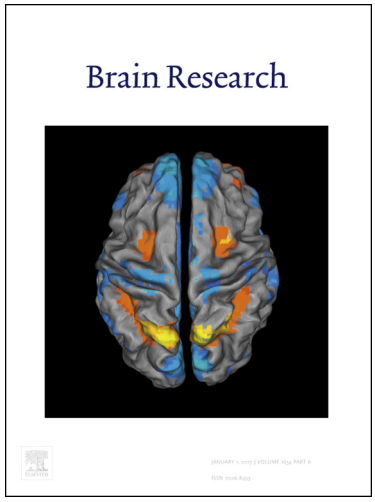

Research report

Comparing dopaminergic dynamics in the dorsolateral striatum between adolescent and adult rats: Effect of an acute dose of WIN55212-2

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Comparing dopaminergic dynamics in the dorsolateral striatum between adolescent and adult rats: Effect of an acute dose of WIN55212-2

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Abbreviations used: DLS: Dorsolateral Striatum; DA: Dopamine; C_{ext}: Extracellular concentration; NAc: Nucleus Accumbens; VTA: Ventral Tegmental Area; DS: Dorsal Striatum; CB1-R: CB1 Receptor; DAT: Dopamine Transporter; KRP: Krebs Ringer Phosphate; AA-KRP: Krebs Ringer Phosphate with 0.2 mM ascorbic acid; PFA: paraformaldehyde; CPu: Caudate putamen; HPLC: High-Performance-Liquid-Chromatography; Ed: Extraction Fraction; SEM: Standard Error of Mean

Abstract

During adolescence dopaminergic neurotransmission shows transient changes until reaching adulthood. The administration of CB1 agonists such as WIN55212-2 during adulthood increases dopamine extracellular levels. However, the effects of acute administration of cannabinoids on nigrostriatal dopamine neurotransmission during adolescence are not fully elucidated. The aim of this research is to compare dorsolateral striatum (DLS) dopamine (DA) dynamics and to study the effect of WIN55212-2 on DLS DA dynamics during adolescence and adulthood. No-net flux microdialysis experiments were carried out in adolescent (post-natal day 35-40) and young-adult (post-natal day 70-75) urethane-anesthetized rats. Basal DA dialysate, DA extraction fraction (Ed) and extracellular concentration of DA (C_{ext}) in DLS were assessed after an acute injection of WIN55212-2 (1.2 mg/kg) or vehicle. An increased basal DA dialysate and DA Ed were observed during adolescence compared to adulthood. Moreover, WIN55212-2 increases DLS DA C_{ext} rising basal DA dialysate in adulthood and decreasing DA Ed in adolescence. Our results suggest that an age-dependent mechanism underlies the effect of WIN 55212-2 on DA balance between release and uptake in DLS.

Keywords: Dopamine, WIN55212-2, Adolescence, no-net flux microdialysis; Dorsolateral Striatum

1. Introduction

Adolescence is a period characterized by gradual physiological and behavioral transitions from childhood to adulthood (Schneider, 2008; Sisk and Foster, 2004; Spear, 2000). During adolescence, neuronal connections overproduced in early life are amply pruned to respond to physiological changes and to adapt to environmental factors (Burke and Miczek, 2014; Casey et al., 2008; De Bellis et al., 2001; Gogtay et al., 2004; Powell, 2006; Schneider, 2008; Teicher et al., 1995). It has been observed that adolescents are more sensitive to rewarding stimuli such as social peer interactions, novelty seeking, and palatable food compared to adults (Adriani et al., 1998; Doremus-Fitzwater and Spear, 2016; Douglas et al., 2004; Friemel et al., 2010). In addition, adolescence has been associated with a transitory changes in the dopaminergic transmission. It has been observed that dopamine (DA) basal extracellular levels in Nucleus Accumbens (NAc) and the firing rate of dopamine neurons from ventral tegmental area (VTA) show an age-dependent U-shaped trajectory. Both DA concentration and firing rate of dopamine neurons present a maximum peak during middle adolescence, followed by a decrease during adulthood (Badanich et al., 2006; McCutcheon and Marinelli, 2009). Similarly, age-dependent differences in nigrostriatal dopaminergic transmission have been observed. A transitory increase in the expression of DA receptors, accompanied by a higher turnover of DA, has been observed in adolescent dorsal striatum (DS) compared to the adult (Naneix et al., 2012). In addition, Nakano and Mizumo (1996) observed a higher DA basal dialysate in adolescent DS compared to adult (Nakano and Mizuno, 1996).

Epidemiological evidence shows that the annual prevalence of cannabis use by the adolescent population is higher compared to the general population of Europe and Americas (UNODC, 2018). Compared to adulthood, cannabis use during adolescence is associated with higher risk of cannabis use dependence (Volkow et al., 2014). This evidence suggests that the neurobiological substrates underlying drug abuse are more susceptible to cannabinoid effects in adolescence compared to adulthood. One of the main neuronal pathways associated with the intake and development of drug addiction is the dopaminergic mesolimbic pathway (Everitt and Robbins, 2016; Robinson et al., 2016). Moreover, recent evidence shows that DA release in DLS can encode drugs abuse related

information. Using self-administration paradigm and fast scan voltammetry recording in freely-moving rat has been observed that habitual cocaine intake is accompanied by a progressive increase in DLS DA release (Willuhn et al., 2012). In addition, repeated exposure to cocaine is associated with increase in DLS neuronal activity induced by a cue in a go/no-go task, suggesting changes in neural process in the striatum after the repeated exposure to the drug (Takahashi et al., 2007). While the effects of cannabinoids on DA transmission have been extensively studied in mesolimbic and nigrostriatal pathways in adult rats, less explored are the effects of cannabinoids on the adolescent nigrostriatal dopaminergic pathway (Covey et al., 2017; French et al., 1997; Higuera-Matas et al., 2010; Pistis et al., 2004; Schneider, 2008).

Cannabinoids modulate different physiological responses in mammals, such as appetite, pain-perception, mood, memory and motivation (Mechoulam and Parker, 2012; Pertwee, 2014). The CB1 receptors (CB1-R) are mainly localized in the central nervous system and their activation is key to modulate the function of different neurotransmitters such as GABA, glutamate, acetylcholine, DA and serotonin (Castillo et al., 2012; Heifets and Castillo, 2009; Kano et al., 2009). In the case of adult dopaminergic transmission, it has been observed that an acute systemic administration of CB1 agonists such as WIN55212-2 produces an increase in dopamine extracellular levels in the NAc (Tanda et al., 1997) and dorsolateral striatum (DLS) (Polissidis et al., 2014, 2013). Also, the acute administration of WIN55212-2 decreases the activity of the DA transporter (DAT) in the DS of adult rodents (Pandolfo et al., 2011; Price et al., 2007). However, the effects of cannabinoids on DA release and DA uptake in the DS during adolescence remains to be addressed. Interestingly, it has been observed that the expression of CB1-R presents transitory changes through development (Rodríguez de Fonseca et al., 1993; Van Waes et al., 2012; Verdurand et al., 2011). The binding of CB1-R increases in parallel with the development until reaching its maximum in adulthood in several areas such as midbrain, hippocampus and striatum (Verdurand et al., 2011). Then, it is possible to suggest that CB1-R agonists could have different effects depending on the current development stage. Specifically, there is no evidence regarding the effect of acute administration of CB1-R agonists on dopaminergic transmission in the nigrostriatal pathway of adolescent rats. The main aim of this research is to compare DLS DA extracellular concentration (C_{ext}) during adolescence and adulthood,

and to study the age-dependent effect of WIN55212-2 in DLS DA release and DA extraction fraction (Ed), an indirect measure of DA uptake (Chefer et al., 2006; Smith and Justice, 1994). Using no-net flux microdialysis, our results show an increased DA release and DA Ed in the DLS of urethane-anesthetized adolescent rats. Moreover, a decrease in DA Ed in DLS of urethane-anesthetized adolescent rats is observed after an acute injection of WIN55212-2, an outcome that was not observed in young-adult rats.

2. Results

2.1. Age-dependent differences in dopaminergic neurotransmission in DLS

2.1.1. Basal and stimulated DA dialysate:

Conventional microdialysis experiments in adolescent and adult rats were carried out to compare age-dependent differences in basal and stimulated DA dialysate in DLS (see Fig 1).

