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REGULAR RESEARCH ARTICLE

Adolescent Exposure to WIN 55212-2 Render the Nigrostriatal Dopaminergic Pathway Activated During Adulthood

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Abstract

Background: During adolescence, neuronal circuits exhibit plasticity in response to physiological changes and to adapt to environmental events. Nigrostriatal dopaminergic pathways are in constant flux during development. Evidence suggests a relationship between early use of cannabinoids and psychiatric disorders characterized by altered dopaminergic systems, such as schizophrenia and addiction. However, the impact of adolescent exposure to cannabinoids on nigrostriatal dopaminergic pathways in adulthood remains unclear. The aim of this research was to determine the effects of repeated activation of cannabinoid receptors during adolescence on dopaminergic activity of nigrostriatal pathways and the mechanisms underlying this impact during adulthood.

Methods: Male Sprague-Dawley rats were treated with 1.2 mg/kg WIN 55212-2 daily from postnatal day 40 to 65. Then no-net flux microdialysis of dopamine in the dorsolateral striatum, electrophysiological recording of dopaminergic neuronal activity, and microdialysis measures of gamma-aminobutyric acid (GABA) and glutamate in substantia nigra par compacta were carried out during adulthood (postnatal days 72–78).

Results: Repeated activation of cannabinoid receptors during adolescence increased the release of dopamine in dorsolateral striatum accompanied by increased population activity of dopamine neurons and decreased extracellular GABA levels in substantia nigra par compacta in adulthood. Furthermore, perfusion of bicuculline, a GABAa antagonist, into the ventral pallidum reversed the increased dopamine neuron population activity in substantia nigra par compacta induced by adolescent cannabinoid exposure.

Conclusions: These results suggest that adolescent exposure to cannabinoid agonists produces disinhibition of nigrostriatal dopamine transmission during adulthood mediated by decreased GABAergic input from the ventral pallidum.

Key Words: Dopamine, adolescence, WIN 55212-2, no-net flux microdialysis, dorsolateral striatum

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Significance Statement

Cannabis intake in teenagers is higher than in the adult population in Europe and the Americas, which results in a higher activation of $CB_{1/2}$ receptors during adolescence. Furthermore, it has been observed that nigrostriatal dopaminergic pathway is maturing during adolescence. However, the consequences of adolescent activation of $CB_{1/2}$ receptor in the adult nigrostriatal dopaminergic pathway remain to be addressed. Using neurochemistry detection and electrophysiological recordings, we observed a disinhibition of nigrostriatal dopaminergic transmission in adult rats after a repeated activation of $CB_{1/2}$ receptor during adolescence. This result and previous evidence suggest that the activated state of dopaminergic nigrostriatal induced by adolescent cannabinoid exposure may be a risk factor that strongly predisposes to major neuropsychiatric disease later in life.

Introduction

Adolescence is characterized by a higher sensitivity to reward and an increase in risk-taking behaviors compared with adulthood (Sturman and Moghaddam, 2011; Doremus-Fitzwater and Spear, 2016). Consequently, this period is associated with the initiation of intake of drugs of abuse such as ethanol, nicotine, and cannabis (Doremus-Fitzwater and Spear, 2016; UNODC, 2018). In the case of cannabis, the annual prevalence of use among the adolescent population is higher than the general population in Europe and the Americas (UNODC, 2018). Furthermore, approximately 17% of those who initiate use of cannabis during adolescence develop cannabis use disorders; in contrast, just 9% who experiment with cannabis in adulthood develop cannabis use disorders (Lopez-Quintero et al., 2011; Volkow et al., 2014). However, the consequences of adolescent exposure to cannabinoids on neurobiological substrates associated with addiction remain unclear.

Dopaminergic transmission in the mesolimbic and nigrostriatal pathways is associated with the development of addictive behavior. Whereas the initial seeking of drugs of abuse is accompanied by an increase in dopaminergic mesolimbic activity (Everitt and Robbins, 2016; Robinson et al., 2016), the transition to a compulsive drug-seeking habit is associated with a progressive increase in dopamine neurotransmission in the nigrostriatal dopamine pathway (Ito et al., 2002; Willuhn et al., 2012; Everitt and Robbins, 2013). The activity of mesolimbic and nigrostriatal dopamine neurons is driven by different inputs (McFarland and Kalivas, 2001; Koob and Volkow, 2016), controlling extracellular dopamine levels in the nucleus accumbens (NAc) and dorsolateral striatum (DLS), respectively (Floresco et al., 2003; Panin et al., 2012). Neuroanatomical evidence has shown that substantia nigra pars compacta (SNc) receives gamma-aminobutyric acid (GABA)ergic input from multiple regions, such as striatum, external globus pallidum, substantia nigra pars reticulata (SNr), and ventral pallidum (VP), while the subthalamic nucleus and pedunculopontine nucleus drive their glutamatergic input on SNc (Watabe-Uchida et al., 2012; Steiner and Tseng, 2017). Of note, a dysfunction in the activity of these inputs can contribute to addictive-like behavior. For instance, inactivation of both subthalamic nucleus or pedunculopontine nucleus decreases drug-seeking of cocaine in adult rats (Corrigall et al., 2002; Baunez et al., 2005), while an imbalance in the activity of striatal medium spiny neurons increases drug-seeking behavior (Yager et al., 2015). Moreover, activation and inhibition of VP GABAergic neurons promote and reduce drug seeking of cocaine, respectively (Root et al., 2015; Farrell et al., 2019; Heinsbroek et al., 2020). Together, these studies suggest that the dysfunction of the nigrostriatal pathway induced by drugs of abuse (Willuhn et al., 2012) is accompanied by changes in the GABAergic and glutamatergic levels in SNc.

Cannabinoids play a role in multiple physiological processes, such as appetite, mood, memory, and motivation (Mechoulam and Parker, 2013; Pertwee, 2014). The CB_1 receptors are crucial to synaptic communication due to their ability to modulate the release of different neurotransmitters, such as GABA, glutamate, and dopamine (Heifets and Castillo, 2009; Kano et al., 2009; Castillo et al., 2012). This receptor is widely expressed in the brain, highlighting regions such as prefrontal cortex, globus pallidum, VP, hippocampus, striatum, ventral tegmental area (VTA), and substantia nigra (Tsou et al., 1998; Egertová and Elphick, 2000; Mátyás et al., 2006, 2008; Davis et al., 2018). In VTA and substantia nigra, the CB_1 receptor is mainly expressed in the axonal terminal region of GABAergic and glutamatergic projections, suggesting an indirect control of dopaminergic transmission (Julian et al., 2003; Yanovsky et al., 2003; Mátyás et al., 2006; Covey et al., 2017; Davis et al., 2018). It has been observed that an acute systemic administration of WIN 55212-2, a $CB_{1/2}$ agonist, increases dopamine extracellular levels in the NAc (Tanda et al., 1997) and DLS (Polissidis et al., 2014) associated with an increase in firing rate of dopamine neurons in the VTA and SNc, respectively (French et al., 1997). This increase in the dopaminergic transmission induced by cannabinoids has also been observed after repeated adolescent exposure. An increase in the firing rate and burst activity of dopamine neurons from VTA is induced by adolescent exposure to Δ^9 tetrahydrocannabinol (Δ^9-THC), the psychoactive compound of cannabis and partial agonist of $CB_{1/2}$ receptors (Renard et al., 2017). In addition, Gomes et al. (2015) showed that adolescent exposure to WIN 55212-2 increases the spontaneous activity of dopaminergic neurons of the VTA in adult rats (Gomes et al., 2015). While it has been suggested that adolescent use of cannabis is a risk factor in developing drug addiction in adults (Schneider, 2008), the studies of consequences of adolescent exposure to cannabinoid have been mainly concerned with the dopaminergic mesolimbic pathway (Pistis et al., 2004; Schneider, 2008; Higuera-Matas et al., 2010; Gomes et al., 2015; Renard et al., 2017).

The aim of this research is to assess the consequences of early exposure to cannabinoid on the nigrostriatal dopaminergic pathway in adulthood. We hypothesized that repeated activation of $CB_{1/2}$ receptors during adolescence would activate the nigrostriatal dopaminergic pathway in adulthood. Microdialysis and single-unit recording experiments showed that adolescent exposure to WIN 55212-2 increases dopamine extracellular levels in DLS and decreases GABA extracellular levels in SNc accompanied by an increase in the population activity of dopamine neurons.

