 10(1), 303-310.

Engeli, E. J., Zoelch, N., Hock, A., Nordt, C., Hulka, L. M., Kirschner, M., ... & Seifritz, E. (2020). Impaired glutamate homeostasis in the nucleus accumbens in human cocaine addiction. Molecular psychiatry, 1-9.

FDA (U.S. Food and drugs administration) (2015). “MEDICATION GUIDE PROVIGIL®C-IV(modafinil).
https://www.fda.gov/downloads/drugs/drugsafety/ucm231722.pdf

FDA (U.S. Food and drugs administration) (2016). https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm269188.htm#2

Ferraro, L., Sergio, T., O’Connor, W.T., Tiziana, A., Francis, R., Kjell, F. (1996). The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. European Journal of Pharmacology, 306, 33-39, https://doi.org/10.1016/0014-2999(96)00182-3

Federici, M., Latagliata, E. C., Rizzo, F. R., Ledonne, A., Gu, H. H., Romigi, A., ... & Mercuri, N. B. (2013). Electrophysiological and amperometric evidence that modafinil blocks the dopamine uptake transporter to induce behavioural activation. Neuroscience, 252, 118-124.

Ferraro, L., Antonelli, T., O'Connor, W. T., Tanganelli, S., Rambert, F. A., & Fuxe, K. (1997). Modafinil: an antinarcoleptic drug with a different neurochemical profile to d-amphetamine and dopamine uptake blockers. Biological psychiatry, 42(12), 1181-1183.

Ferraro, L., Antonelli, T., O'Connor, W. T., Tanganelli, S., Rambert, F. A., & Fuxe, K. (1998). The effects of modafinil on striatal, pallidal and nigral GABA and glutamate release in the conscious rat: evidence for a preferential inhibition of striato-pallidal GABA transmission. Neuroscience letters, 253(2), 135-138.

Ferris, M. J., España, R. A., Locke, J. L., Konstantopoulos, J. K., Rose, J. H., Chen, R., & Jones, S. R. (2014). Dopamine transporters govern diurnal variation in extracellular dopamine tone. Proceedings of the National Academy of Sciences, 111(26), E2751-E2759.

Field, E. F., & Pellis, S. M. (1994). Differential effects of amphetamine on the attack and defense components of play fighting in rats. Physiology & behaviour, 56(2), 325-330.

Freese, L., Signor, L., Machado, C., Ferigolo, M., & Barros, H., Tannhauser. M., (2012). Non-medical use of methylphenidate: a review. Trends in Psychiatry and Psychotherapy, 34(2), 110-115. https://doi.org/10.1590/S2237-60892012000200010

Ford, C. P., Gantz, S. C., Phillips, P. E., & Williams, J. T. (2010). Control of extracellular dopamine at dendrite and axon terminals. Journal of Neuroscience, 30(20), 6975-6983.

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

Greenhill, L. L., Biederman, J., Boellner, S.W., Rugino, T.A., Sangal, R.B., Earl, C.Q., Jiang, J.G., Swanson, J. M., (2006) A Randomized, Double-Blind, Placebo-Controlled Study of Modafinil Film-Coated Tablets in Children and Adolescents with Attention-Deficit/Hyperactivity Disorder. Journal of the American Academy of Child & Adolescent Psychiatry 45, 503–511

Goez, H. R., Scott, O., Nevo, N., Bennett-Back, O., & Zelnik, N. (2012). Using the test of variables of attention to determine the effectiveness of modafinil in children with attention-deficit hyperactivity disorder (ADHD): a prospective methylphenidate-controlled trial. Journal of child neurology, 27(12), 1547-1552.

González, B., Jayanthi, S., Gomez, N., Torres, O. V., Sosa, M. H., Bernardi, A., ... & Bisagno, V. (2018). Repeated methamphetamine and modafinil induce differential cognitive effects and specific histone acetylation and DNA methylation profiles in the mouse medial prefrontal cortex. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 82, 1-11.

Haber, S. N. (2003). The primate basal ganglia: parallel and integrative networks. Journal of chemical neuroanatomy, 26(4), 317-330.

Insel, T. R., & Fernald, R. D. (2004). How the brain processes social information: searching for the social brain. Annu. Rev. Neurosci., 27, 697-722.

Kahbazhi, M., Ghoreishi, A., Rahiminejad, F., Mohammadi, M. R., Kamalipour, A., & Akhondzadeh, S. (2009). A randomized, double-blind and placebo-controlled trial of modafinil in children and adolescents with attention deficit and hyperactivity disorder. Psychiatry research, 168(3), 234-237.

Kalivas, P. W., McFarland, K. R. I. S. T. A., Bowers, S., Szumlinski, K., Xi, Z. X., & Baker, D. (2003). Glutamate transmission and addiction to cocaine. Annals of the New York Academy of Sciences, 1003(1), 169-175.

Kalivas, P. W., Sorg, B. A., & Hooks, M. S. (1993). The pharmacology and neural circuitry of sensitization to psychostimulants. Behavioural pharmacology.

Kalivas, P. W., & Duffy, P. (1998). Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. Journal of neurochemistry, 70(4), 1497-1502.

Kallweit U, Bassetti CL. (2017) Pharmacological management of narcolepsy with and without cataplexy. Expert Opin Pharmacother;18(8):809-817. doi:10.1080/14656566.2017.1323877

Kennerley, S. W., & Walton, M. E. (2011). Decision making and reward in frontal cortex: complementary evidence from neurophysiological and neuropsychological studies. Behavioural neuroscience, 125(3), 297.

Khan, Z. U., Mrzljak, L., Gutierrez, A., De La Calle, A., & Goldman-Rakic, P. S. (1998). Prominence of the dopamine D2 short isoform in dopaminergic pathways. Proceedings of the national academy of sciences, 95(13), 7731-7736.

Khan, Z. U., Gutiérrez, A., Martín, R., Peñafiel, A., Rivera, A., & De La Calle, A. (1998). Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. Journal of Comparative Neurology, 402(3), 353-371.

Kim D. (2012). Practical use and risk of modafinil, a novel waking drug. Environmental health and toxicology, 27, e2012007. https://doi.org/10.5620/eht.2012.27.e2012007

Krishnan, R., & Chary, K. V. (2015). A rare case modafinil dependence. Journal of pharmacology & pharmacotherapeutics, 6(1), 49.