Basal DA dialysate was higher in adolescent rats compared to adult rats (Fig 2 Adolescent group: 0.89 ± 0.18 nM; n= 6; vs Adult group: 0.52 ± 0.04 nM; n= 5; $p< 0.05$ according to unpaired t-test). The perfusion of 40 mM K^+-KRP increased extracellular concentration of DA in both groups (Fig 2 Basal Adolescent group: 0.89 ± 0.18 nM; n= 6; vs 40 mM K^+-KRP adolescent group: 37.16 ± 7.42 nM; n= 6; $p< 0.01$ according to paired t-test; Basal Adult group: 0.52 ± 0.04 nM; n= 5; vs 40 mM K^+-KRP adult group: 22.37 ± 8.95 nM; n= 5; $p< 0.05$ according to paired t-test). No significant difference in high potassium-stimulated DA dialysate was observed between adolescent and adult rats (Fig 2 Adolescent group: 37.16 ± 7.42 nM; n= 6; vs Adult group: 22.37 ± 8.95 nM; n= 5; $p> 0.05$ according to unpaired t-test).

2.1.2. DA extraction fraction and extracellular concentration:

No-net flux microdialysis experiments in adolescent and adult rats were carried out to compare age-dependent differences in DA C_{ext} and Ed in DLS.

As previously shown (fig 2), basal DA dialysate in adolescent rats was significantly higher than in adult rats (Fig 3a adolescent group: 0.69 ± 0.05 nM; n= 6; vs adult group: 0.53 ± 0.04 nM; n= 5; $p< 0.05$ according to unpaired t-test). Interestingly, adolescent rats showed a higher DA Ed compared to adult rats (Fig 3b adolescent group: 0.56 ± 0.06 nM; n= 6; vs adult group: 0.41 ± 0.06 nM; n= 5; $p< 0.05$ according to unpaired t-test), suggesting a transitory increase in DAT activity during adolescence. Consequently, DA C_{ext} remained constant in both adolescence and adulthood (Fig 3c adolescent group: 1.23 ± 0.14 nM; n= 6; vs adult group: 1.36 ± 0.14 nM; n= 5; $p> 0.05$ according to unpaired t-test). Neither the perfusion of ascorbic acid nor systemic acute injection of vehicle modified basal DA dialysate (Figure supplementary 1).

2.2. Effects of acute exposure of WIN55212-2 in DLS dopamine dynamics

No-net flux microdialysis were carried out in adolescent and adult rats to assess age-dependent consequences of acute systemic injection of WIN55212-2 in DLS DA C_{ext} and Ed.

2.2.1. Adolescent Rats:

The acute intraperitoneal (ip) injection of WIN55212-2 did not modify basal DA dialysate in DLS compared to vehicle group (Fig 4a vehicle group: 0.69 ± 0.05 nM; n= 6; vs WIN group: 0.67 ± 0.04 nM; n= 5; $p > 0.05$ according to unpaired t-test). However, the acute injection of WIN was accompanied by a significant decrease of DA Ed compared to vehicle group (Fig 4b vehicle group: 0.59 ± 0.05; n= 6; vs WIN group: 0.39 ± 0.05; n= 5; $p < 0.05$ according to unpaired t-test). Consequently, an acute injection of WIN55212-2 significantly increased the C_{ext} compared to vehicle injection (Fig 4c vehicle group: 1.23 ± 0.14 nM; n= 6; vs WIN group: 1.91 ± 0.37 nM; n= 5; $p < 0.05$ according to unpaired t-test).

2.2.2. Adult Rats:

Contrary to what was observed in adolescent rats, an acute ip injection of WIN increased basal DA dialysate in DLS compared to vehicle group (Fig 5a vehicle group: 0.53 ± 0.04 nM; n= 5; vs WIN group: 0.80 ± 0.06 nM; n= 5; $p < 0.05$ according to unpaired t-test). The acute injection of WIN55212-2 did not modify DA Ed compared to vehicle injection (Fig 5b vehicle group: 0.412 ± 0.056; n= 5; vs WIN group: 0.418 ± 0.065; n= 5; $p > 0.05$ according to unpaired t-test). Consequently, the acute injection of WIN55212-2 was accompanied by a significant increase of C_{ext} compared to an acute injection of vehicle (Fig 5c vehicle group: 1.36 ± 0.14 nM; n= 5; vs WIN group: 2.05 ± 0.26 nM; n= 5; $p < 0.05$ according to unpaired t-test).

3. Discussion

Transitory changes in DA neurotransmission have been observed between adolescence and adulthood (Badanich et al., 2006; Matthews et al., 2013; McCutcheon and Marinelli, 2009; Nakano and Mizuno, 1996). Accordingly, our results show age-dependent differences in homeostatic control of DLS DA extracellular levels. Conventional microdialysis experiments show a higher DLS basal DA dialysate in adolescent rats compared to adult rats, without differences in high potassium-stimulated DA dialysate. Moreover, no-net flux microdialysis experiments in DLS indicate that DA Ed during adolescence is higher than in adulthood. Consequently, age-dependent differences in DLS DA C_{ext} were not observed. Interestingly, the results show an age-dependent difference after an acute administration of WIN55212-2. A significant increase in DLS basal DA dialysate after an acute injection of WIN55212-2 is only observed in adult rats. Adolescence is accompanied by a significant decrease in DLS DA Ed after an acute injection of WIN55212-2, which is not observed in adult rats. Consequently, the increase of DLS DA C_{ext} induced by WIN55212-2 depends on mechanisms that change with age: a decrease in DA Ed in adolescent rats and an increase in DA release in adult rats. In addition, our results suggest that the DLS DAT activity shows an age-dependent vulnerability to the effects of WIN55212-2.

Contributing to evidence showing transitory changes in dopaminergic neurotransmission associated with the transition from adolescence to adulthood (McCutcheon et al., 2012; McCutcheon and Marinelli, 2009; Naneix et al., 2012), a higher basal DLS DA dialysate is observed in adolescent compared to adult rats (Fig. 2a). The high potassium perfusion increases DA dialysate in a similar magnitude in adolescent and adult rats (Fig 2b), suggesting no age-dependent differences in vesicular DA storage (Castañeda et al., 1988; Kantor et al., 1999). Taken together, it is possible to suggest that the increased basal DA release observed in adolescent rats is associated to an increased electrical activity of DA neurons from SNc (Tepper et al., 1990).

Using various experimental approaches, it has been demonstrated that there is a more efficient uptake of DA during adolescence (Stamford, 1989; Volz et al., 2009). However, the evidence about DAT expression in the striatal region is not conclusive. Binding essays (Tarazi et al., 1998) have demonstrated that the transition from adolescence to adulthood is

accompanied by a systematic increase in DAT expression in DLS. On the other hand, western blot experiments have shown contradictory results in DAT expression in DS. While Matthews et al. (2013) observed a lower expression of DAT (Matthews et al., 2013), Volz et al. (2009) showed a higher DAT expression in adolescent compared to adult rats (Volz et al., 2009). In this sense, our results using no-net flux microdialysis show an increased DA Ed during adolescence compared to adulthood. This data is in agreement with results showed by Volz et al. (2009), suggesting that higher activity of DAT in adolescent rats is associated with higher DAT expression in adolescent DS (Volz et al., 2009).

The DA C_{ext} is an estimated concentration of DA in the synaptic cleft, which depends on basal DA release and uptake, associated with DA Ed (Chefer et al., 2006; Smith and Justice, 1994). As mentioned before, a higher basal DA dialysate in DLS is observed in adolescent rats compared to adult rats. Moreover, a higher DA Ed in DLS is observed during adolescence. Consequently, no significant differences were observed in C_{ext} of DA in DLS of adolescent rats compared to adult rats. Thus, the higher dopaminergic nigrostriatal release observed during adolescence is tuned by an increase in DAT activity, preserving DA C_{ext} at homeostatic levels similar to adulthood. Interestingly, a transitory increase of DA C_{ext} during adolescence was observed in NAc, without significant changes in DA Ed (Badanich et al., 2006). Thus, the lower DA C_{ext} observed during adulthood in NAc would depend on a late decrease on DA release.

The significant increase in basal dialysate of DA in DLS of adult rats induced by CB1 agonist is in line with previous pre-clinical evidence. An increase in basal DA release in DS (Polissidis et al., 2014, 2013) associated with an increase of neuronal activity of DA neuron from substantia nigra pars compacta (French et al., 1997) has been observed after an acute administration of WIN55212-2 in adult rats. The increase in neuronal activity of DA neurons from SNc induced by WIN55212-2 is mediated by a decrease in gabaergic transmission (Yanovsky et al., 2003). In contrast, an acute administration of WIN55212-2 is not accompanied by significant changes in basal DA dialysate in the DLS of adolescent rats. To our knowledge, this is the first evidence related to the acute effects of WIN55212-2 in DA release in the DLS of adolescent rats. A lower expression of CB1-R in adolescent

midbrain (Verdurand et al., 2011) could explain the lack of effect of WIN55212-2 exposure in basal DA dialysate.