METHODS

All procedures were carried out accordance with the guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals* (8th edition) and the principles presented in the "Guidelines for

the Use of Animals in Neuroscience Research" by the Society for Neuroscience. The microdialysis protocols were approved by the local bioethics committee, verifying that it complies with the basic principles set forth in Chilean Law 20.380 on Animal Protection 2009 (ID project: 160816013). Electrophysiological experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Animals

In microdialysis experiments, adolescent male Sprague-Dawley (post-natal day [PD] 33) rats were obtained from the Animal Care Faculty of the Biological Sciences, Pontificia Universidad Católica de Chile (Charles River, Wilmington, MA; RRID: RGD_728193) under veterinarian supervision. Rats were maintained in Animal Care of the Department of Pharmacy, Pontificia Universidad Católica de Chile. In electrophysiological experiments, adolescent male Sprague-Dawley rats were obtained from Envigo (Indianapolis, IN). All rats were housed in groups of 2 per cage and kept at room temperature between 22°C and 24°C on a 12-hour-light/-dark cycle (lights on at 7:00 AM EST) with access to food and water ad libitum. Rats were handled for 1 week before starting the treatment. A total of 75 adolescent rats were divided into 2 treatment groups: 38 rats for vehicle treatment and 37 rats for WIN 55212-2 treatment. All vehicle and treatment protocols were performed in parallel.

Reagents

The $CB_{1/2}$ agonist, WIN 55212-2 mesylate (CAS no. 131543-23-2), was emulsified in 2% Tween 80 (CAS no. 9005-65-6) in saline solution 0.9% at a concentration of 1.2 mg/mL. The GABAa antagonist bicuculline methyl bromide (CAS no. 66016-70-4) (0.1 µg) was mixed fresh in Dulbecco's buffer (MDL no. MFCD00131855) before starting recording. Reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Treatment

Adolescent rats were daily injected i.p. with WIN 55212-2 ($CB_{1/2}$ agonist) at a dose of 1.2 mg/kg (WIN) or with vehicle (2% Tween 80 in saline solution 0.9%) at a volume of 1 mL/kg from PD 40 to PD 65, similar to previously described (Gomes et al., 2015). Previous evidence has shown that cannabinoid exposure during this range of age modifies behavioral tests, such as object recognition, social interaction, and amphetamine-induced locomotion in adult rats (Schneider and Koch, 2003, 2005; Gomes et al., 2015). All the experiments were carried out between PD 72 and PD 78 (Figure 1).

No-Net Flux Microdialysis

To assess the effects of adolescent WIN 55212-2 exposure in nigrostriatal dopaminergic transmission, adult rats were anesthetized with urethane 1.5 g/kg i.p., and no-net flux microdialysis experiments in DLS were carried out as described in previous publications (Azocar et al., 2019; Pérez-Valenzuela et al., 2019). Urethane was chosen due to the extended half-life (Gumbleton and Benet, 1991). In addition, urethane does not modify basal and stimulated dopamine dialysate in striatum (Tepper et al., 1991; Howard and Feigenbaum, 1997). Anesthetized rats were placed in a stereotaxic apparatus, the skull was exposed, and a hole was drilled. A concentric microdialysis probe (CMA 11 Microdialysis, Holliston, MA) with an in vitro recovery rate higher than 10% was lowered vertically into the DLS using the coordinates +1.2 AP, +3.6 ML relative to bregma, and –4.8 DV from the dura (Paxinos and Watson, 2009). Body temperature was maintained by a thermostatically controlled electric heating pad. The probe was perfused for 40 minutes with Krebs–Ringer phosphate buffer with 0.2 mM acid ascorbic (AA-KRP) using a Harvard infusion pump (Harvard Apparatus, Holliston, MA) at a rate of 2 µL/min to allow equilibration. The composition of the AA-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). The probe was then randomly perfused with 5 different concentrations of dopamine—0.0, 5.0, 10.0, 20.0, and 40.0 nM—in AA-KRP to determine dopamine basal dialysate, dopamine extracellular concentration (C_{ext}), and extraction fraction (Ed), an indirect measure of dopamine uptake (Smith and Justice, 1994). After a stabilization period of 20 minutes, 3 consecutive samples were collected every 5 minutes for each concentration of dopamine. Perfusion samples were collected in 2 µL of perchloric acid (0.2 N) and maintained on ice (4°C) until quantification.

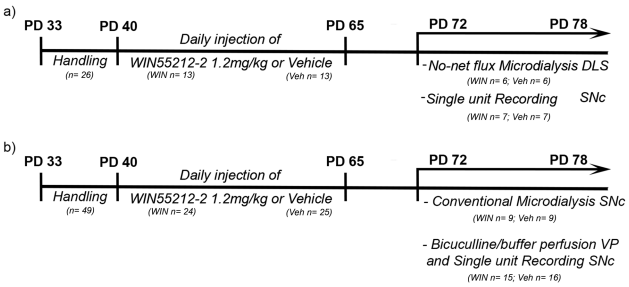

Figure 1. Treatment protocol. All adolescent rats (post-natal day [PD] 33) were handled for 1 week before treatment. Adolescent rats were then injected with WIN 55212-2 or vehicle once daily for 25 days (between PD 40 and PD 65) and all experiments were carried out between PD 72 and PD 78. (a) To assess the effects of adolescent WIN 55212-2 exposure on nigrostriatal dopaminergic transmission, no-net flux microdialysis experiments in DLS, and single-unit recording in substantia nigra pars compacta (SNc) were carried out at least a week after treatment. (b) To study the mechanism underlying the facilitation of nigrostriatal dopaminergic pathway activity induced by WIN 55212-2, microdialysis experiments in SNc were performed to quantify glutamate and gamma-aminobutyric acid (GABA). To determine the role of the ventral pallidum (VP), single-unit recording in SNc was carried out after a local perfusion of bicuculline in VP in adult rats.

Conventional Microdialysis

Conventional microdialysis experiments were carried out in SNc to determine basal dialysate levels of glutamate and GABA. Adult rats were anesthetized with urethane 1.5 g/kg i.p. and placed in a stereotaxic apparatus, the skull was exposed, and a hole was drilled. Then, a concentric microdialysis probe (CMA 11 Microdialysis, Holliston, MA) with an in vitro recovery rate higher than 10% was lowered diagonally into the SNc using a 40° angle from the horizontal axis at the following coordinates: –4.9 AP, +7.6 ML relative to bregma, and –8.0 DV from the dura (Paxinos and Watson, 2009). Body temperature was maintained by a thermostatically controlled electric heating pad. The probe was perfused for 40 minutes with KRP buffer at a rate of 2 µL/min using a Harvard infusion pump (Harvard Apparatus). After a stabilization period using KRP, 3 consecutive samples were collected every 5 minutes for the determination of GABA and glutamate basal dialysate. Perfusion samples were collected in 2 µL perchloric acid (0.2 N) and maintained on ice (4°C) until quantification.

Analysis of Dialysate Samples

Dopamine was quantified using a high-performance-liquid-chromatography (HPLC) system with an amperometric detector as described previously (Escobar et al., 2012). Twelve (10 µL of sample plus 2 µL PCA 0.2 N) µL of the collected samples was injected into an HPLC system (BASi America, West Lafayette, IN) with the following configuration: a pump (Jasco LC-Net II/ADC, Tokyo, Japan), a UNIJET LC column (part no. MF-8954, BASi), and an amperometric detector (LC4C, BASi America). The mobile phase contained 100 mM NaH_2PO_4, 1.0 mM ethylenediaminetetraacetic acid, 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 dopamine was 6 minutes.

Glutamate and GABA were quantified using an HPLC system with a fluorescence detector as described previously (Sotomayor-Zárate et al., 2010). Briefly, 12 µL of the sample of dialysis perfusate and PCA were mixed with 12 µL KRP and 4 µL borate buffer (pH 10.8), and then the mixture was derivatized by adding 4µL fluorogenic reagent (20 mg orthophthaldehyde and 10µL β-mercaptoethanol in 5mL ethanol). At 90 seconds after derivatization, samples were injected into an HPLC system with the following configuration: quaternary gradient pump (Jasco Co. Ltd.), a C-18 reverse phase column (Kromasil, Eka Chemicals, Bohus, Sweden), and a fluorescence detector (Jasco Co. Ltd.). A mobile phase containing 0.1 M NaH_2PO_4 and 23% CH_3CN (pH 5.7) was pumped for 20 minutes. The flow rate of the mobile phase was set at 1.2 mL/min, and the retention time for glutamate was 3 minutes and for GABA was 16 minutes.