Ko, J. (2017). Neuroanatomical substrates of rodent social behaviour: the medial prefrontal cortex and its projection patterns. Frontiers in neural circuits, 11, 41.

Kohut, S. J., Hiranita, T., Hong, S. K., Ebbs, A. L., Tronci, V., Green, J., ... & Katz, J. L. (2014). Preference for distinct functional conformations of the dopamine transporter alters the relationship between subjective effects of cocaine and stimulation of mesolimbic dopamine. Biological psychiatry, 76(10), 802-809.

Korotkova, T. M., Klyuch, B. P., Ponomarenko, A. A., Lin, J. S., Haas, H. L., & Sergeeva, O. A. (2007). Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors. Neuropharmacology, 52(2), 626-633.

Kumar, R. (2008). Approved and investigational uses of modafinil. Drugs, 68(13), 1803-1839.

L'Aurelle, A. J., Furman, C. A., Zhang, M., Guptaroy, B., & Gnegy, M. E. (2005). Rapid delivery of the dopamine transporter to the plasmalemmal membrane upon amphetamine stimulation. Neuropharmacology, 49(6), 750-758.

Lee, E., Rhim, I., Lee, J. W., Ghim, J. W., Lee, S., Kim, E., & Jung, M. W. (2016). Enhanced neuronal activity in the medial prefrontal cortex during social approach behaviour. Journal of Neuroscience, 36(26), 6926-6936.

Le Moine C, Bloch B (1995a). D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol.;355(3):418-426. doi:10.1002/cne.903550308.

Le Moine, C. and Bloch, B. (1995b). D1 and D2 dopamine receptor gene expression in the rat striatum: Sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAS in distinct neuronal populations of the dorsal and ventral striatum. J. Comp. Neurol., 355: 418-426. doi:10.1002/cne.903550308.

Li, J., Yang, X., Zhou, F., Liu, C., Wei, Z., Xin, F., ... & Becker, B. (2020). Modafinil enhances cognitive, but not emotional conflict processing via enhanced inferior frontal gyrus activation and its communication with the dorsomedial prefrontal cortex. Neuropsychopharmacology, 45(6), 1026-1033.

Liu, Y., Aragona, B. J., Young, K. A., Dietz, D. M., Kabbaj, M., Mazei-Robison, M., ... & Wang, Z. (2010). Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. Proceedings of the National Academy of Sciences, 107(3), 1217-1222.

Leuzinger-Bohleber, M. (2010). Psychoanalytic Preventions/Interventions and Playing “Rough-and-Tumble” Games: Alternatives to Medical Treatments of Children Su. International Journal of Applied Psychoanalytic Studies, 7(4), 332-338.

Loland, C. J., Mereu, M., Okunola, O. M., Cao, J., Prisinzano, T. E., Mazier, S., ... & Newman, A. H. (2012). R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. Biological psychiatry, 72(5), 405-413.

López-Pérez, S. J., Vergara, P., Ventura-Valenzuela, J. P., Ureña-Guerrero, M. E., Segovia, J., & Beas-Zárate, C. (2005). Modification of dopaminergic markers expression in the striatum by neonatal exposure to glutamate during development. International Journal of Developmental Neuroscience, 23(4), 335–342. http://doi.org/10.1016/j.ijdevneu.2004.12.010.

Lu, L., Grimm, J. W., Shaham, Y., & Hope, B. T. (2003). Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. Journal of Neurochemistry, 85(6), 1604–1613. https://doi.org/10.1046/j.1471-4159.2003.01824.x.

Malison, R. T., Best, S. E., McCance, E., Laruelle, M., Baldwin, R. M., Seibyl, J. S., ... & Zoghbi, S. S. (1995). Euphorigenic doses of cocaine reduce [123 I] β-CIT SPECT measures of dopamine transporter availability in human cocaine addicts. Psychopharmacology, 122(4), 358-362.

Malison, R. T., McCance, E., Carpenter, L. L., Baldwin, R. M., Seibyl, J. P., Price, L. H., ... & Innis, R. B. (1998). [123I] β-CIT SPECT imaging of dopamine transporter availability after mazindol administration in human cocaine addicts. Psychopharmacology, 137(4), 321-325.

Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L. J., & Trezza, V. (2016). Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social

Play Behaviour in Rats. Neuropsychopharmacology, 41(9), 1–9. http://doi.org/10.1038/npp.2016.22.

Mereu, M., Bonci, A., Newman, A. H., & Tanda, G. (2013). The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. Psychopharmacology, 229(3), 415-434.

Mignot, E., Nishino, S., Guilleminault, C., Dement, W. C. Modafinil Binds to the Dopamine Uptake Carrier Site with Low Affinity (1994). Sleep, 17, 5, 436–437, https://doi.org/10.1093/sleep/17.5.436

Minzenberg, M. J., & Carter, C. S. (2008). Modafinil: a review of neurochemical actions and effects on cognition. Neuropsychopharmacology, 33(7), 1477-1502.

Mitler, M.M., Harsh, J., Hirshkowitz, M., Guilleminault, C., 2000. Long-term efficacy and safety of modafinil (PROVIGIL((R))) for the treatment of excessive daytime sleepiness associated with narcolepsy. Sleep Med. 1 (3), 231–243.

Mortensen, O. V., & Amara, S. G. (2003). Dynamic regulation of the dopamine transporter. European journal of pharmacology, 479(1-3), 159-170.

Muñoz-Villegas, P., Rodríguez, V. M., Giordano, M., & Juárez, J. (2017). Risk-taking, locomotor activity and dopamine levels in the nucleus accumbens and medial prefrontal cortex in male rats treated prenatally with alcohol. Pharmacology Biochemistry and Behavior, 153, 88-96.

Myrick, H., Malcolm, R., Taylor, B., & LaROWE, S. T. E. V. E. N. (2004). Modafinil: preclinical, clinical, and post-marketing surveillance—a review of abuse liability issues. Annals of Clinical Psychiatry, 16(2), 101-109.

Nader, M. A., Morgan, D., Gage, H. D., Nader, S. H., Calhoun, T. L., Buchheimer, N., … Mach, R. H. (2006). PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nature Neuroscience, 9(8), 1050–1056. https://doi.org/10.1038/nn1737

Nicola, S. M., Surmeier, D. J., & Malenka, R. C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annual review of neuroscience, 23(1), 185-215.