The no-net flux microdialysis experiments show age-dependent effects of cannabinoids on DA uptake. The acute exposure to WIN55212-2 is accompanied by a significant decrease in DA Ed in adolescent rats, a result that was not observed in adult rats. While there is no evidence related to the effects of WIN55212-2 on DA uptake in adolescent rats, an inhibitory effect of WIN55212-2 on DA uptake has been observed in adult rats. Interestingly, the inhibitory effect of WIN55212-2 on DA uptake has been shown independent of CB1-R activation (Price et al., 2007). In fact, it has been proposed that WIN55212-2 interacts directly with the DAT protein (Pandolfo et al., 2011; Price et al., 2007; Steffens and Feuerstein, 2004). Whether a similar mechanism than the one observed in adult rats underlies an inhibitory effect of WIN55212-2 in DA uptake in adolescent rats remains to be addressed. The age-dependent effects observed under our experimental conditions could be explained by lower glycosylation of DAT in adolescent rats. (Patel et al., 1994). It has been described that the higher glycosylation of DAT observed in adult rats attenuates the inhibitory effect of drugs on DA uptake (Li et al., 2004). It is tempting to suggest that the lower dose used in our experiment could explain the lack of inhibitory effects of WIN55212-2 in DA Ed observed in adult rats. Further experiments using higher doses of WIN55212-2 are necessary to address this proposal.

A significant increase in DLS DA C_{ext} is observed after an acute administration of WIN55212-2 in adult and adolescent rats. Interestingly, age-dependent mechanisms underlie the increase in DA C_{ext}. An increase in DA release contributes to the increase in DA C_{ext} induced by WIN55212-2 during adulthood, meanwhile a decrease in DA uptake is associated with the increase in DA C_{ext} in DLS of adolescent rats.

In summary, our results show that adolescence is accompanied by a higher basal DA release and also a higher DA Ed in the DLS compared to adulthood. The acute exposure to WIN55212-2 increases DA release in adulthood and decreases DA Ed in adolescence, resulting in an increase in DA C_{ext} in both groups. These findings suggest that an increased DAT activity is opposed to a higher DA release in adolescent rats, preserving the DLS DA C_{ext} at homeostatic levels similar to those observed in adult rats. Moreover, an age-

dependent mechanism underlies the effect of WIN 55212-2 on DA C_{ext} in DLS suggesting a high vulnerability to inhibitory effects of cannabinoids on DAT activity during adolescence.

4. Experimental Procedure

The study was not pre-registered. All procedures were in strict accordance with the guidelines published in the "NIH Guide for the Care and Use of Laboratory Animals" (8°Edition) and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. Also, the protocols were approved by the local bioethics committees, verifying that it complies with the basic principles set forth in Chilean Law 20.380 on Animal Protection 2009 (ID project: 160816013). The rats were identified with markings on the tail and numbered accordingly. Using pseudo randomization (random number generator) the adult and adolescent animals were divided into two experimental groups (vehicle exposure and WIN55212-2 exposure). All control and experimental protocols were performed in parallel. A priori statistical power, effect, and sample size calculations were performed by [G*power (Faul et al., 2007), α=0.05; power >0.8] based on preliminary results of this study. The results obtained suggested a sample size of n=5/group for the experiment. Exact numbers for all experiments are provided in the figure legends and results section.

4.1. Animals

Adult (post-natal day from 72 to 78) and adolescent (post-natal day from 35 to 40) male Sprague-Dawley rats were grown from the Animal Care Faculty of the Biological Sciences, Pontificia Universidad Católica de Chile (Charles River; Wilmington, MA, USA; RRID: RGD_728193), under the supervision of a veterinarian. One week before microdialysis experiments, rats were maintained in the Animal Care Faculty of the Department of Pharmacy, Pontificia Universidad Católica de Chile, following the instruction of a protocol approved by the veterinarian. Rats with similar age were housed in groups of three per cage and kept at room temperature between 22–24°C on a 12 h light/dark cycle (lights on at 7:00 EST) with access to food and water *ad libitum*. Rats were handled for one week before starting the experiments. A total of 11 adolescent rats and 10 adult rats were divided into

two groups; 6 adolescent rats and 5 adult rats for vehicle treatment, and 5 adolescent rats and 5 adult rats for WIN55212-2 treatment. No animals died during the experiments.

4.2. Reagents

The $CB_{1/2}$ agonist, WIN55212-2 mesylate, was purchased from Medchemexpress (Monmouth Junction, NJ, USA). WIN55212-2 was emulsified in 2% Tween 80, then diluted in saline solution (NaCl 0.9%) at concentration of 1.2 mg/mL and sonicated for 5 min. Urethane and Tween 80 were obtained from Sigma Aldrich (St. Louis, MO, USA). The compounds of Krebs-Ringer phosphate buffer (NaCl, KCl, $CaCl_2$, NaH_2PO_4, Na_2HPO_4 and ascorbic acid) and the compounds of the mobile phase (octane-1-sulfonic acid sodium salt, acetonitrile and EDTA) were purchased from Merck (Darmstadt, Germany).

4.3. Microdialysis experiments

Adult and adolescent rats were anesthetized with urethane 1.5 g/kg ip and placed in a stereotaxic apparatus. Urethane was chosen due to the extended half-life (Gumbleton and Benet, 1991). In addition, urethane does not modify basal and stimulated DA dialysate (Howard and Feigenbaum, 1997; Tepper et al., 1991). The skull of the rat was exposed, and a hole was drilled targeting the DLS. A concentric microdialysis probe (CMA 11 Microdialysis, Holliston, MA) was lowered into the DLS using the following coordinates: for adult rats +1.2 AP, -3.6 ML and -4.8 DV relative to bregma and for adolescent rats +1.0 AP, -3.6 ML and -4.6 DV relative to bregma (Paxinos and Whaton, 2009). Body temperature was maintained by a thermostatically controlled electric heating pad. The microdialysis probe was perfused for 40 minutes to allow equilibration with Krebs–Ringer Phosphate (KRP) buffer at a rate of 2 µL/min using a Harvard infusion pump (Harvard Apparatus, Holliston, MA). The composition of the KRP was 120 mM NaCl, 2.4 mM KCl, 1.2 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 1.4 mM Na_2HPO_4, and 0.2 mM of ascorbic acid (pH 7.4). Perfusion samples were collected every 5 min in 2 µL of perchloric acid (0.2 N) and maintained on ice (4°C) until DA determination. To determine the DA release induced by depolarization a conventional microdialysis was carried out, while, a no-net flux microdialysis was carried out to determine the effect of acute exposure to WIN55212-2 on dopaminergic presynaptic dynamics. Fifteen minutes after conventional microdialysis, adult

and adolescent rats were exposed to an acute dose ip of 1.2 mg/kg of WIN55212-2 or vehicle solution (2% tween 80, dissolved in saline solution). Rats were separated in 4 experimental groups: Adult Vehicle (n= 5), Adult WIN (n= 5), Adolescent Vehicle (n= 6) and Adolescent WIN (n= 5). A random number generator method was applied for pharmacological treatment. In the conventional microdialysis, after 40 minutes of stabilization, three consecutive samples were collected every 5 min for determination of an average DA basal level. DA-evoked release was stimulated during 5 min using a 40 mM K^+-KRP. Subsequently, N-KRP with 0.2 mM acid ascorbic (AA-KRP) was perfused during fifteen minutes prior to an acute injection of WIN55212-2 or vehicle. No-net flux microdialysis was carried out twenty minutes after the injection according to Azocar et al. (2018) (Azocar et al., 2018). The probe was randomly perfused with five different concentrations of dopamine: 0.0, 5.0, 10.0, 20.0 and 40.0 nM in AA-KRP to determine DA C_{ext} and Ed, an indirect measure of DA uptake (Smith and Justice, 1994). A random number generator method was applied for perfusion of the different concentrations of DA. After a stabilization period of 20 minutes, three consecutive samples were collected every 5 minutes for each concentration of DA. The end of the microdialysis procedure was considered the endpoint of the experiments.