Single-Unit Recording

To assess the effects of adolescent WIN 55212-2 exposure on nigrostriatal dopaminergic transmission, single-unit recordings in SNc were carried out after at least a week of treatment. Adult rats were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) for a non-survival procedure (Field et al., 1993). The anesthesia was monitored by assessing the foot pinch reflex and maintained throughout the experiment with additional doses of chloral hydrate (approximately 50 mg/kg, i.p.). Chloral hydrate was used in the recording experiments since it does not modify the percentage of spikes per bursts in bursting dopamine neurons in the SNc (Kelland et al., 1990). Anesthetized rats were placed in a stereotaxic apparatus (Stoelting), and the body temperature was maintained by a thermostatically controlled electric heating pad. A hole was drilled in the coordinates AP –4.9, ML +2.2 from bregma. The electrodes were pulled from Omegadot 2.0-mm glass tubing on a Narishige P-5 vertical electrode puller and the tip has broken back under microscopic control and filled with 2 M NaCl containing 2% Pontamine Sky Blue dye. The impedance of the electrodes was tested in situ ranging from 6 to 15 MΩ. The recording procedure was based on (Gomes et al., 2015). Six vertical tracks separated by 200 mm were sampled at the following coordinates: AP –4.9 to –5.1, ML 2.0 to 2.4 from bregma, and –6.5 to n–9.0 DV from brain surface (see Figure 2c). Single-unit activity was filtered using a high-pass filter at 30 Hz and low-pass at 10 kHz. Only stable spontaneous neuronal activity (at least 1–3 minutes) with a signal-to-noise ratio greater than 3:1 was analyzed. Dopamine neurons were identified according to the following criteria: (1) location; (2) an action potential duration >2.2 ms; (3) slow firing rate (<10 Hz); and (4) irregular and burst firing patterns, with the start of burst characterized by an interspike interval <80 ms and burst termination defined as a subsequent inter-spike interval >160 ms (Grace and Bunney, 1983; Ungless and Grace, 2012). At the end of the experiment, the electrode placement was marked using electrophoretic ejection of Pontamine Sky Blue dye from the tip of the electrode (20 µA constant negative current, 30 minutes).

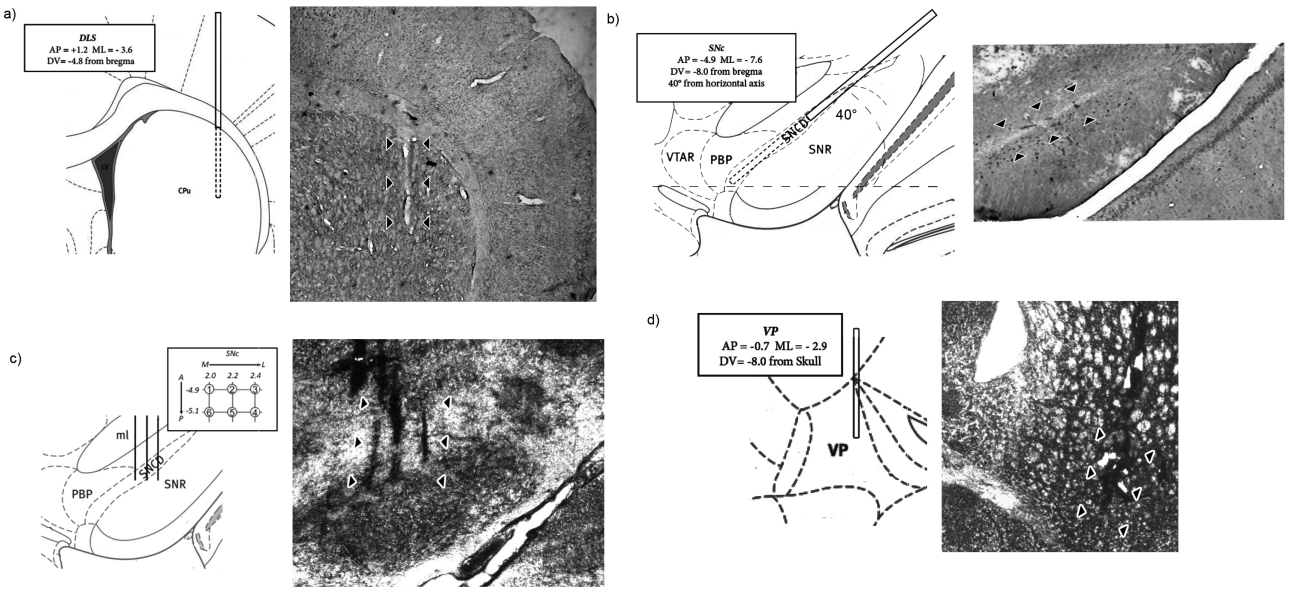

Figure 2. Representative anatomical placements (left side) and histology example of placements (right side) of a microdialysis probe, electrode, and cannula guide. The microdialysis probe was lowered into the (a) dorsolateral striatum (DLS) and (b) substantia nigra par compacta (SNc) using the coordinates: (a) 1.2 mm anterior to bregma, 3.6 mm lateral, 4.8 mm below dura, and (b) 4. mm posterior to bregma, 7.6 mm lateral, and 8.0 mm below dura using an angle of 40° from horizontal axis according to Paxinos and Watson (2009). The electrode was lowered in a preset 6-track pattern, with each track separated by 200 µm in the (c) SNc of each rat at the following the coordinates: AP –4.9 to –5.1, ML 2.0 to 2.4 from bregma, and –6.5 to –9.0 DV from the brain surface. A guide cannula was lowered in (d) ventral pallidum using the following coordinates: AP –0.7, ML +2.9 from bregma, DV –6.0 from the skull. A 30-gauge injection cannula protruding 2.0 mm past the end of the guide was used to perfuse a solution of bicuculline at a rate of 0.1 µL/min over 5 minutes. Diagrams were adapted from Paxinos and Watson (2009).

Pharmacological Manipulations

To study the mechanism underlying the effects of adolescent exposure to WIN 55212-2 on nigrostriatal dopaminergic transmission, bicuculline methyl bromide (0.1 µg) was mixed fresh in 0.5 µL Dulbecco's buffer and infused into the VP through a 30-gauge injection cannula protruding 2.0 mm past the end of the guide at an injection volume of 0.1 µL every 1 minute. The cannula was lowered to the following coordinate: AP –0.7, ML +2.9 from bregma, DV –6.0 from the skull (Paxinos and Watson, 2009). Ten minutes after the perfusion of bicuculline, a single-unit recording was carried out. The doses used in this study have been shown to not alter the basal activity of dopamine neuron (Floresco et al., 2003).

Histology

Rats were decapitated under deep anesthesia (urethane 1.5 g/kg i.p. in microdialysis experiments and chloral hydrate 400 mg/kg i.p. in single-unit recording), and brains were extracted and cleaned with NaCl 0.9%. Brains were stored in 4% paraformaldehyde. At least 2 days before slicing, the brains were cryoprotected using a solution of 30% sucrose. To assess the location of the probe and electrode, brains were frozen and sliced coronally into 50-mm-thick sections. Slices were stained with cresyl violet, and the probe/electrode placement was localized using the atlas of brain rat (Paxinos and Watson, 2009). Only data from correct probe/electrode placements were subjected to further analysis (Figure 2).

Data Analysis

The concentrations of dopamine, GABA, and glutamate were calculated considering the area under the curve of the chromatogram provided by Chromgraph software. No-net flux microdialysis data were analyzed as described by (Chefer et al., 2005, 2006). The amount of dopamine gained or lost from the probe during the no-net flux microdialysis ($C_{in} - C_{out}$) was calculated for each animal at each dopamine perfusion concentration (C_{in}: 0.0, 5.0, 10.0, 20.0, and 40.0 nM). The net change in dopamine ($C_{in} - C_{out}$) was plotted against C_{in} and subjected to linear regression (Figure 3a). The point at which no dopamine was gained or lost ($C_{in} - C_{out} = 0$) represents an estimate of dopamine C_{ext}. The slope of the linear regression line represents the Ed, an indirect measure of dopamine transporter (DAT) activity. Basal dialysate dopamine levels were calculated for each animal as the average of the 3 basal samples ($C_{in} = 0$).