Niesink, R. J., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. Neuropharmacology, 28(4), 411-418.

Nijhof, S. L., Vinkers, C. H., van Geelen, S. M., Duijff, S. N., Achterberg, E. M., Van Der Net, J., ... & van der Brug, A. W. (2018). Healthy play, better coping: The importance of play for the development of children in health and disease. Neuroscience & Biobehavioral Reviews, 95, 421-429.

Nguyen, T. L., Tian, Y. H., You, I. J., Lee, S. Y., & Jang, C. G. (2011). Modafinil-induced conditioned place preference via dopaminergic system in mice. Synapse, 65(8), 733-741.

Nicolaidis, S., & De Saint Hilaire, Z. (1993). Nonamphetamine awakening agent modafinil induces feeding changes in the rat. Brain Research Bulletin, 32(2), 87–90. http://doi.org/10.1016/0361-9230(93)90060-O

Northcutt, K. V., & Nguyen, J. M. (2014). Female juvenile play elicits Fos expression in dopaminergic neurons of the VTA. Behavioural Neuroscience, 128(2), 178.

Panksepp, J., Siviy, S., & Normansell, L. (1984). The psychobiology of play: theoretical and methodological perspectives. Neuroscience & Biobehavioural Reviews, 8(4), 465-492.

Panksepp, J., Burgdorf, J., Turner, C., & Gordon, N. (2003). Modeling ADHD-type arousal with unilateral frontal cortex damage in rats and beneficial effects of play therapy. Brain and Cognition, 52(1), 97-105.

Paterson, N. E., Fedolak, A., Olivier, B., Hanania, T., Ghavami, A., & Caldarone, B. (2010). Psychostimulant-like discriminative stimulus and locomotor sensitization properties of the wake-promoting agent modafinil in rodents. Pharmacology Biochemistry and Behavior, 95(4), 449-456.

Paxinos and Watson (2007). The Rat Brain in Stereotaxic Coordinates, 6th Edition. Academic Press

Pellis, S. M., Castañeda, E., McKenna, M. M., Tran-Nguyen, L. T., & Whishaw, I. Q. (1993). The role of the striatum in organizing sequences of play fighting in neonatally dopamine-depleted rats. Neuroscience letters, 158(1), 13-15.

Pellis, S. M., & McKenna, M. (1995). What do rats find rewarding in play fighting? - An analysis using drug-induced non-playful partners. Behavioural Brain Research, 68(1), 65–73. https://doi.org/10.1016/0166-4328(94)00161-8

Pellis, S. M., Field, E. F., Smith, L. K., & Pellis, V. C. (1997). Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. Neuroscience & Biobehavioural Reviews, 21(1), 105-120.

Pellis, S. M., Pellis, V. C., & Himmler, B. T. (2014). How play makes for a more adaptable brain: a comparative and neural perspective. American Journal of Play, 7(1), 73-98.

Polanczyk, G., De Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: a systematic review and meta regression analysis. American journal of psychiatry, 164(6), 942-948.

Polanczyk, G. V., Willcutt, E. G., Salum, G. A., Kieling, C., & Rohde, L. A. (2014). ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. International journal of epidemiology, 43(2), 434-442.

Qu, W. M., Huang, Z. L., Xu, X. H., Matsumoto, N., & Urade, Y. (2008). Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. Journal of Neuroscience, 28(34), 8462-8469.

Reith, M. E., Blough, B. E., Hong, W. C., Jones, K. T., Schmitt, K. C., Baumann, M. H., ... & Katz, J. L. (2015). Behavioural, biological, and chemical perspectives on atypical agents targeting the dopamine transporter. Drug and alcohol dependence, 147, 1-19.

Renard, G. M., Sotomayor-Zarate, R., Blanco, E. H. and Gysling, K. (2014), Withdrawal from chronic amphetamine reduces dopamine transmission in the rat lateral septum. Journal of Neuroscience Research, 92: 937-943. doi:10.1002/jnr.23369

Riddle, E. L., Hanson, G. R., & Fleckenstein, A. E. (2007). Therapeutic doses of amphetamine and methylphenidate selectively redistribute the vesicular monoamine transporter 2. European journal of pharmacology, 571(1), 25–28. doi: 10.1016/j.ejphar.2007.05.044

Robinson, D. L., Zitzman, D. L., & Williams, S. K. (2011). Mesolimbic dopamine transients in motivated behaviours: focus on maternal behaviour. Frontiers in psychiatry, 2, 23.

Robinson, D. L., Zitzman, D. L., Smith, K. J., & Spear, L. P. (2011). Fast dopamine release events in the nucleus accumbens of early adolescent rats. Neuroscience, 176, 296-307.

Rowley, H. L., Kulkarni, R. S., Gosden, J., Brammer, R. J., Hackett, D., & Heal, D. J. (2014). Differences in the neurochemical and behavioural profiles of lisdexamfetamine methylphenidate and modafinil revealed by simultaneous dual probe microdialysis and locomotor activity measurements in freely moving rats. Journal of Psychopharmacology, 28(3), 254–269. https://doi.org/10.1177/0269881113513850

Rudebeck, P. H., Bannerman, D. M., & Rushworth, M. F. S. (2008). The contribution of distinct subregions of the ventromedial frontal cortex to emotion, social behaviour, and decision making. Cognitive, Affective, & Behavioural Neuroscience, 8(4), 485-497.

Rugino, T. A., & Samsock, T. C. (2003). Modafinil in children with attention-deficit hyperactivity disorder. Pediatric Neurology, 29(2), 136-142.

Rushworth, M. F., Noonan, M. P., Boorman, E. D., Walton, M. E., & Behrens, T. E. (2011). Frontal cortex and reward-guided learning and decision-making. Neuron, 70(6), 1054-1069.

Russo, S. J., & Nestler, E. J. (2013). The brain reward circuitry in mood disorders. Nature Reviews Neuroscience, 14(9), 609-625.

Sabeti, J., Gerhardt, G. A., & Zahniser, N. R. (2002). Acute cocaine differentially alters accumbens and striatal dopamine clearance in low and high cocaine locomotor responders: behavioural and electrochemical recordings in freely moving rats. Journal of Pharmacology and Experimental Therapeutics, 302(3), 1201-1211.