4.4. Histology

After microdialysis experiments, rats were decapitated under deep anesthesia (urethane 1.5 g/kg ip), and brains were extracted and cleaned with a saline solution (NaCl 0.9%). Brains were stored in 4% paraformaldehyde (PFA). At least two days before slicing, the brains were cryoprotected using a solution of sucrose 30%. To assess the location of probes, brains were frozen and coronally sliced in sections of 50 mm. Slices were stained with cresyl violet and the probe placement, observed in a light microscope, was localized using the atlas of Paxinos & Watson for rats (2009)(Paxinos and Whaton, 2009). Only data coming from correct probe placements were considered for further analysis (figure 1).

4.5. Analysis of dialysate samples

Quantification of DA was carried out as described previously (Escobar et al., 2012). Twelve μL of the collected samples were injected in a Rheodyne injector valve to a High-

Performance-Liquid-Chromatography (HPLC) system (BASi America, West Lafayette, IN, USA) with the following configuration: a pump (Jasco LC-Net II/ADC), a UNIJET ™ LC column (part number: MF-8954, BASi) and an amperometric detector (LC4C, BASi America). The mobile phase contained 100 mM NaH_2PO_4, 1.0 mM EDTA, 1.0 mM octane-1-sulfonic acid sodium salt, and 5% acetonitrile (pH 3.0), and it was pumped at a flow rate of 700 µl/min. The potential of the amperometric detector was set at 650 mV. Under these experimental conditions, the retention time for DA was 6 min. The technician was blinded to the experimental group during the measurement of the samples in the HPLC.

4.6. Data Analysis

No-net flux microdialysis data were analyzed as described by Chefer et al. 2005, 2006 (Chefer et al., 2006, 2005). The amount of DA gained or lost from the probe during the no-net flux microdialysis (C_{in}-C_{out}) was calculated for each animal at each DA perfusion concentration (C_{in}: 0.0, 5.0, 10.0, 20.0 and 40.0 nM). The net change in DA (C_{in}–C_{out}) was plotted against C_{in} and subjected to linear regression. The point when no DA was gained or lost (C_{in}-C_{out}=0) represents an estimate of DA extracellular concentration (C_{ext}). The slope of the linear regression line represents the Ed, an indirect measure of DA transporter (DAT) activity. Basal dialysate DA levels were calculated for each animal as the average of the three basal samples (C_{in}=0). All statistical analyses were performed using Prism 5.0 GraphPad Software. Data points outside the 95% confidence interval are treated as outliers and could be excluded from the data analysis. Neither point was excluded from the data analysis. Normality was checked with the Kolmogorov–Smirnov test. Resultant data was analyzed by unpaired t-test when appropriate. All data was reported as mean ± SEM.

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The authors have no conflict of interest.

Figures Captions

Figure 1: Anatomical placements of microdialysis probe. The microdialysis probe was implanted in the dorsolateral striatum (DLS) using the coordinates: (a) for adolescent 1.0 mm anterior to bregma, 3.6 mm lateral, and 4.6 mm below dura and (b) for adult 1.2 mm anterior to bregma, 3.6 mm lateral, and 4.8 mm below dura according to the Atlas of Paxinos and Watson (2009). Left. Photo of representative microdialysis probe placement in the DLS of (a) adolescent rats and (b) adult rats (upper panel), and map showing the placement of the microdialysis probe (black line) of the representative photo (lower panel). Right. Representative anatomical placements of tips of the microdialysis probe (black dots) of (a) adolescent and (b) adult rats. Diagrams were adapted from Paxinos and Watson (2009). CPu: Caudate putamen

Figure 2: Age-dependent differences in DLS basal and stimulated DA dialysate. In *vivo* conventional microdialysis in anesthetized rats was carried out in adolescent (n= 6) and adult (n= 5). Data correspond to mean ± SEM. (a) Basal dopamine (DA) dialysate levels. *$p<0.05$ compared with adolescent group according to paired t-test. (b) stimulated DA dialysate. #$p<0.05$ ## $p<0.01$ compared with respect basal group according to paired t-test.

Figure 3: Age-dependent differences in DLS dopaminergic dynamics. In *vivo* microdialysis no-net flux in anesthetized rats was carried out in adolescent (n= 6) and adult (n= 5). Data correspond to mean ± SEM. (a) Basal dopamine (DA) dialysate levels. *$p<0.05$ compared with adolescent group according to unpaired t-test. (b) Extraction fraction. *$p<0.05$ compared with adolescent group according to unpaired t-test. (c) Extracellular dopamine concentration (DA C_{ext}).

Figure 4: Effect of an acute injection of WIN55212-2 in DLS dopaminergic dynamics in adolescence. In vivo no-net flux microdialysis in anesthetized animals were carried out in vehicle (n= 6) and WIN55212-2 exposed rats (n= 5). Data correspond to mean ± SEM. (a) Basal dopamine (DA) dialysate levels. (b) Extraction fraction. *$p<0.05$ compared with vehicle group according to unpaired t-test. (c) DA C_{ext}. *$p<0.05$ compared with vehicle group according to unpaired t-test.

Figure 5: Effect of an acute injection of WIN55212-2 in DLS dopaminergic dynamics in adulthood. In vivo no-net flux microdialysis in adult anesthetized animals were carried out in vehicle (n=5 rats) and WIN55212-2 exposed rats (n=5 rats). Data correspond to mean ± SEM. (a) Basal dopamine (DA) dialysate levels. *p<0.05 compared with vehicle group according to unpaired t-test. (b) Extraction fraction. (c) DA C_{ext}. *p<0.05 compared with vehicle group according to unpaired t-test.

Figure Supplementary 1: DLS basal DA dialysate using KRP and after an acute injection of vehicle using AA-KRP. In *vivo* conventional microdialysis in anesthetized rats weres carried out in adolescent (n= 6) and adult (n= 5). Data correspond to mean ± SEM. n.s. p > 0.05 according to paired t-test.

References

Adriani, W., Chiarotti, F., Laviola, G., 1998. Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. Behav. Neurosci. 112, 1152–66.

Azocar, V., Sepúlveda, G., Ruiz, C., Aguilera, C., Andés, E., Fuentealba, J., 2018. The blocking of kappa-opioid receptor reverses the changes in dorsolateral striatum dopamine dynamics during the amphetamine sensitization. J. Neurochem. 0–3. https://doi.org/10.1111/jnc.14612

Badanich, K.A., Adler, K.J., Kirstein, C.L., 2006. Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi. Eur. J. Pharmacol. 550, 95–106. https://doi.org/10.1016/j.ejphar.2006.08.034

Burke, A.R., Miczek, K.A., 2014. Stress in adolescence and drugs of abuse in rodent models: Role of dopamine, CRF, and HPA axis. Psychopharmacology (Berl). 231, 1557–1580. https://doi.org/10.1007/s00213-013-3369-1

Casey, B.J., Getz, S., Galvan, A., 2008. The adolescent brain. Dev. Rev. 28, 62–77. https://doi.org/10.1016/j.dr.2007.08.003

Castañeda, E., Becker, J.B., Robinson, T.E., 1988. The long-term effects of repeated amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro. Life Sci. 42, 2447–2456. https://doi.org/10.1016/0024-3205(88)90343-8

Castillo, P.E., Younts, T.J., Chávez, A.E., Hashimotodani, Y., 2012. Endocannabinoid Signaling and Synaptic Function. Neuron 76, 70–81. https://doi.org/10.1016/j.neuron.2012.09.020

Chefer, V.I., Czyzyk, T., Bolan, E.A., Moron, J., Pintar, J.E., Shippenberg, T.S., 2005. Endogenous-Opioid Receptor Systems Regulate Mesoaccumbal Dopamine Dynamics and Vulnerability to Cocaine. https://doi.org/10.1523/JNEUROSCI.0854-05.2005

Chefer, V.I., Zapata, A., Shippenberg, T.S., Bungay, P.M., 2006. Quantitative no-net-flux microdialysis permits detection of increases and decreases in dopamine uptake in mouse nucleus accumbens. J. Neurosci. Methods 155, 187–193. https://doi.org/10.1016/j.jneumeth.2005.12.018

Covey, D.P., Mateo, Y., Sulzer, D., Cheer, J.F., Lovinger, D.M., 2017. Endocannabinoid modulation of dopamine neurotransmission. Neuropharmacology 124, 52–61. https://doi.org/10.1016/j.neuropharm.2017.04.033

De Bellis, M.D., Keshavan, M.S., Beers, S.R., Hall, J., Frustaci, K., Masalehdan, A., Noll, J., Boring, A.M., 2001. Sex differences in brain maturation during childhood and adolescence. Cereb. Cortex 11, 552–7.