Each dopamine neuron record was analyzed using Neuroexplorer software, and the following parameters were determined: (1) the number of spontaneously active dopamine neurons per track, (2) average firing rate (number of spikes/time of the record), and (3) percentage of spikes in bursts (number of spikes in burst/total of spikes in record × 100). Statistical analyses were performed using Prism 5.0 GraphPad software. Data points outside of the 95% confidence interval were treated as outliers and could be excluded from the data analysis, although none were excluded in these studies. Normality was checked using the Kolmogorov–Smirnov test. Resultant data were analyzed by 2-way ANOVA, Sidak post-test, and 2-tailed unpaired *t* test when appropriate. All data are reported as mean ± SEM.

RESULTS

Effects of Repeated Exposure to WIN 55212-2 in Adolescence on DLS Dopamine Dynamics of Adult Rats

Adolescent exposure to WIN 55212-2 significantly increased basal dopamine dialysate in DLS of adult rats (Figure 3b; vehicle group: 0.55 ± 0.11 nM, n = 6 vs WIN group: 1.00 ± 0.12 nM, n = 6; $P = .018$, unpaired *t* test). The adolescent exposure to WIN did not modify dopamine Ed compared with the vehicle group (Figure 3c; vehicle group: 0.38 ± 0.07, n = 6 vs WIN group: 0.42 ± 0.06, n = 6; $P = .51$, unpaired *t* test). Consequently, dopamine C_{ext} was higher in adult rats treated with WIN 55212-2 during adolescence compared with treated rats with vehicle (Figure 3d; vehicle group: 1.51 ± 0.13 nM, n = 6 vs WIN group: 2.42 ± 0.35 nM, n = 6; $P = .033$, unpaired *t* test).

Effects of Repeated Exposure to WIN 55212-2 During Adolescence on SNc Dopamine Neuron Activity

The adolescent administration of WIN 55212-2 increased significantly the spontaneous activity of dopamine neurons of the SNc (Figure 4b; vehicle group: 0.86 ± 0.09 cells/track, n = 7 vs WIN group: 1.29 ± 0.17 cells/track, n = 7; $P = .046$, unpaired *t* test). Differences in firing rate were not observed between groups treated with WIN 55212-2 and vehicle (Figure 4c; vehicle group: 3.10 ± 0.42 Hz, n = 36 neurons vs WIN group: 3.54 ± 0.51 Hz, n = 54 neurons; $P = .60$, unpaired *t* test). Adolescent exposure of WIN 55212-2 did not modify the percentage of spikes per burst compared with the vehicle group (Figure 4d; vehicle group: 17.55 ± 4.00%, n = 36 vs WIN group: 17.29 ± 3.43%, n = 54; $P = .96$, unpaired *t* test).

Effects of Repeated Exposure to WIN 55212-2 During Adolescence on Dialysate Levels of GABA and Glutamate in SNc of Adult Rats

Repeated exposure of WIN 55212-2 during adolescence decreased significantly basal GABA dialysate in SNc of adult rats (Figure 5a; vehicle group: 0.071 ± 0.006 µM, n = 9 vs WIN group: 0.051 ± 0.005 µM, n = 9; $P = .03$, unpaired *t* test). Adolescent exposure of WIN 55212-2 did not modify basal glutamate dialysate levels compared with the vehicle group (Figure 5b; vehicle group: 0.65 ± 0.10 µM, n = 9 vs WIN group: 0.57 ± 0.08 µM, n = 9; $P = .54$, unpaired *t* test).

Role of VP GABAergic Transmission on Dopamine Neuron Activity in SNc of Adult Rats After Adolescent Exposure to WIN 55212-2

A 2-way ANOVA, with adolescent treatment and VP perfusion as 2 independent factors, showed no significant effect of WIN 55212-2 treatment during adolescence ($F_{1,27} = 0.997$; $P = .327$) and bicuculline perfusion into the VP ($F_{1,27} = 1.447$; $P = .235$) on dopamine population activity. The interaction between these variables was significant ($F_{1,27} = 6.535$; $P = .017$) (Figure 6a). Reproducing the results observed in Figure 4b, adolescent exposure to WIN 55212-2 increased significantly population activity of dopamine neurons in adult rats after a buffer perfusion into the VP (Figure 6a; buffer/vehicle group: 0.905 ± 0.11 cells/track, n = 7 vs buffer/WIN group: 1.39 ± 0.15 cells/track, n = 7; $P = .046$, Sidak post-test). As observed in VTA (Floresco et al., 2003), changes in dopamine neuron population activity were

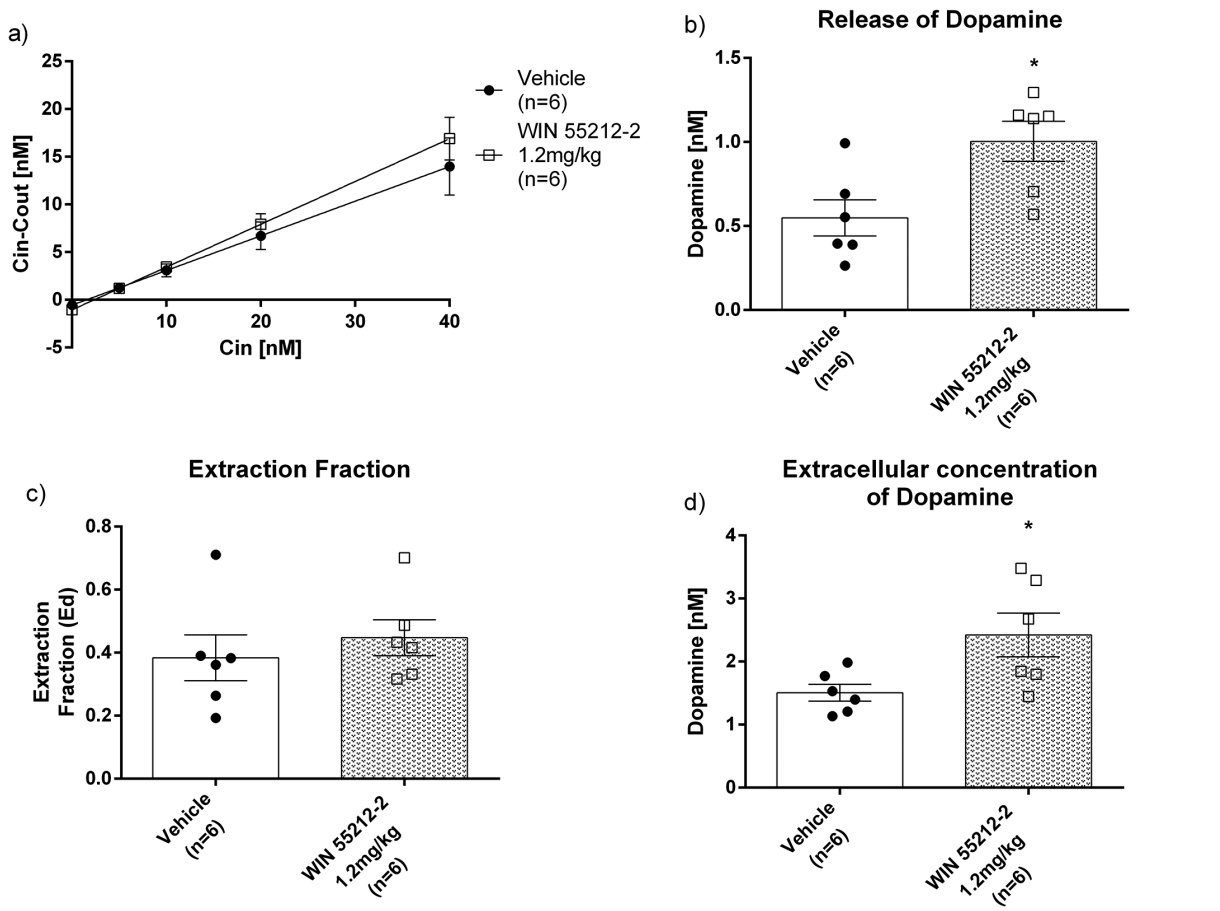

Figure 3. Adolescent exposure to WIN 55212-2 increased dopamine release and dopamine extracellular concentration (C_{ext}) in DLS in adult rats. In vivo no-net flux microdialysis in anesthetized adult rats was carried out in vehicle (n = 6) and WIN 55212-2 adolescent exposed rats (n = 6). (a) The plot of the net change in dopamine ($C_{in} - C_{out}$) was plotted against perfusion concentration (C_{in}) and the average linear regression of adult rats exposed to vehicle (black circle) or WIN 55212-2 (white square) during adolescence. (b) Basal dopamine dialysate levels. *P < .05 compared with vehicle group; unpaired *t* test. (c) Extraction fraction. (d) Dopamine C_{ext}. *P < .05 compared with vehicle group; unpaired *t* test. Data correspond to mean ± SEM.