Sabeti, J., Gerhardt, G. A., & Zahniser, N. R. (2003). Individual differences in cocaine-induced locomotor sensitization in low and high cocaine locomotor-responding rats are associated with differential inhibition of dopamine clearance in nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics, 305(1), 180-190.

Sandoval, V., Riddle, E. L., Ugarte, Y. V., Hanson, G. R., & Fleckenstein, A. E. (2001). Methamphetamine-induced rapid and reversible changes in dopamine transporter function: an in vitro model. Journal of Neuroscience, 21(4), 1413-1419.

Schmitt, K. C., & Reith, M. E. (2010). Regulation of the dopamine transporter: aspects relevant to psychostimulant drugs of abuse. Annals of the New York Academy of Sciences, 1187(1), 316-340.

Schmitt, K. C., & Reith, M. E. (2011). The atypical stimulant and nootropic modafinil interact with the dopamine transporter in a different manner than classical cocaine-like inhibitors. PloS one, 6(10), e25790.

Schmitt, K. C., Rothman, R. B., & Reith, M. E. (2013). Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. Journal of Pharmacology and Experimental Therapeutics, 346(1), 2-10.

Scoriels, L., Jones, P. B., & Sahakian, B. J. (2013). Modafinil effects on cognition and emotion in schizophrenia and its neurochemical modulation in the brain. Neuropharmacology, 64, 168–184. http://doi.org/http://dx.doi.org/10.1016/j.neuropharm.2012.07.011

Siciliano, C. A., Calipari, E. S., Ferris, M. J., & Jones, S. R. (2015). Adaptations of presynaptic dopamine terminals induced by psychostimulant self-administration. ACS chemical neuroscience, 6(1), 27-36.

Singh I, Bard I, Jackson J (2014) Robust Resilience and Substantial Interest: A Survey of Pharmacological Cognitive Enhancement among University Students in the UK and Ireland. PLoS ONE 9(10): e105969. doi: 10.1371/journal.pone.0105969

Siviy, S. M., Baliko, C. N., & Bowers, K. S. (1997). Rough-and-tumble play behaviour in Fischer-344 and Buffalo rats: Effects of social isolation. Physiology & Behaviour, 61(4), 597-602.

Siviy, S. M., Crawford, C. A., Akopian, G., & Walsh, J. P. (2011). Dysfunctional play and dopamine physiology in the Fischer 344 rat. Behavioural Brain Research, 220(2), 294-304.

Siviy, S. M. (2020). How strain differences could help decipher the neurobiology of mammalian playfulness: what the less playful Fischer 344 rat can tell us about play. International Journal of Play, 9(1), 9-24.

Spencer, R. C., Klein, R. M., & Berridge, C. W. (2012). Psychostimulants act within the prefrontal cortex to improve cognitive function. Biological Psychiatry, 72(3), 221–227.http://doi.org/10.1016/j.biopsych.2011.12.002

Stewart, J., & Vezina, P. (1989). Microinjections of Sch-23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systematic amphetamine. Brain research, 495(2), 401-406.

Striplin, C. D., & Kalivas, P. W. (1992). Correlation between behavioural sensitization to cocaine and G protein ADP-ribosylation in the ventral tegmental area. Brain Research, 579(2), 181–186. https://doi.org/10.1016/0006-8993(92)90049-F

Sotomayor R, Forray MI, Gysling K. (2005) Acute morphine administration increases extracellular DA levels in the rat lateral septum by decreasing the GABAergic inhibitory tone in the ventral tegmental area. Journal of Neuroscience Research; 81(1):132-9.

Tanda, G., Newman, A. H., & Katz, J. L. (2009). Discovery of drugs to treat cocaine dependence: behavioural and neurochemical effects of atypical dopamine transport inhibitors. Advances in pharmacology 57, 253 - 289. Academic Press.

Teter, C. J., McCabe, S. E., LaGrange, K., Cranford, J. A. and Boyd, C. J. (2006), Illicit Use of Specific Prescription Stimulants Among College Students: Prevalence, Motives, and Routes of Administration. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 26: 1501–1510. doi:10.1592/phco.26.10.1501

Teter, C. J., Falone, A. E., Cranford, J. A., Boyd, C. J., & McCabe, S. E. (2010). Nonmedical use of prescription stimulants and depressed mood among college students: Frequency and routes of administration. Journal of Substance Abuse Treatment, 38(3), 292–298. http://doi.org/10.1016/j.jsat.2010.01.005

Thiel, K. J., Okun, A. C. & Neisewander, J. L. (2008). Social reward-conditioned place preference: A model revealing an interaction between cocaine and social context rewards in rats. Drug and Alcohol Dependence 96, 202–212

Thomas, R., Sanders, S., Doust, J., Beller, E., & Glasziou, P. (2015). Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics, 135(4), e994-e1001.

Thor, D. H., & Holloway, W. R. (1983). Play soliciting in juvenile male rats: Effects of caffeine, amphetamine and methylphenidate. Pharmacology, Biochemistry and Behaviour, 19(4), 725–727. http://doi.org/10.1016/0091-3057(83)90352-0

Thor, D. H., & Holloway, W. R. (1986). Testosterone dependent effects of methylphenidate on social investigatory behaviour of the rat. Physiology and Behaviour, 37(6), 869–873. http://doi.org/10.1016/S0031-9384(86)80006-3

Trezza, V., Damsteegt, R., & Vanderschuren, L. J. (2009). Conditioned place preference induced by social play behavior: parametrics, extinction, reinstatement and disruption by methylphenidate. European Neuropsychopharmacology, 19(9), 659-669.

Trezza, V., & Vanderschuren, L. J. (2008). Cannabinoid and opioid modulation of social play behaviour in adolescent rats: differential behavioural mechanisms. European Neuropsychopharmacology, 18(7), 519-530.

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: pharmacological insights into social reward mechanisms. Trends in Pharmacological Sciences, 31(10), 463–469. http://doi.org/10.1016/j.tips.2010.06.008.

Trezza, V., Damsteegt, R., Manduca, A., Petrosino, S., Van Kerkhof, L. W., Pasterkamp, R. J., ... & Vanderschuren, L. J. (2012). Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. Journal of Neuroscience, 32(43), 14899-14908.