Doremus-Fitzwater, T.L., Spear, L.P., 2016. Reward-centricity and attenuated aversions: An adolescent phenotype emerging from studies in laboratory animals. Neurosci. Biobehav. Rev. 70, 121–134. https://doi.org/10.1016/j.neubiorev.2016.08.015

Douglas, L.A., Varlinskaya, E.I., Spear, L.P., 2004. Rewarding properties of social interactions in adolescent and adult male and female rats: impact of social versus isolate housing of subjects and partners. Dev. Psychobiol. 45, 153–62. https://doi.org/10.1002/dev.20025

Escobar, A. del P., Cornejo, F.A., Andrés, E., Fuentealba, J.A., 2012. Repeated treatment with the kappa opioid receptor agonist U69593 reverses enhanced K + induced dopamine release in the nucleus accumbens, but not the expression of locomotor sensitization in amphetamine-sensitized rats. Neurochem. Int. 60, 344–349. https://doi.org/10.1016/j.neuint.2012.01.014

Everitt, B.J., Robbins, T.W., 2016. Drug Addiction: Updating Actions to Habits to Compulsions Ten Years On. Annu. Rev. Psychol. 67, 150807174122003. https://doi.org/10.1146/annurev-psych-122414-033457

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191. https://doi.org/10.3758/BF03193146

French, E.D., Dillon, K., Wu, X., 1997. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport 8, 649–652. https://doi.org/10.1097/00001756-199702100-00014

Friemel, C.M., Spanagel, R., Schneider, M., 2010. Reward sensitivity for a palatable food reward peaks during pubertal developmental in rats. Front. Behav. Neurosci. 4. https://doi.org/10.3389/fnbeh.2010.00039

Gogtay, N., Giedd, J.N., Lusk, L., Hayashi, K.M., Greenstein, D., Vaituzis, A.C., Nugent, T.F., Herman, D.H., Clasen, L.S., Toga, A.W., Rapoport, J.L., Thompson, P.M., 2004. Dynamic mapping of human cortical development during childhood through early adulthood. Proc. Natl. Acad. Sci. 101, 8174–8179. https://doi.org/10.1073/pnas.0402680101

Gumbleton, M., Benet, L.Z., 1991. Drug Metabolism and Laboratory Anesthetic Protocols in the Rat: Examination of Antipyrine Pharmacokinetics. Pharm. Res. 08, 544–546. https://doi.org/10.1023/A:1015827917684

Heifets, B.D., Castillo, P.E., 2009. Endocannabinoid Signaling and Long-Term Synaptic Plasticity. Annu. Rev. Physiol. 71, 283–306. https://doi.org/10.1146/annurev.physiol.010908.163149

Higuera-Matas, A., Botreau, F., Del Olmo, N., Miguéns, M., Olías, Ó., Montoya, G.L., García-Lecumberri, C., Ambrosio, E., 2010. Periadolescent exposure to cannabinoids alters the striatal and hippocampal dopaminergic system in the adult rat brain. Eur. Neuropsychopharmacol. 20, 895–906. https://doi.org/10.1016/j.euroneuro.2010.06.017

Howard, S.G., Feigenbaum, J.J., 1997. Effect of γ-hydroxybutyrate on central dopamine release in vivo: A microdialysis study in awake and anesthetized animals. Biochem. Pharmacol. 53, 103–110. https://doi.org/10.1016/S0006-2952(96)00664-8

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., Watanabe, M., 2009. Endocannabinoid-Mediated Control of Synaptic Transmission. Physiol. Rev. 89, 309–380. https://doi.org/10.1152/physrev.00019.2008

Kantor, L., Hewlett, G.H.K., Gnegy, M.E., 1999. Enhanced Amphetamine- and K+-Mediated Dopamine Release in Rat Striatum after Repeated Amphetamine: Differential Requirements for Ca2+- and Calmodulin-Dependent Phosphorylation and Synaptic Vesicles. J. Neurosci. 19, 3801–3808. https://doi.org/10.1523/JNEUROSCI.19-10-03801.1999

Li, L.-B., Chen, N., Ramamoorthy, S., Chi, L., Cui, X.-N., Wang, L.C., Reith, M.E.A., 2004. The Role of *N* -Glycosylation in Function and Surface Trafficking of the Human Dopamine Transporter. J. Biol. Chem. 279, 21012–21020. https://doi.org/10.1074/jbc.M311972200

Matthews, M., Bondi, C., Torres, G., Moghaddam, B., 2013. Reduced presynaptic dopamine activity in adolescent dorsal striatum. Neuropsychopharmacology 38, 1344–1351. https://doi.org/10.1109/SIBGRAPI.2017.31

McCutcheon, J.E., Conrad, K.L., Carr, S.B., Ford, K.A., McGehee, D.S., Marinelli, M., 2012. Dopamine neurons in the ventral tegmental area fire faster in adolescent rats than in adults. J. Neurophysiol. 108, 1620–1630. https://doi.org/10.1152/jn.00077.2012

McCutcheon, J.E., Marinelli, M., 2009. Age matters. Eur. J. Neurosci. 29, 997–1014. https://doi.org/10.1111/j.1460-9568.2009.06648.x

Mechoulam, R., Parker, L.A., 2012. The Endocannabinoid System and the Brain. https://doi.org/10.1146/annurev-psych-113011-143739

Nakano, M., Mizuno, T., 1996. Age-related changes in the metabolism of neurotransmitters in rat striatum: A microdialysis study. Mech. Ageing Dev. 86, 95–104. https://doi.org/10.1016/0047-6374(95)01680-5

Naneix, F., Marchand, A.R., Di Scala, G., Pape, J.-R., Coutureau, E., 2012. Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. J. Neurosci. 32, 16223–32. https://doi.org/10.1523/JNEUROSCI.3080-12.2012

Pandolfo, P., Silveirinha, V., Santos-Rodrigues, A. Dos, Venance, L., Ledent, C., Takahashi, R.N., Cunha, R.A., Köfalvi, A., 2011. Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. Eur. J. Pharmacol. 655, 38–45. https://doi.org/10.1016/j.ejphar.2011.01.013

Patel, A.P., Cerruti, C., Vaughan, R.A., Kuhar, M.J., 1994. Developmentally regulated glycosylation of dopamine transporter. Dev. Brain Res. 83, 53–58. https://doi.org/10.1016/0165-3806(94)90178-3

Paxinos, G., Whaton, C., 2009. The Rat Brain, Sixth edit. ed. San Diego.

Pertwee, R.G., 2014. Handbook of cannabis.

Pistis, M., Perra, S., Pillolla, G., Melis, M., Muntoni, A.L., Gessa, G.L., 2004. Adolescent

exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. Biol. Psychiatry 56, 86–94. https://doi.org/10.1016/j.biopsych.2004.05.006

Polissidis, A., Chouliara, O., Galanopoulos, A., Naxakis, G., Papahatjis, D., Papadopoulou-Daifoti, Z., Antoniou, K., 2014. Cannabinoids negatively modulate striatal glutamate and dopamine release and behavioural output of acute d-amphetamine. Behav. Brain Res. 270, 261–269. https://doi.org/10.1016/j.bbr.2014.05.029

Polissidis, A., Galanopoulos, A., Naxakis, G., Papahatjis, D., Papadopoulou-Daifoti, Z., Antoniou, K., 2013. The cannabinoid CB1 receptor biphasically modulates motor activity and regulates dopamine and glutamate release region dependently. Int. J. Neuropsychopharmacol. 16, 393–403. https://doi.org/10.1017/S1461145712000156

Powell, K., 2006. How does the teenage brain work? Nature 442, 865–867. https://doi.org/10.1038/442865a

Price, D.A., Owens, W.A., Gould, G.G., Frazer, A., Roberts, J.L., Daws, L.C., Giuffrida, A., 2007. CB1-independent inhibition of dopamine transporter activity by cannabinoids in mouse dorsal striatum. J. Neurochem. 101, 389–396. https://doi.org/10.1111/j.1471-4159.2006.04383.x

Robinson, M.J.F., Fischer, A.M., Ahuja, A., Lesser, E.N., Maniates, H., 2016. Roles of "Wanting"; and "Liking" in Motivating Behavior: Gambling, Food, and Drug Addictions. Curr. Top. Behav. Neurosci. 27, 105–36. https://doi.org/10.1007/7854_2015_387

Rodríguez de Fonseca, F., Ramos, J.A., Bonnin, A., Fernández-Ruiz, J.J., 1993. Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport 4, 135–8.