not observed in adult rats treated with vehicle after bicuculline perfusion (Figure 6a; buffer/vehicle group: 0.905 ± 0.11 cells/track, n = 7 vs bicuculline/vehicle group: 1.09 ± 0.16 cells/track, n = 9; *P* = .57, according to Sidak post-test). Perfusion of bicuculline into the VP reversed the increase in dopamine neuron population activity induced by adolescent exposure to WIN 55212-2 (Figure 6a; bicuculline/vehicle group: 1.09 ± 0.16 cells/track, n = 9 vs bicuculline/WIN group: 0.88 ± 0.10 cells/track, n = 8; *P* = .45, Sidak post-test; buffer/WIN group: 1.39 ± 0.15 cells/track, n = 7 vs bicuculline/WIN group: 0.88 ± 0.10 cells/track, n = 8; *P* = .028, Sidak post-test).

In addition, a 2-way ANOVA showed no significant effect of adolescent treatment of WIN55212-2 ($F_{1187} = 1.615$; *P* = .205) and bicuculline perfusion into the VP ($F_{1187} = 3.570$; *P* = .06) on dopamine neuron firing rate. The interaction was considered not significant ($F_{1187} = 0.065$; *P* = .799) (Figure 6b). Also, a nonsignificant effect of WIN 55212-2 treatment during adolescence ($F_{1187} = 1.79$; *P* = .183) and bicuculline perfusion into the VP ($F_{1187} = 0.21$; *P* = .650) was observed on dopamine neuron burst firing pattern. The interaction was considered not significant ($F_{1187} = 0.001$; *P* = .980) (Figure 6c).

Discussion

Cannabinoid use in the adolescent population has increased significantly in recent years across the world (UNODC, 2018). However, information regarding its long-term effects on brain activity is still sparse. Our study reveals that adolescent exposure to the cannabinoid agonist WIN 55212-2 renders the nigrostriatal pathway activated during adulthood. Electrophysiological and neurochemical approaches show an increase in the number of active dopamine neurons in the SNc accompanied by a significant increase in the dopamine C_{ext} in DLS of adult rats. Interestingly, blocking GABAa receptors in the VP reverses the increase in dopamine neuron population activity in the SNc of adult rats treated with WIN 55212-2 during adolescence. The consequences of the VP-nigrostriatal circuit dysfunction induced by adolescent exposure to WIN 55212-2 on the limbic/cognitive/motor process during adulthood remains to be addressed.

The no-net flux experiments indicate that adolescent WIN 55212-2 is accompanied by an increase in DLS dopamine release without significant changes in Ed in the DLS of adult rats. An increase in dopamine release is consistent with previous evidence showing an increase of dopamine turnover in DLS of adult rats after repeated treatment with WIN 55212-2 (2 mg/kg) during early adolescence (PD 35–48) (Bortolato et al., 2014). Although an inhibitory effect of acute WIN 55212-2 on dopamine uptake has been observed in adolescent (Pérez-Valenzuela et al., 2019) and adult (Pandolfo et al., 2011) rats, no changes in dopamine Ed in DLS was evident in adult rats, suggesting that adolescent

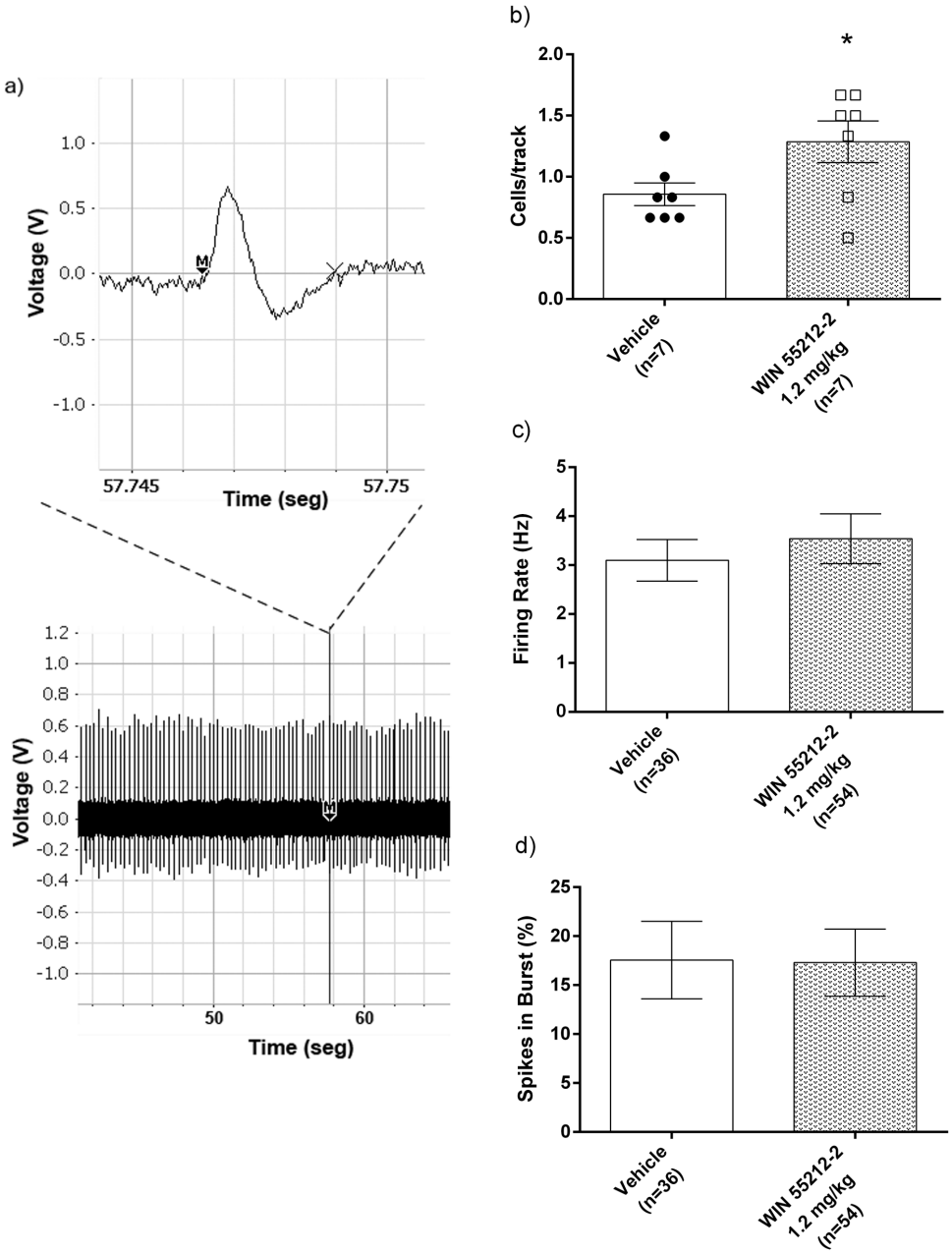

Figure 4. Adolescent exposure to WIN 55212-2 increased substantia nigra pars compacta (SNc) dopamine neuron population activity in adult rats. In vivo single-unit recording in anesthetized adult animals was carried out in vehicle (n = 7) and WIN 55212-2 adolescent exposed rats (n = 7). (a) Example of action potential (top) and a trace of SNc dopamine neuron (bottom). (b) Population activity of dopamine neurons. *$P < .05$ compared with vehicle group; unpaired *t* test. (c) Average firing rate. (d) Percentage of spike in a burst. Data correspond to mean ± SEM.

exposure to WIN 55212-2 is not accompanied by enduring modifications in DAT activity. In line with our observations, previous experiments of radioligand binding have shown that treatment with Δ^9-THC during early adolescence (PD 28–38) did not modify the binding of DAT in the dorsal striatum of adult male rats (Higuera-Matas et al., 2010). Collectively, the no-net flux experiments support the idea that the increase in dopamine C_{ext} after adolescent exposure to WIN 55212-2 depends more on neuronal excitability than on pre-synaptic control involving dopamine uptake.