Todtenkopf, M. S., Carreiras, T., Melloni Jr, R. H., & Stellar, J. R. (2002). The dorsomedial shell of the nucleus accumbens facilitates cocaine-induced locomotor activity during the induction of behavioral sensitization. Behavioural brain research, 131(1-2), 9-16.

US Modafinil in Narcolepsy Multicenter Study Group (2000) Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology 54, 1166–1175.

Van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology, 34(2), 129-138.

Vanderschuren, L. J., Niesink, R. J., Spruijt, B. M., & Van Ree, J. M. (1995). Influence of environmental factors on social play behaviour of juvenile rats. Physiology & behaviour, 58(1), 119-123.

Vanderschuren, L. J., & Trezza, V. (2013). What the laboratory rat has taught us about social play behaviour: role in behavioural development and neural mechanisms. In The neurobiology of childhood (pp. 189-212). Springer, Berlin, Heidelberg.

Vanderschuren, L. J. M. J., Trezza, V., Griffioen-Roose, S., Schiepers, O. J. G., Van Leeuwen, N., De Vries, T. J., & Schoffelmeer, A. N. M. (2008). Methylphenidate disrupts social play behaviour in adolescent rats. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 33(12), 2946–56. http://doi.org/10.1038/npp.2008.10

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. Neuroscience and Biobehavioural Reviews, 70, 86–105. http://doi.org/10.1016/j.neubiorev.2016.07.025

van Kerkhof, L. W., Trezza, V., Mulder, T., Gao, P., Voorn, P., Vanderschuren, L. J., & Amsterdam, V. U. (2012). Cellular activation in limbic brain systems during social play behaviour in adolescent rats. The brain at play: neural substrates of social play behaviour in adolescent rats, 29.

van Kerkhof, L. W., Damsteegt, R., Trezza, V., Voorn, P., & Vanderschuren, L. J. (2013). Social play behaviour in adolescent rats is mediated by functional activity in medial prefrontal cortex and striatum. Neuropsychopharmacology, 38(10), 1899-1909.

van Kerkhof, L. W., Trezza, V., Mulder, T., Gao, P., Voorn, P., & Vanderschuren, L. J. (2014). Cellular activation in limbic brain systems during social play behaviour in rats. Brain Structure and Function, 219(4), 1181-1211.

Varlinskaya, E. I., & Spear, L. P. (2008). Social interactions in adolescent and adult Sprague–Dawley rats: impact of social deprivation and test context familiarity. Behavioural brain research, 188(2), 398-405.

Varlinskaya, E. I., Spear, L. P., & Spear, N. E. (1999). Social Behaviour and Social Motivation in Adolescent Rats. Physiology & Behaviour, 67(4), 475–482. https://doi.org/10.1016/s0031-9384(98)00285-6

Velásquez VB, Zamorano GA, Martínez-Pinto J, Bonansco C, Jara P, Torres GE, Renard GM and Sotomayor-Zárate R (2019). Programming of Dopaminergic Neurons by Early Exposure to Sex Hormones: Effects on Morphine-Induced Accumbens Dopamine Release, Reward, and Locomotor Behaviour in Male and Female Rats. Front. Pharmacol. 10:295. doi: 10.3389/fphar.2019.00295

Volkow, N. D., Fowler, J. S., Wang, G. -J, Hitzemann, R., Logan, J., Schlyer, D. J., … Wolf, A. P. (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse, 14(2), 169–177. https://doi.org/10.1002/syn.890140210

Volkow, N. D., Koob, G. F., & McLellan, A. T. (2016). Neurobiologic advances from the brain disease model of addiction. New England Journal of Medicine, 374(4), 363-371.

Volkow, N. D., & Morales, M. (2015). The brain on drugs: from reward to addiction. Cell, 162(4), 712-725.

Volkow, N. D., Wang, G, J., Fowler, J, S., Gatley, S, J., Logan, J., Ding, Y., Hitzemann, R. and Pappas, N. Dopamine Transporter Occupancies in the Human Brain Induced by Therapeutic Doses of Oral Methylphenidate (1998). American Journal of Psychiatry, 155:10, 1325-1331

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Ding, Y. S., Sedler, M., … Pappas, N. (2001). Low level of brain dopamine D2 receptors in methamphetamine abusers: Association with metabolism in the orbitofrontal cortex. American Journal of Psychiatry, 158(12), 2015–2021. https://doi.org/10.1176/appi.ajp.158.12.2015

Volkow, N. D., Fowler, J. S., Logan, J., Alexoff, D., Zhu, W., Telang, F., … Apelskog-Torres, K. (2009). Effects of modafinil on dopamine and dopamine transporters in the male human brain: Clinical implications. Jama, 301(11), 1148–1154. http://doi.org/10.1001/jama.2009.351

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. Journal of Neuroscience, 31(21), 7811-7816.

Yang, P. B., Swann, A. C., & Dafny, N. (2007). Chronic administration of methylphenidate produces neurophysiological and behavioural sensitization. Brain research, 1145, 66-80.

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'shea, D. J., ... & Stehfest, K. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature, 477(7363), 171-178.

Wang, Z. et al. (1999) Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (Microtus ochrogaster): A mechanism for pair bonding? Behavioural Neuroscience 113, 602–611

Willford, J. A., Segar, T. M., Hansen-Trench, L. S., & Barron, S. (1999). The effects of neonatal cocaine exposure on a play-rewarded spatial discrimination task in juvenile rats. Pharmacology Biochemistry and Behaviour, 62(1), 137-143.

Williams, J. M., & Galli, A. (2006). The dopamine transporter: a vigilant border control for psychostimulant action. In Neurotransmitter Transporters (pp. 215-232). Springer, Berlin, Heidelberg.

Wuo-Silva, R., Fukushiro, D. F., Borçoi, A. R., Fernandes, H. A., Procópio-Souza, R., Hollais, A. W., ... & Saito, L. P. (2011). Addictive potential of modafinil and cross-sensitization with cocaine: A pre-clinical study. Addiction biology, 16(4), 565-579.

Zahm, D. S. (2000). An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neuroscience & Biobehavioural Reviews, 24(1), 85-105.