Schneider, M., 2008. Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict. Biol. 13, 253–263. https://doi.org/10.1111/j.1369-1600.2008.00110.x

Sisk, C.L., Foster, D.L., 2004. The neural basis of puberty and adolescence. Nat. Neurosci. 7, 1040–1047. https://doi.org/10.1038/nn1326

Smith, A.D., Justice, J.B., 1994. The effect of inhibition of synthesis, release, metabolism and uptake on the microdialysis extraction fraction of dopamine. J. Neurosci. Methods 54, 75–82. https://doi.org/10.1016/0165-0270(94)90161-9

Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations, Neuroscience and Biobehavioral Reviews. https://doi.org/10.1016/S0149-7634(00)00014-2

Stamford, J.A., 1989. Development and Ageing of the Rat Nigrostriatal Dopamine System Studied with Fast Cyclic Voltammetry. J. Neurochem. 52, 1582–1589. https://doi.org/10.1111/j.1471-4159.1989.tb09212.x

Steffens, M., Feuerstein, T.J., 2004. Receptor-independent depression of DA and 5-HT uptake by cannabinoids in rat neocortex - Involvement of Na+/K+-ATPase. Neurochem. Int. 44, 529–538. https://doi.org/10.1016/j.neuint.2003.08.009

Takahashi, Y., Roesch, M.R., Stalnaker, T.A., Schoenbaum, G., 2007. Cocaine exposure shifts the balance of associative encoding from ventral to dorsolateral striatum. Front. Integr. Neurosci. 1, 11. https://doi.org/10.3389/neuro.07.011.2007

Tanda, G., Pontieri, F.E., Di Chiara, G., 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science (80-.). 276, 2048–2050. https://doi.org/10.1126/science.276.5321.2048

Tarazi, F., Tomasini, E.C., Baldessarini, R.J., 1998. Postnatal development of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. Neurosci. Lett. 254, 21–24. https://doi.org/10.1016/S0304-3940(98)00644-2

Teicher, M.H., Andersen, S.L., Hostetter, J.C., 1995. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Dev. Brain Res. 89, 167–172. https://doi.org/10.1016/0165-3806(95)00109-Q

Tepper, J.M., Creese, I., Schwartz, D.H., 1991. Stimulus-evoked changes in neostriatal dopamine levels in awake and anesthetized rats as measured by microdialysis. Brain Res. 559, 283–292. https://doi.org/10.1016/0006-8993(91)90013-L

Tepper, J.M., Trent, F., Nakamura, S., 1990. Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons. Dev. Brain Res. 54, 21–33. https://doi.org/10.1016/0165-3806(90)90061-3

UNODC, 2018. World Drug Report, World Drug Report 2018.

Van Waes, V., Beverley, J.A., Siman, H., Tseng, K.Y., Steiner, H., 2012. CB1 cannabinoid receptor expression in the striatum: Association with corticostriatal circuits and developmental regulation. Front. Pharmacol. 3 MAR, 1–8. https://doi.org/10.3389/fphar.2012.00021

Verdurand, M., Nguyen, V., Stark, D., Zahra, D., Gregoire, M.-C., Greguric, I., Zavitsanou, K., 2011. Comparison of Cannabinoid CB $_1$ Receptor Binding in Adolescent and Adult Rats: A Positron Emission Tomography Study Using [^{18} F]MK-9470. Int. J. Mol. Imaging 2011, 1–11. https://doi.org/10.1155/2011/548123

Volkow, N.D., Baler, R.D., Compton, W.M., Weiss, S.R.B., 2014. Adverse Health Effects of Marijuana Use. N. Engl. J. Med. 370, 2219–2227. https://doi.org/10.1056/NEJMra1402309

Volz, T.J., Farnsworth, S.J., Rowley, S.D., Hanson, G.R., Annette, E., 2009. Age-dependent differences in dopamine transporter and vesicular monoamine transporter-2 function and their implications for methamphetamine neurotoxicity. Synapse 63, 147–151. https://doi.org/10.1002/syn.20580.AGE-DEPENDENT

Willuhn, I., Burgeno, L.M., Everitt, B.J., Phillips, P.E.M., 2012. Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use. Proc. Natl. Acad. Sci. U. S. A. 109, 20703–8. https://doi.org/10.1073/pnas.1213460109

Yanovsky, Y., Mades, S., Misgeld, U., 2003. Retrograde signaling changes the venue of postsynaptic inhibition in rat substantia nigra. Neuroscience 122, 317–328.

https://doi.org/10.1016/S0306-4522(03)00607-9

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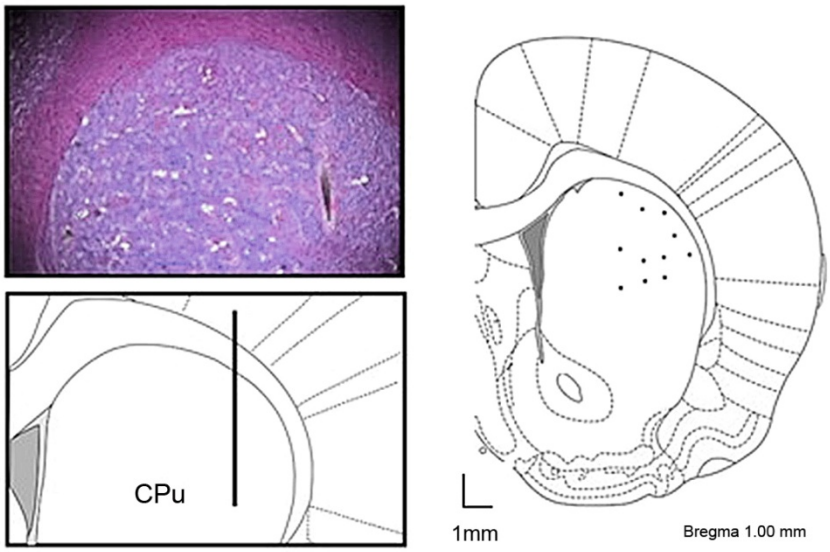

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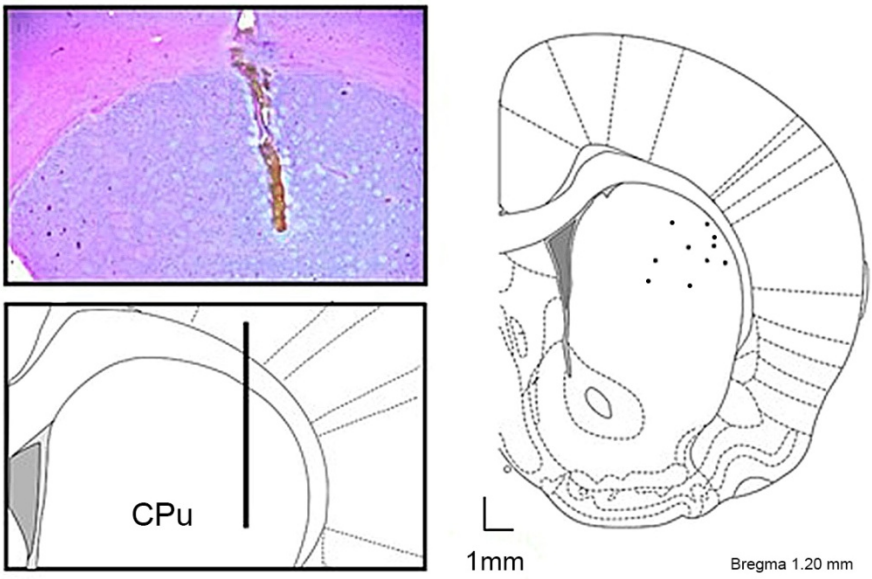