Studies have consistently reported that an increase in the activity of mesencephalic dopamine neurons produces an increase in dopamine efflux in the terminal regions (Floresco et al., 2003; Panin et al., 2012). Simultaneous microdialysis and extracellular recording experiments showed that an increase in firing rate and burst firing of SNc dopamine neurons increases basal dopamine dialysate in the DLS (Panin et al., 2012). In addition, evidence indicates that an increase in the proportion of spontaneous firing dopamine neurons produces an increase of basal dopamine dialysate (Floresco et al., 2003). Supporting the rise in dopamine C_{ext} in DLS, in vivo single-unit recordings showed that adolescent exposure to WIN 55212-2 increases the population activity of dopamine neurons in the SNc without significant changes in firing rate or burst activity. A similar consequence of adolescent exposure to cannabinoids has been observed in VTA dopamine neurons (Gomes et al., 2015; Renard et al., 2017).

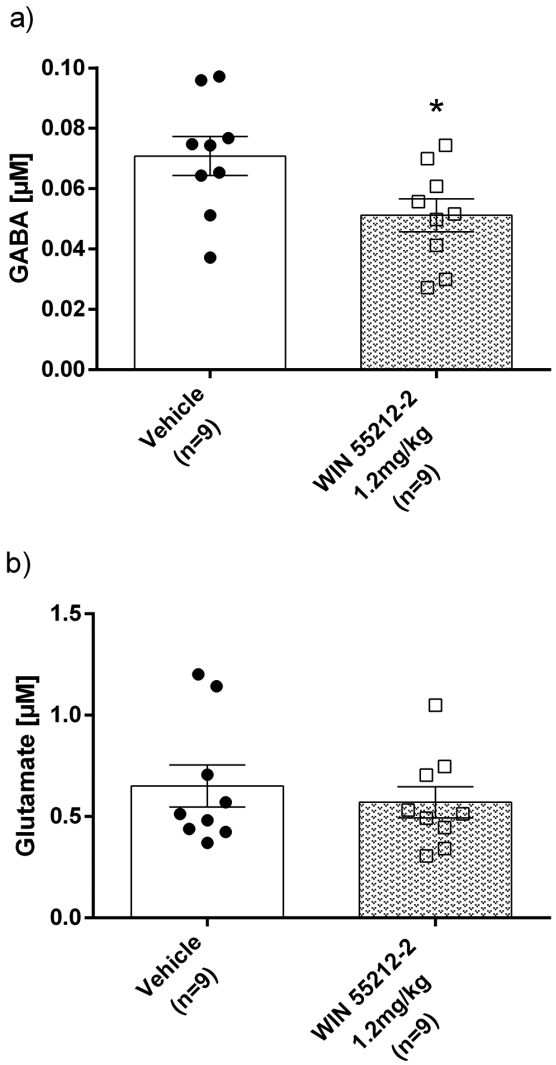

Figure 5. Adolescent exposure to WIN 55212-2 decreased basal gamma-aminobutyric acid (GABA) dialysate in the substantia nigra pars compacta (SNc) of adult rats. In vivo conventional microdialysis in anesthetized adult rats was carried out following vehicle (n=9) or WIN 55212-2 exposure during adolescence (n=9). Data correspond to mean±SEM. (a) Basal GABA dialysate. *P<.05 compared with vehicle group; unpaired *t* test. (b) Basal glutamate dialysate.

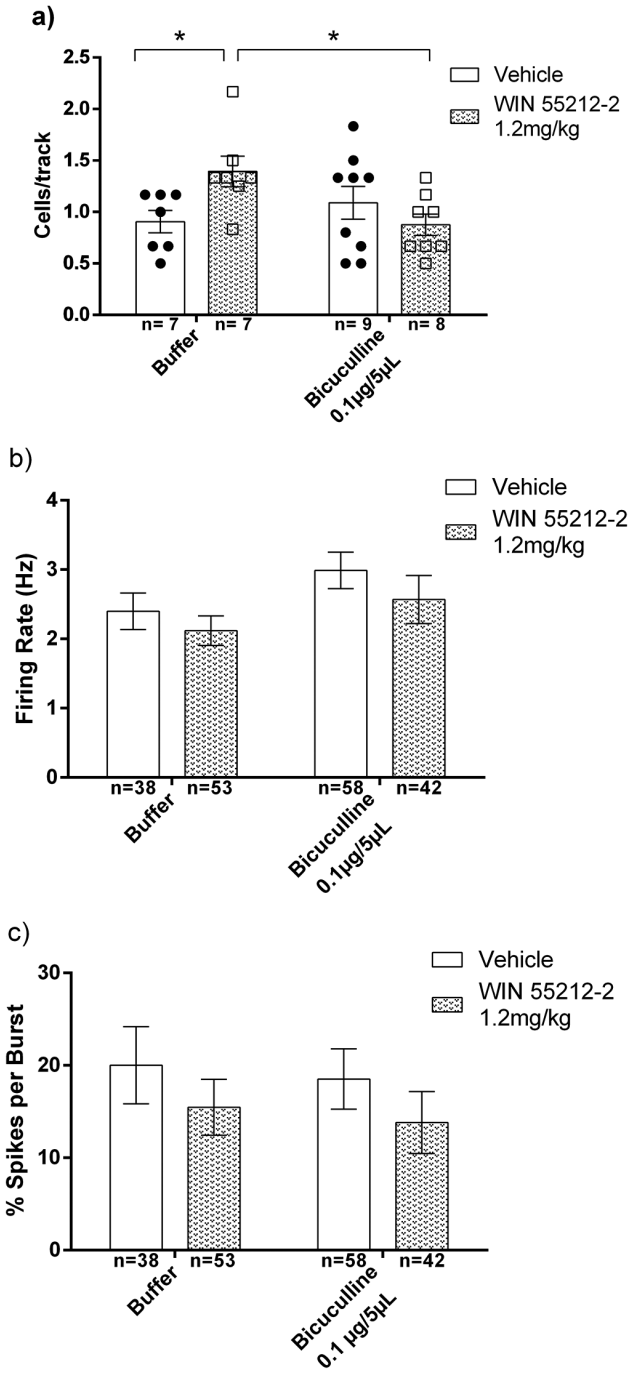

Figure 6. Local perfusion of bicuculline into the ventral pallidum (VP) reversed the increase in dopamine neuron spontaneous activity induced by adolescent exposure to WIN 55212-2. Local perfusion of bicuculline (0.1 µg/0.5 µL) or buffer solution was carried out in VP 10 minutes before in vivo single-unit recording. Recordings were performed in anesthetized adult rats following vehicle (buffer/vehicle n=7 and bicuculline/vehicle n=9) and WIN 55212-2 adolescent exposure (buffer/WIN n=7 and bicuculline/WIN n=8). Data correspond to mean±SEM. (a) Population activity of dopamine neurons. *P<.05 compared with buffer/WIN group, Sidak post-test. (b) Average firing rate. (c) Q1262039 Percentage of spike in a burst.

Here, Gomes et al. (2015) showed that intermittent exposure to WIN 552112-2 during adolescence (PD 40–65) produces an increase in VTA DA neuron spontaneous activity (Gomes et al., 2015). Furthermore, Renard et al. (2017) showed that adolescent exposure to Δ^{9}-THC increases the firing rate, bursting activity, and population activity of VTA dopamine neurons (Renard et al., 2017). Together, our results indicate that the increase in dopamine C_{ext} in DLS of adult rats after adolescent exposure to WIN 55212-2 depends on the facilitation of dopamine release supported by an increase in the number of spontaneously active dopamine neurons in the SNc.