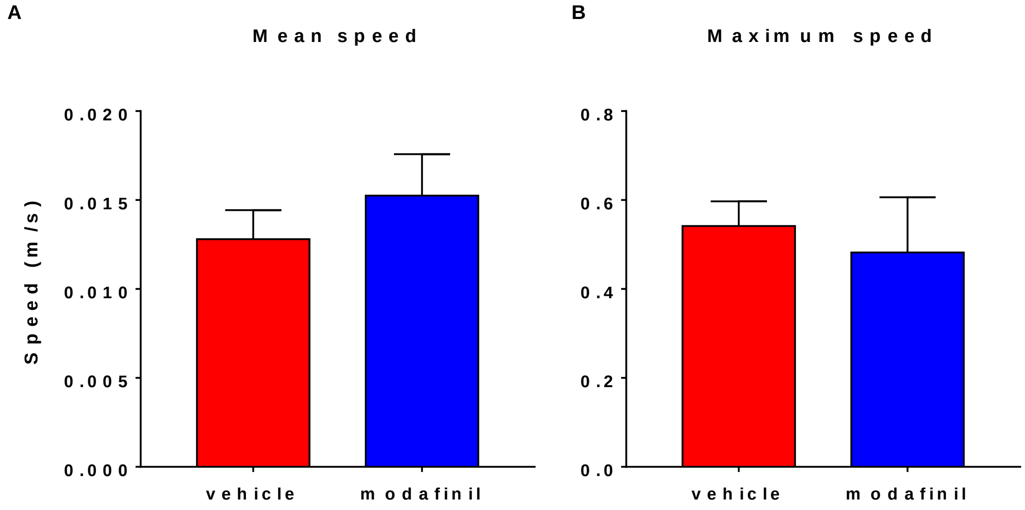
Zahniser, N. R., & Sorkin, A. (2004). Rapid regulation of the dopamine transporter: role in stimulant addiction? Neuropharmacology, 47, 80-91.

Zapata, A., Chefer, V. I., Ator, R., Shippenberg, T. S., & Rocha, B. A. (2003). Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after intravenous cocaine self-administration in mice. European Journal of Neuroscience, 17(3), 590-596.

Zell, V., Steinkellner, T., Hollon, N. G., Warlow, S. M., Souter, E., Faget, L., ... & Hnasko, T. S. (2020). VTA Glutamate Neuron Activity Drives Positive Reinforcement Absent Dopamine Co-release. Neuron.

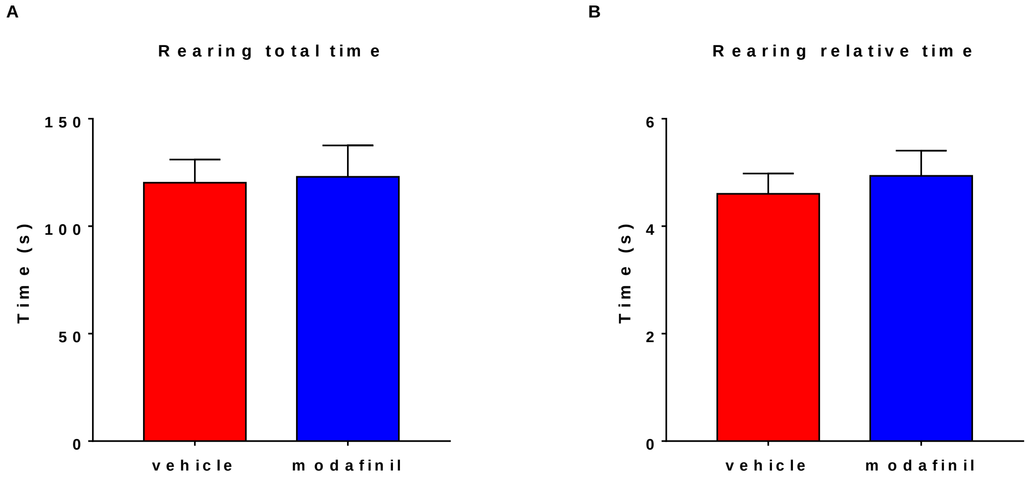
Annexes

1. Basal horizontal locomotor activity



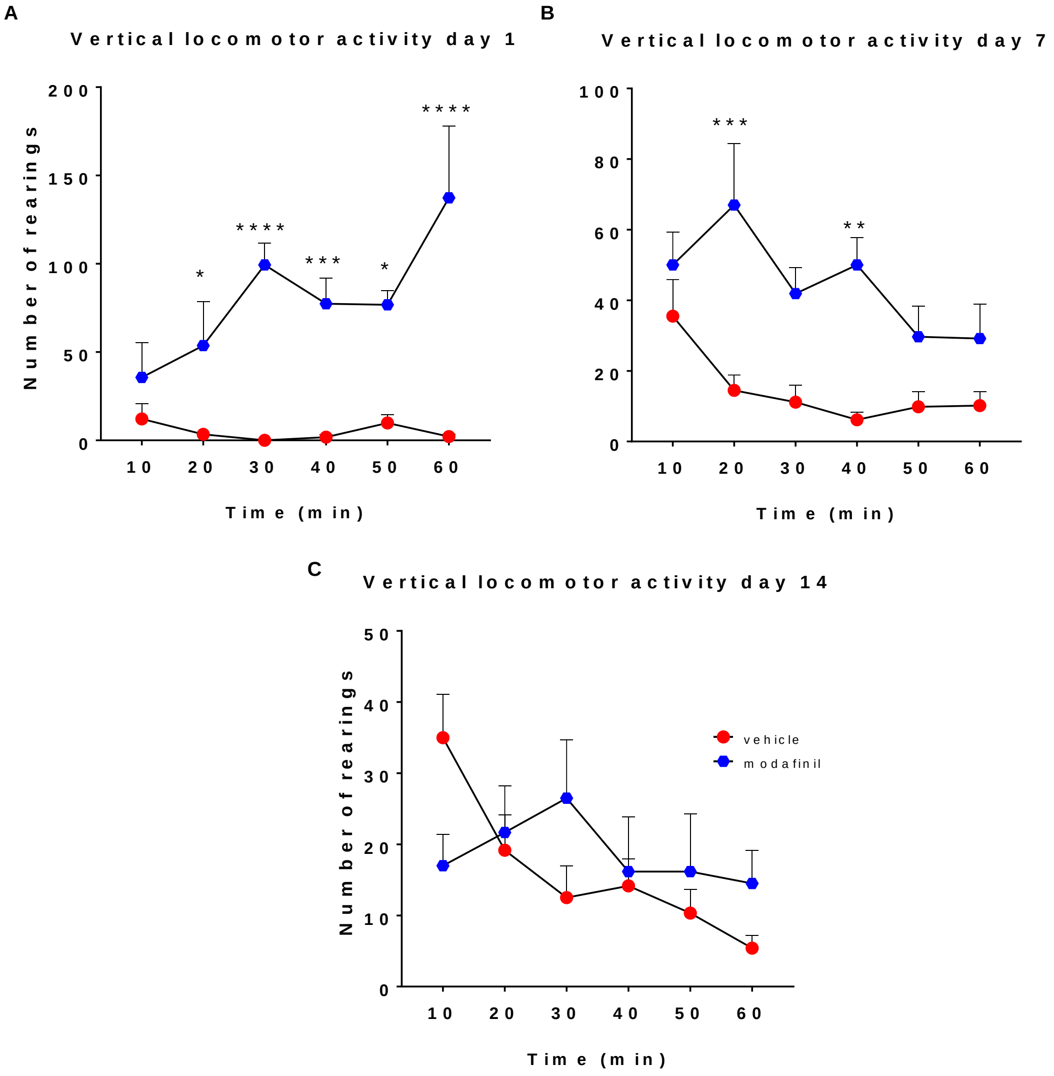
Annexe 1. Basal horizontal locomotor activity measured at PND 36 in young and healthy rats after 14 days of treatment with MOD. (A) Mean speed reached in the 10 minutes basal locomotor test; vehicle (n=10), modafinil (n=8); p= 0.346 (Unpaired Mann-Whitney w/Welch correction) **(B)** Maximum speed reached in 10 minutes basal locomotor test. vehicle (n=10) modafinil (n=8); p= 0.671 (Unpaired T test w/Welch correction). All data are represented as mean ± SEM.