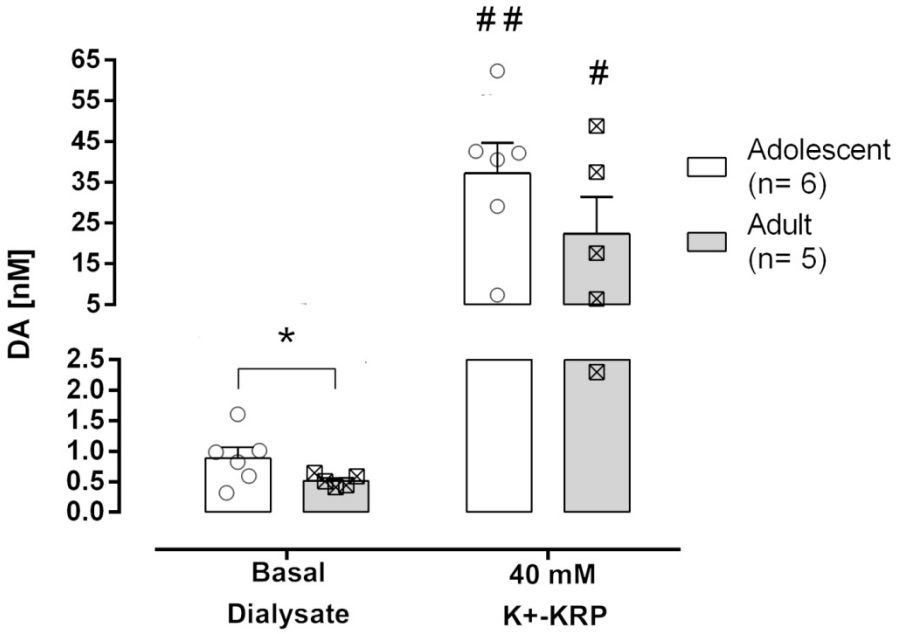

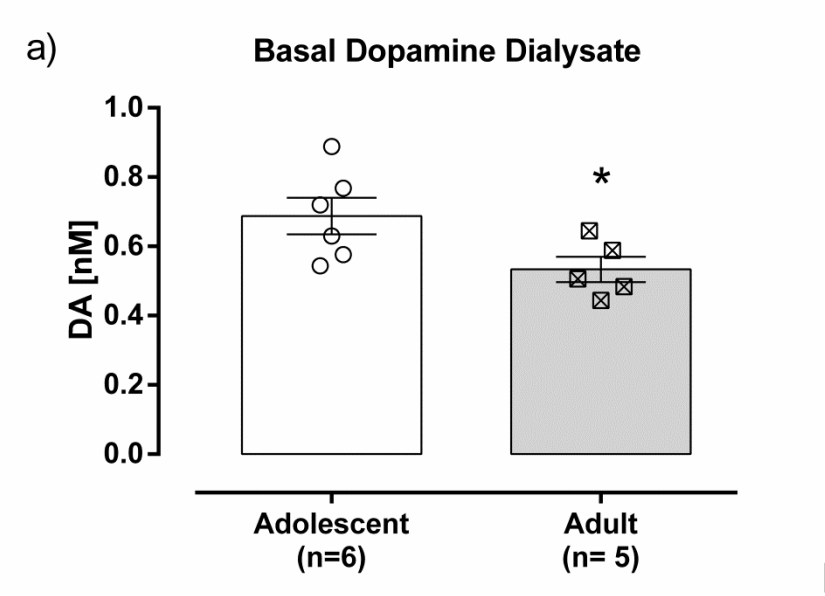

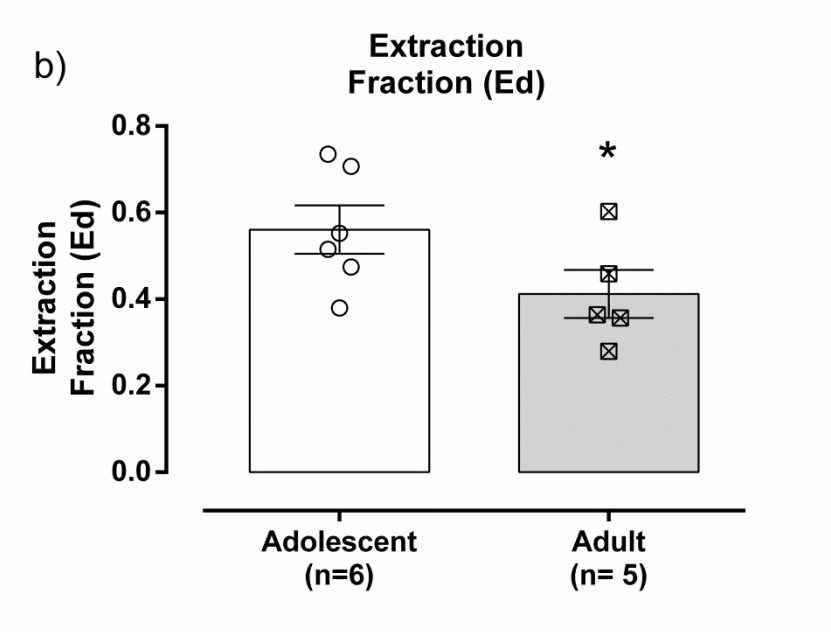

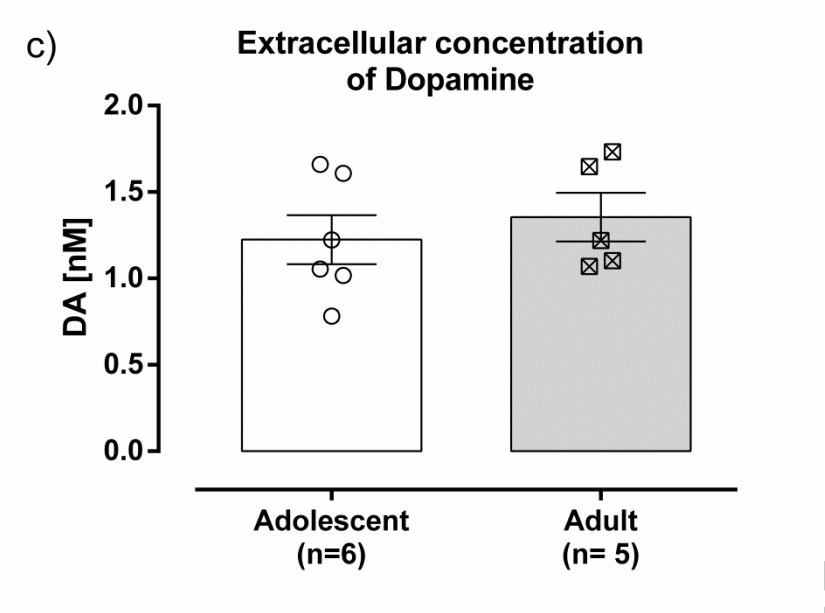

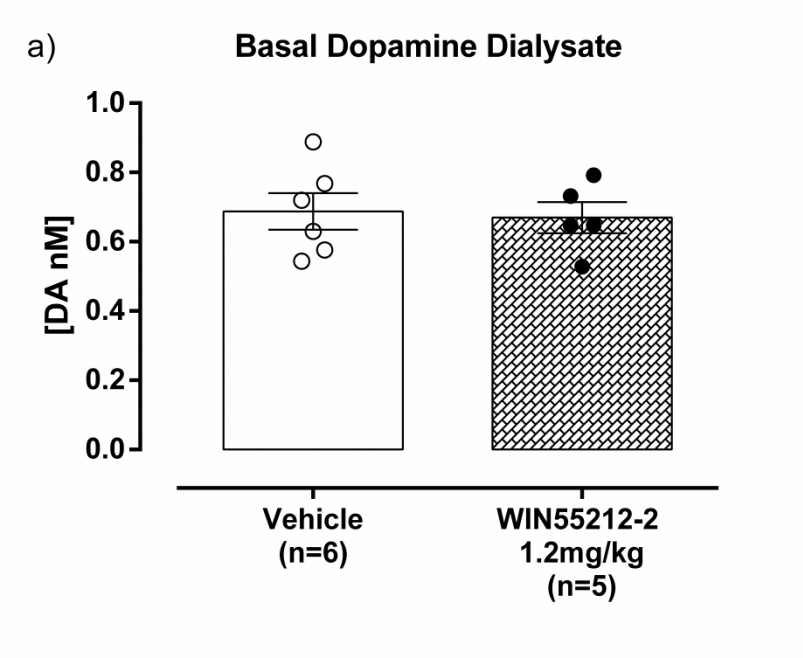

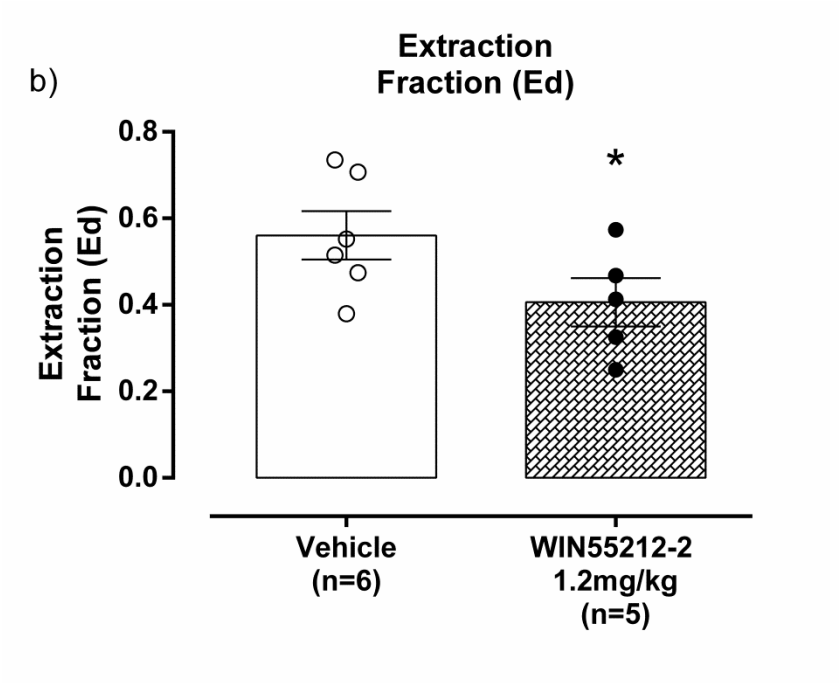

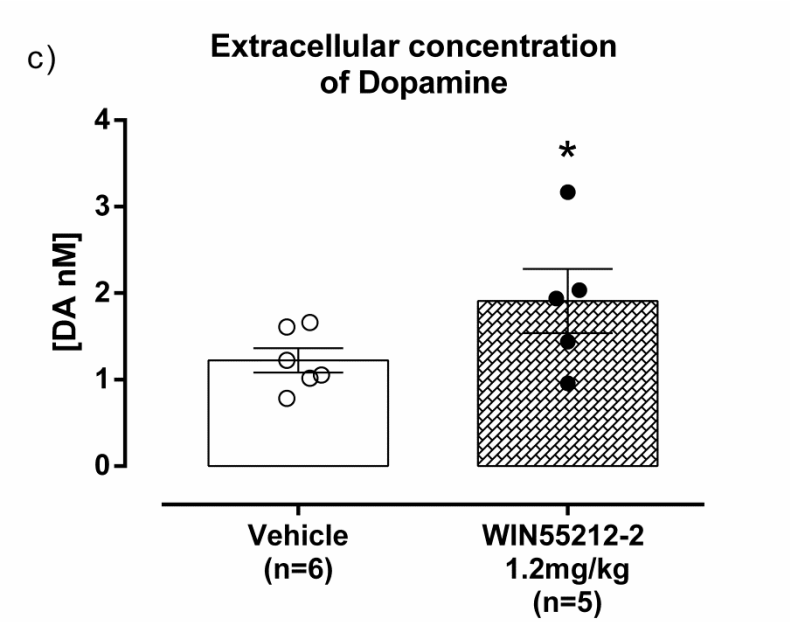

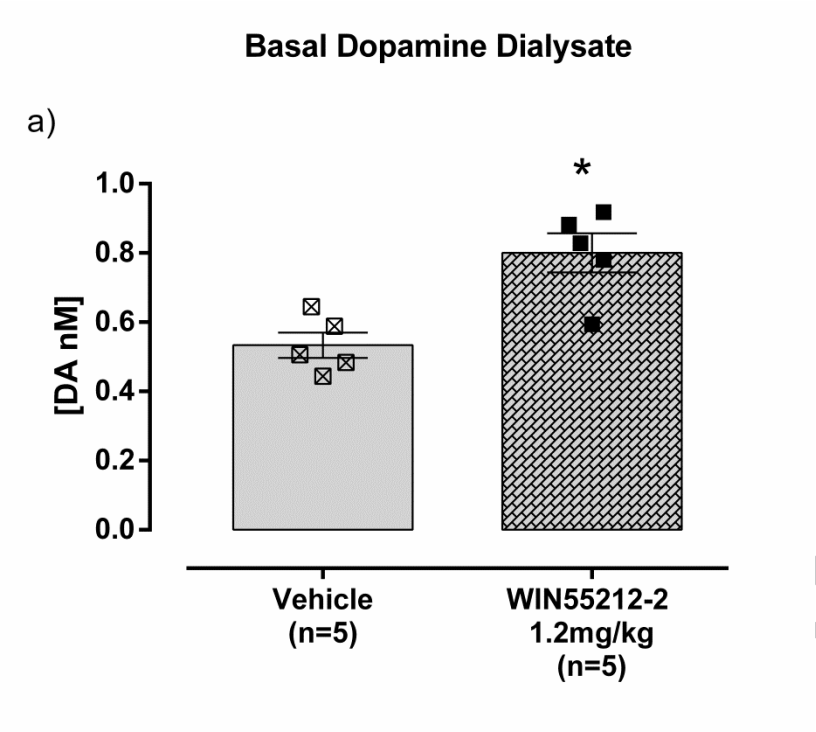