It has been suggested that the number of spontaneously active dopamine neurons is regulated by GABA neurotransmission, while the burst pattern of dopamine neurons depends on glutamate neurotransmission (Floresco et al., 2003). Consistent with our recording data, microdialysis experiments showed a decrease in GABA extracellular concentration without changes in glutamate extracellular concentration in SNc of adult rats exposed to WIN 55212-2 during adolescence. Interestingly, a decrease in GABA neurotransmission in the adult medial prefrontal cortex has been observed after adolescent exposure to cannabinoid in males (Cass et al., 2014) and female (Zamberletti et al., 2014) rats. Accordingly, the decrease of GABA concentration in SNc along with attenuation of cortical inhibitory

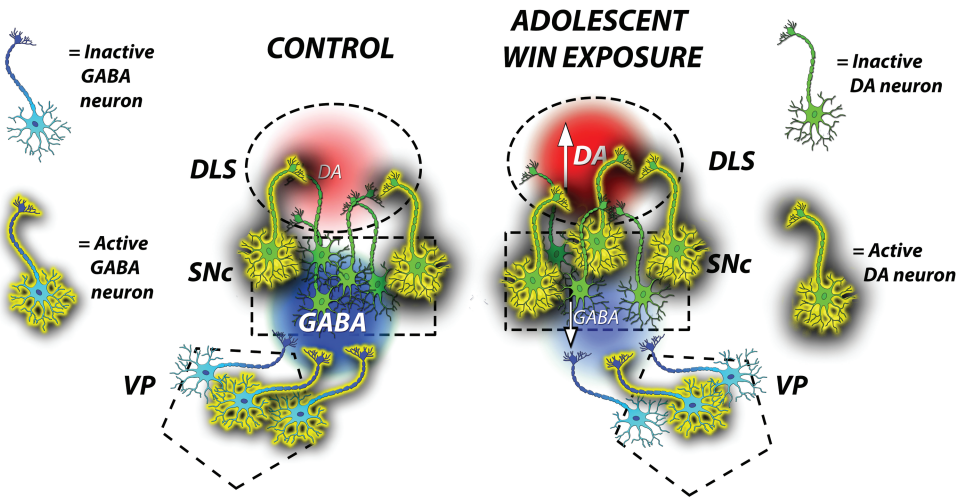

Figure 7. Adolescent WIN 55212-2 actives adult nigrostriatal dopamine neurons. Alterations in the dopaminergic neuron activity in substantia nigra pars compact (SNc) have been associated with changes in the dopamine (DA) concentrations in the dorsolateral striatum (DLS). A significant increase in population activity of DA neurons associated with a significant decrease in gamma-aminobutyric acid (GABA) concentration in SNc, and an increase in DA extracellular concentration (C_{ext}) in the DLS was observed in adult rats after an adolescent exposure to WIN 55212-2. An attenuation of GABA inhibitory tone on DA neurons in SNc driven by ventral pallidum (VP) neurons could contribute to the nigrostriatal pathway activation during adulthood after an adolescent exposure to WIN 55212-2.

neurotransmission observed after adolescent exposure to cannabinoids (Cass et al., 2014) suggests a higher vulnerability in GABA neurotransmission during adolescence.

The extracellular GABA levels in the SNc are regulated by the endocannabinoid system. It has been described that the CB_1 receptor is expressed in GABA inputs from dorsal striatum (Davis et al., 2018) and SNr (Freestone et al., 2014) onto SNc dopaminergic neurons. An increase in GABA postsynaptic currents is observed after the blocking of CB_1 receptors, indicating an inhibitory tonic control of endocannabinoids on GABA levels (Freestone et al., 2014). Although the possible contribution of the striatal and SNr GABA inputs to our results cannot be ruled out, the repeated exposure to cannabinoids during the adolescence induces desensitization of CB_1 receptors (Rubino et al., 2008), a result that would not account for the decrease in GABA extracellular levels observed in the SNc after adolescent exposure to WIN-55212-2.

As mentioned before, the SNc also receive monosynaptic inputs from the VP. Although cutting-edge neuroanatomical techniques have shown relatively low labeling between these regions, this does not imply a functional weakness (Watabe-Uchida et al., 2012). In fact, it has been shown that VP neurons modulate the population activity of dopamine neurons in the SNc (Bortz and Grace, 2018). Interestingly, CB_1 receptors are expressed in terminal axons forming mainly inhibitory synapsis in the VP (Pickel et al., 2012), suggesting that repeated exposure to WIN 55212 could modify the VP control on dopamine neurons in the SNc. Therefore, single-unit recording experiments were carried out to assess if the activated state in the dopamine neurons of SNc after the adolescent cannabinoid exposure depends on VP control. Consistent with VTA observations (Floresco et al., 2003), bicuculline perfusion into the VP did not modify the population activity of SNc dopamine neurons in adult vehicle rats. Interestingly, blocking GABAa receptors in the VP reversed the increase in population activity in the SNc after adolescent WIN 55212-2 exposure. Our results suggest that the adolescence exposure to cannabinoid disinhibits SNc dopamine neurons, decreasing GABA inhibitory tone driven by VP. While acute exposure to WIN 55212-2 decreased the GABA extracellular levels in VP (Caillé and Parsons, 2006), the desensitization of CB_1 signaling after repeated exposure to WIN 55212-2 (Rubino and Parolaro, 2008; Rubino et al., 2008) would increase the GABA tone in the VP, disinhibiting the nigrostriatal dopaminergic pathway.

To our knowledge, this study is the first evidence that shows the vulnerability and consequences of adolescent repeated exposure to cannabinoids in the nigrostriatal dopaminergic transmission of adult rats. Several questions remain unanswered at present. Further research is necessary to assess whether other GABA inputs contribute to the disinhibition of the nigrostriatal pathway after adolescent exposure to WIN 55212-2. Microdialysis experiments should be carried out to determine the effect of VP activation on the GABA and dopamine levels in the nigrostriatal pathway. Although previous evidence suggests an age-related vulnerability, it remains to be addressed whether these effects are observed during adult exposure to WIN 55212-2 and during long withdrawal after adolescent exposure to cannabinoids.

In summary, our results show that adolescent exposure to WIN 55212-2 increases the dopamine C_{ext} in DLS accompanied by an increase in the population activity of dopamine neurons in SNc (Figure 7). In addition, this increase in dopamine neuron activity was reversed after blocking GABAa receptors in the VP. These pieces of evidence suggest that repeated activation of CB_1 receptors during adolescence is accompanied by long-term consequences on the nigrostriatal dopaminergic pathway. As recently suggested, the activated state of dopaminergic nigrostriatal induced by adolescent cannabinoid exposure is a risk factor that could contribute to compulsive drug-seeking habit behavior during adulthood (Giuliano et al., 2019).

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Statement of Interest

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References

Azocar VH, Sepúlveda G, Ruiz C, Aguilera C, Andrés ME, Fuentealba JA (2019) The blocking of kappa-opioid receptor reverses the changes in dorsolateral striatum dopamine dynamics during the amphetamine sensitization. J Neurochem 148:348–358.

Baunez C, Dias C, Cador M, Amalric M (2005) The subthalamic nucleus exerts opposite control on cocaine and "natural" rewards. Nat Neurosci 8:484–489.

Bortolato M, Bini V, Frau R, Devoto P, Pardu A, Fan Y, Solbrig MV (2014) Juvenile cannabinoid treatment induces frontostriatal gliogenesis in Lewis rats. Eur Neuropsychopharmacol 24:974–985.

Bortz DM, Grace AA (2018) Medial septum differentially regulates dopamine neuron activity in the rat ventral tegmental area and substantia nigra via distinct pathways. Neuropsychopharmacology 43:2093–2100.

Caillé S, Parsons LH (2006) Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. Neuropsychopharmacology 31:804–813.

Cass DK, Flores-Barrera E, Thomases DR, Vital WF, Caballero A, Tseng KY (2014) CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. Mol Psychiatry 19:536–543.

Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y (2012) Endocannabinoid signaling and synaptic function. Neuron 76:70–81.

Chefer VI, Czyzyk T, Bolan EA, Moron J, Pintar JE, Shippenberg TS (2005) Endogenous kappa-opioid receptor systems regulate mesoaccumbal dopamine dynamics and vulnerability to cocaine. J Neurosci 25:5029–5037.

Chefer VI, Zapata A, Shippenberg TS, Bungay PM (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.

Corrigall WA, Coen KM, Zhang J, Adamson L (2002) Pharmacological manipulations of the pedunculopontine tegmental nucleus in the rat reduce self-administration of both nicotine and cocaine. Psychopharmacology (Berl) 160:198–205.

Covey DP, Mateo Y, Sulzer D, Cheer JF, Lovinger DM (2017) Endocannabinoid modulation of dopamine neurotransmission. Neuropharmacology 124:52–61.

Davis MI, Crittenden JR, Feng AY, Kupferschmidt DA, Naydenov A, Stella N, Graybiel AM, Lovinger DM (2018) The cannabinoid-1 receptor is abundantly expressed in striatal striosomes and striosome-dendron bouquets of the substantia nigra. Plos One 13:e0191436.