2. Basal vertical locomotor activity



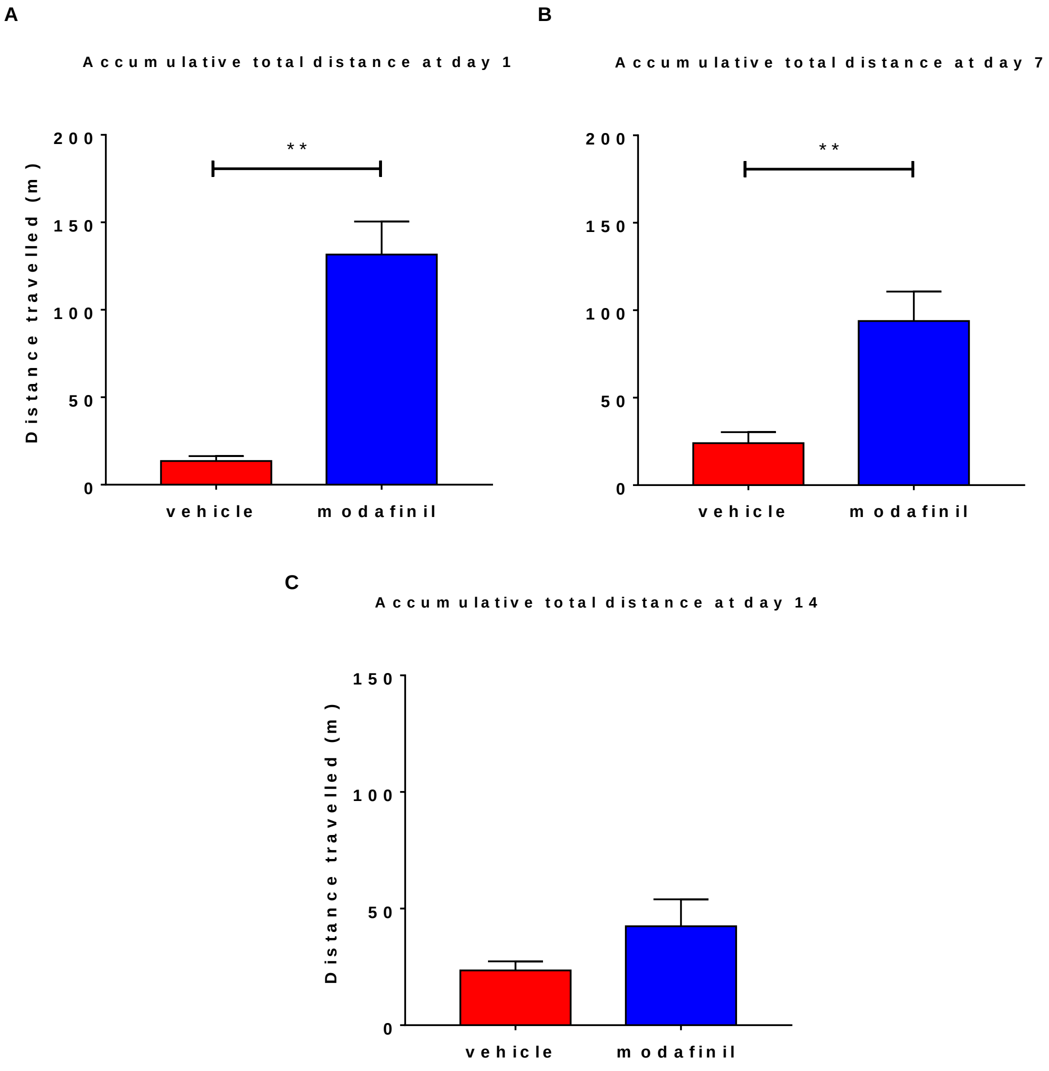
Annexe 2. Basal vertical locomotor activity measured at PND 36 in young and healthy rats after 14 days of treatment with MOD. (A) Total time rats displayed rearing in the 10 minutes basal locomotor test; vehicle (n=17) modafinil (n=13), p= 0.497 (Unpaired T test w/Welch correction). **(B)** Rearing relative time measured as the total number of rearing divided by the total time engaged in rearing; vehicle (n=17) modafinil (n=13), p=0.586. All data are represented as mean ± SEM.