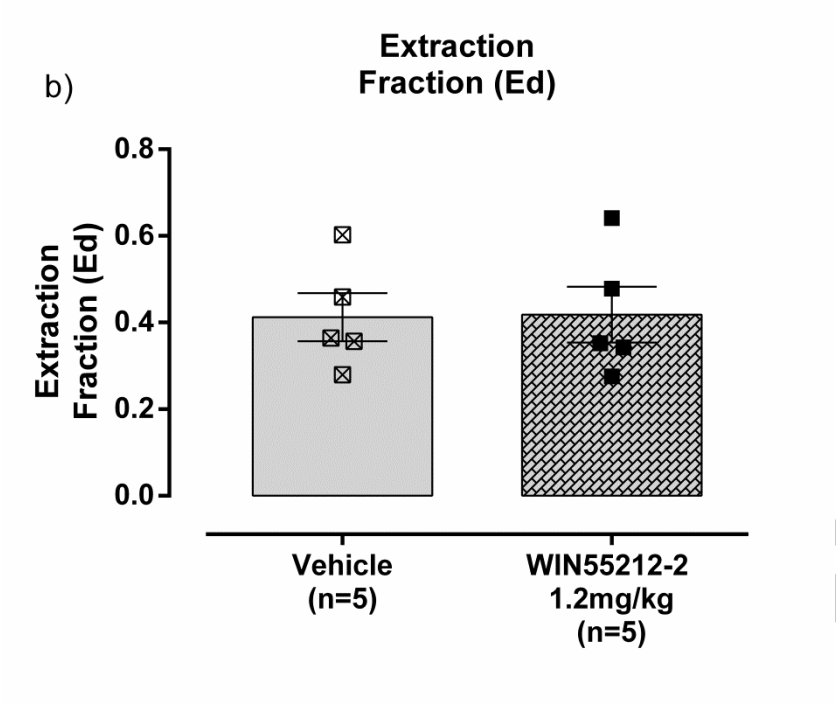

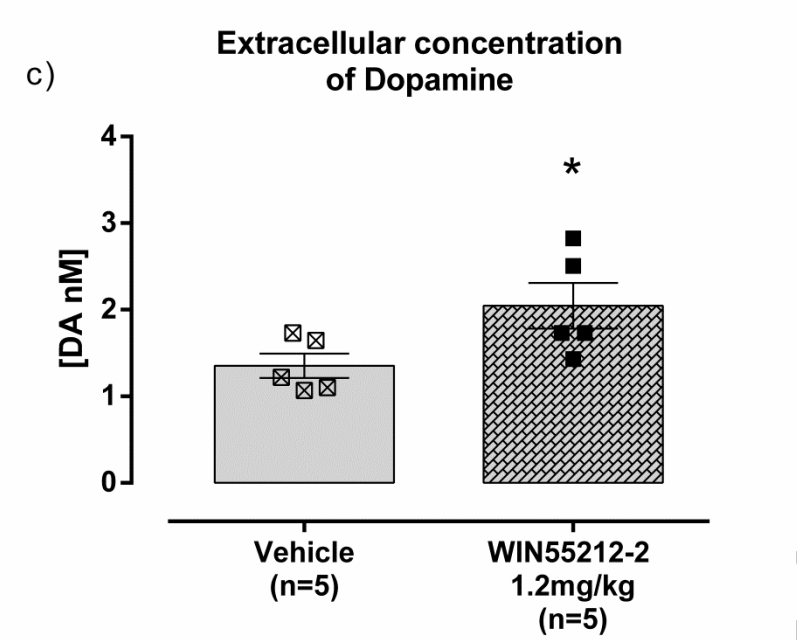

Highlights

- Adolescent rats show higher basal DA dialysate in DLS compared to adult rats.
- Adolescent rats show higher DA extraction fraction in DLS compared to adult rats.
- WIN55212-2 decreases DA extraction fraction in DLS during adolescence.