Doremus-Fitzwater TL, Spear LP (2016) Reward-centricity and attenuated aversions: an adolescent phenotype emerging from studies in laboratory animals. Neurosci Biobehav Rev 70:121–134.

Egertová M, Elphick MR (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol 422:159–171.

Escobar AP, Cornejo FA, Andrés ME, Fuentealba JA (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.

Everitt BJ, Robbins TW (2013) From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. Neurosci Biobehav Rev 37:1946–1954.

Everitt BJ, Robbins TW (2016) Drug addiction: updating actions to habits to compulsions ten years on. Annu Rev Psychol 67:23–50.

Farrell MR, Ruiz CM, Castillo E, Faget L, Khanbijian C, Liu S, Schoch H, Rojas G, Huerta MY, Hnasko TS, Mahler SV (2019) Ventral pallidum is essential for cocaine relapse after voluntary abstinence in rats. Neuropsychopharmacology 44:2174–2185.

Field KJ, White WJ, Lang CM (1993) Anaesthetic effects of chloral hydrate, pentobarbitone and urethane in adult male rats. Lab Anim 27:258–269.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968–973.

Freestone PS, Guatteo E, Piscitelli F, di Marzo V, Lipski J, Mercuri NB (2014) Glutamate spillover drives endocannabinoid production and inhibits GABAergic transmission in the substantia nigra pars compacta. Neuropharmacology 79:467–475.

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

Giuliano C, Belin D, Everitt BJ (2019) Compulsive alcohol seeking results from a failure to disengage dorsolateral striatal control over behavior. J Neurosci 39:1744–1754.

Gomes FV, Guimarães FS, Grace AA (2015) Effects of pubertal cannabinoid administration on attentional set-shifting and dopaminergic hyper- responsivity in a developmental disruption model of schizophrenia. Int J Neuropsychopharmacol 18:1–10.

Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons–1. Identification and characterization. Neuroscience 10:301–315.

Gumbleton M, Benet LZ (1991) Drug metabolism and laboratory anesthetic protocols in the rat: examination of antipyrine pharmacokinetics. Pharm Res 8:544–546.

Heifets BD, Castillo PE (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol 71:283–306.

Heinsbroek JA, Bobadilla AC, Dereschewitz E, Assali A, Chalhoub RM, Cowan CW, Kalivas PW (2020) Opposing regulation of cocaine seeking by glutamate and GABA neurons in the ventral pallidum. Cell Rep 30:2018–2027.e3.

Higuera-Matas A, Botreau F, Del Olmo N, Miguéns M, Olías Ó, Montoya GL, 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.

Howard SG, Feigenbaum JJ (1997) Effect of gamma-hydroxybutyrate on central dopamine release in vivo.

A microdialysis study in awake and anesthetized animals. Biochem Pharmacol 53:103–110.

Ito R, Dalley JW, Robbins TW, Everitt BJ (2002) Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J Neurosci 22:6247–6253.

Julian MD, Martin AB, Cuellar B, Rodriguez De Fonseca F, Navarro M, Moratalla R, Garcia-Segura LM (2003) Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. Neuroscience 119:309–318.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89:309–380.

Kelland MD, Chiodo LA, Freeman AS (1990) Anesthetic influences on the basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons. Synapse 6:207–209.

Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. The Lancet Psychiatry 3:760–773.

Lopez-Quintero C, de los Cobos JP, Hasin DS, Okuda M, Wang S, Grant BF, Blanco C (2011) Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the national epidemiologic survey on alcohol and related conditions (NESARC). Drug Alcohol Depend 115:120–130.

Mátyás F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF (2006) Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. Neuroscience 137:337–361.

Mátyás F, Urbán GM, Watanabe M, Mackie K, Zimmer A, Freund TF, Katona I (2008) Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. Neuropharmacology 54:95–107.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.

Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol 64:21–47.

Pandolfo P, Silveirinha V, dos Santos-Rodrigues A, Venance L, Ledent C, Takahashi RN, Cunha RA, Köfalvi A (2011) Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. Eur J Pharmacol 655:38–45.

Panin F, Cathala A, Piazza PV, Spampinato U (2012) Coupled intracerebral microdialysis and electrophysiology for the assessment of dopamine neuron function in vivo. J Pharmacol Toxicol Methods 65:83–92.

Paxinos G, Watson C (2009) The rat brain in stereotaxic coordinates. Compact 6th ed. New York: Elsevier Science.

Pérez-Valenzuela E, Castillo-Faúndez R, Fuentealba JA (2019) Comparing dopaminergic dynamics in the dorsolateral striatum between adolescent and adult rats: effect of an acute dose of WIN55212-2. Brain Res 1719:235–242.

Pertwee RG (2014) Handbook of cannabis. Oxford: Oxford University Press.

Pickel VM, Shobin ET, Lane DA, Mackie K (2012) Cannabinoid-1 receptors in the mouse ventral pallidum are targeted to axonal profiles expressing functionally opposed opioid peptides and contacting N-acylphosphatidylethanolamine-hydrolyzing phospholipase D terminals. Neuroscience 227:10–21.

Pistis M, Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL (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.

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.

Renard J, Rosen LG, Loureiro M, De Oliveira C, Schmid S, Rushlow WJ, Laviolette SR (2017) Adolescent cannabinoid exposure induces a persistent sub-cortical hyper-dopaminergic state and associated molecular adaptations in the prefrontal cortex. Cereb Cortex 27:1297–1310.

Robinson MJF, Fischer AM, Ahuja A, Lesser EN, Maniates H (2016) Roles of "wanting"; and "liking" in motivating behavior: gambling, food, and drug addictions. Curr Top Behav Neurosci 27:105–136.

Root DH, Melendez RI, Zaborszky L, Napier TC (2015) The ventral pallidum: subregion-specific functional anatomy and roles in motivated behaviors. Prog Neurobiol 130:29–70.

Rubino T, Parolaro D (2008) Long lasting consequences of cannabis exposure in adolescence. Mol Cell Endocrinol 286:108–113.

Rubino T, Vigano' D, Realini N, Guidali C, Braida D, Capurro V, Castiglioni C, Cherubino F, Romualdi P, Candeletti S, Sala M, Parolaro D (2008) Chronic Δ9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology 33:2760–2771.

Schneider M (2008) Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict Biol 13:253–263.

Schneider M, Koch M (2003) Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology 28:1760–1769.

Schneider M, Koch M (2005) Deficient social and play behavior in juvenile and adult rats after neonatal cortical lesion: effects of chronic pubertal cannabinoid treatment. Neuropsychopharmacology 30:944–957.

Smith AD, Justice JB (1994) The effect of inhibition of synthesis, release, metabolism and uptake on the microdialysis extraction fraction of dopamine. J Neurosci Methods 54:75–82.

Sotomayor-Zárate R, Araya KA, Pereira P, Blanco E, Quiroz G, Pozo S, Carreño P, Andrés ME, Forray MI, Gysling K (2010) Activation of GABA-B receptors induced by systemic amphetamine abolishes dopamine release in the rat lateral septum. J Neurochem 114:1678–1686.

Steiner H, Tseng KY (2017) Handbook of basal ganglia structure and function. London: Academic Press.

Sturman DA, Moghaddam B (2011) The neurobiology of adolescence: changes in brain architecture, functional dynamics, and behavioral tendencies. Neurosci Biobehav Rev 35:1704–1712.

Tanda G, Pontieri FE, Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048–2050.

Tepper JM, Creese I, Schwartz DH (1991) Stimulus-evoked changes in neostriatal dopamine levels in awake and anesthetized rats as measured by microdialysis. Brain Res 559:283–292.

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Ungless MA, Grace AA (2012) Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. Trends Neurosci 35:422–430.

UNODC (2018) World drug report. Available at: https://www.unodc.org/wdr2018. Accessed 3 June 2020.

Volkow ND, Baler RD, Compton WM, Weiss SR (2014) Adverse health effects of marijuana use. N Engl J Med 370:2219–2227.

Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N (2012) Whole-brain mapping of direct inputs to midbrain dopamine neurons. Neuron 74:858–873.

Willuhn I, Burgeno LM, Everitt BJ, Phillips PE (2012) Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use. Proc Natl Acad Sci U S A 109:20703–20708.

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: role in drug addiction. Neuroscience 301:529–541.

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

Zamberletti E, Beggiato S, Steardo L Jr, Prini P, Antonelli T, Ferraro L, Rubino T, Parolaro D (2014) Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol Dis 63:35–47.