3. Vertical and horizontal locomotor activity induced by modafinil



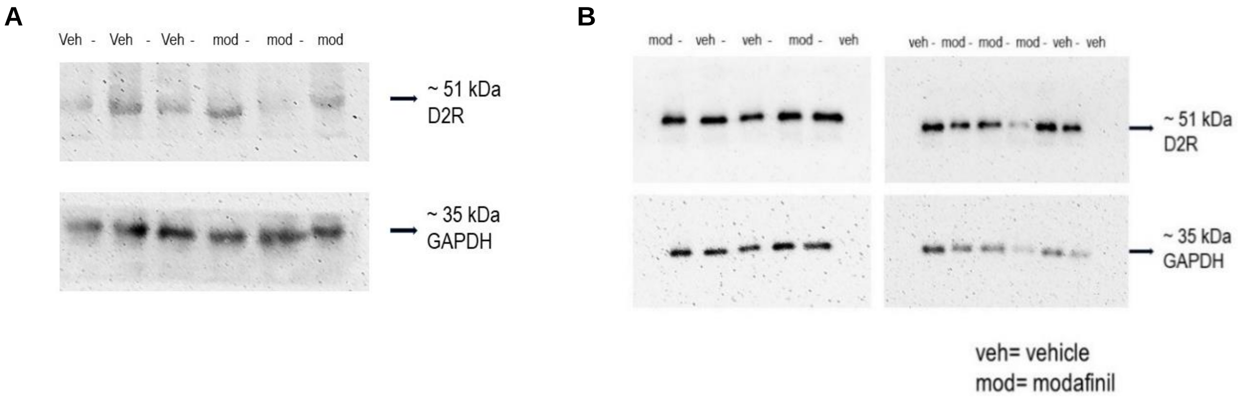
Annexe 3. Effects on vertical locomotor activity induced by MOD trough entire treatment measured in locomotor activity test of 60 minutes. (A) Number of rearing in locomotor test after first administration. Vehicle and modafinil groups (n=6); Interaction (F (5, 55) = 4.052), p=0.003; Time factor (F (5, 55) = 2.887), p=0.022; treatment factor (F (1, 55) = 90.03), p<0.0001; ***p<0.0001; ****p<0.0007; *p<0.05 (Two-Way ANOVA Sidak's multiple comparison test).

(B) Number of rearing in locomotor test after day 7 of administration; Interaction (F (5, 60) = 1.613), p=0.170; Time factor (F (5, 60) = 2.8), p=0.024; treatment factor (F (1, 60) = 37.63), p<0.0001; **p=0.003; *p<0.05 (Two-Way ANOVA Sidak's multiple comparison test). **(C)** Number of rearing in locomotor test after day 14 of administration; Interaction (F (5, 59) = 1.829), p=0.121; Time factor (F (5, 59) = 1.9), p=0.093; treatment factor (F (1, 59) = 0.59), p=0.442 (Two-Way ANOVA Sidak's multiple comparison test).



Annexe 3.1. Effects on horizontal locomotor activity induced by MOD trough entire treatment measured in locomotor activity test of 60 minutes. (A) Number of rearing in locomotor test after first administration. Vehicle and modafinil groups (n=6); Interaction (F (5, 55) = 4.052), p=0.003; Time factor (F (5, 55) = 2.887), p=0.022; treatment factor (F (1, 55) = 90.03), p<0.0001; ***p<0.0001; ****p<0.0007; *p<0.05 (Two-Way ANOVA Sidak's multiple comparison test). (B) Number of rearing in locomotor test after day 7 of administration; Interaction (F (5, 60) = 1.613), p=0.170; Time factor (F (5, 60) = 2.8), p=0.024; treatment factor (F (1, 60) = 37.63), p<0.0001; **p=0.003; *p<0.05 (Two-Way ANOVA Sidak's multiple comparison test). (C) Number of rearing in locomotor test after day 14 of administration; Interaction (F (5, 59) = 1.829), p=0.121; Time factor (F (5, 59) = 1.9), p=0.093; treatment factor (F (1, 59) = 0.59), p=0.442 (Two-Way ANOVA Sidak's multiple comparison test).

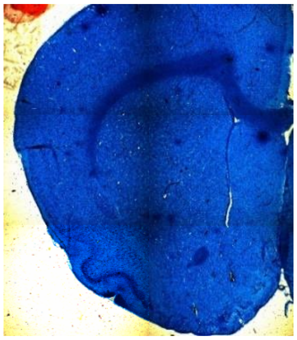
4. Western blots for D2R expression

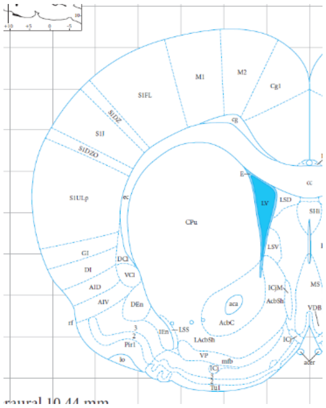


Annexe 4. Blots examples for expression of dopamine receptor type 2 in Prefrontal cortex and Nucleus accumbens after 14 days of treatment. Data is presented as arbitrary units of D_2R immunoreactivity normalized to GAPDH immunoreactivity. **(A)** D_2R expression in PFC **(B)** D_2R expression in NAc. We used Image J for images analysis.

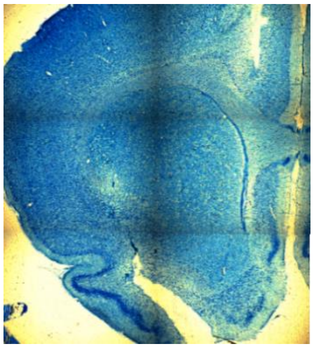
5. Probe localization NAc examples

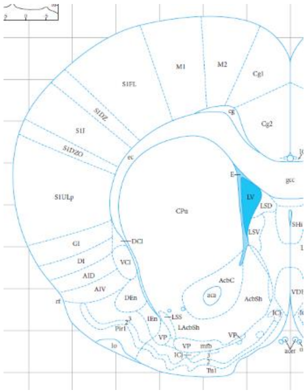
Bregma 1.44 mm





Bregma 1.68 mm





Bregma 2.28 mm