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Behavioral and Neurochemical Changes in Mesostriatal Dopaminergic Regions of the Rat after Chronic Administration of the Cannabinoid Receptor Agonist WIN55,212-2

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Abstract

Background: The endocannabinoid system interacts extensively with other neurotransmitter systems and has been implicated in a variety of functions, including regulation of basal ganglia circuits and motor behavior. The present study examined the effects of repeated administration of the nonselective cannabinoid receptor 1 agonist WIN55,212-2 on locomotor activity and on binding and mRNA levels of dopamine receptors and transporters and $GABA_A$ receptors in mesostriatal dopaminergic regions of the rat.

Methods: Rats received systemic injections of WIN55,212-2 (0, 0.1, 0.3, or 1 mg/kg, intraperitoneally) for 20 consecutive days. Locomotor activity was measured on days 1, 10, and 20. Following the last measurement, rats were euthanized and prepared for in vitro binding and in situ hybridization experiments.

Results: Acutely, 0.3 and 1 mg/kg of WIN55,212-2 produced hypolocomotion, which was sustained for the next 2 measurements, compared to vehicle. Repeated administration of WIN55,212-2 decreased the mRNA levels of the D2 autoreceptors in substantia nigra and ventral tegmental area and increased D1 receptor mRNA and binding in nucleus accumbens. Furthermore, both dopamine receptor and transporter binding and mRNA levels were decreased in substantia nigra. Moreover, repeated administration of WIN55,212-2 decreased $GABA_A$ receptor binding levels in dorsal striatum and substantia nigra.

Conclusions: Our data indicate that chronic WIN55,212-2 administration results in sustained effects on locomotor activity, similar to those observed after acute administration, and modulates the dopaminergic and GABAergic systems in a region-, dose-, and neurotransmitter-selective manner

Keywords: WIN55,212-2, locomotor activity, dopamine transporter, dopamine receptors, GABAA receptor

Introduction

The rewarding and motor effects produced by Δ^9-tetrahydrocannabinol (Δ^9-THC) and synthetic cannabinoid agonists are mediated primarily by cannabinoid 1 receptors (CB1R) (Ameri, 1999). CB1R are abundantly expressed in the basal ganglia (Herkenham et al., 1991a), a brain region involved in motor control (Martín et al., 2008; Morera-Herreras et al., 2008). It has been shown that systemic administration of Δ^9-THC and other CB1R agonists exerts biphasic effects on motor activity, with low doses increasing motor activation and higher doses producing hypolocomotion or even catalepsy (Sañudo-Peña et al., 2000; Drews et al., 2005; Shi et al., 2005; Rodvelt et al., 2007; Polissidis et al., 2010, 2013; Katsidoni et al., 2013).

It has been suggested that cannabinoids elicit their pharmacological effects in part through activation of dopaminergic neurons in the brain and more specifically the mesostriatal dopaminergic system with cell bodies located within the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc) (French et al., 1997; Rodríguez De Fonseca et al., 2001; Pan et al., 2008; Morera-Herreras et al., 2008) by enhancing dopamine release in their respective dopamine terminal fields, the nucleus accumbens (NAc) (Tanda et al., 1997) and striatum (Taylor et al., 1988).

Interestingly, CB1Rs do not appear to be expressed at dopaminergic terminals in these main dopaminoceptive regions (Herkenham et al., 1991a, 1991b; Mailleux and Vanderhaeghen, 1992, 1993; Matsuda et al., 1993; Westlake et al., 1994; Julian et al., 2003) but rather on presynaptic GABAergic interneurons (Katona et al., 2000) and glutamatergic projecting neurons (Hermann et al., 2002), indicating that the effects of cannabinoids on dopamine neurotransmission are mainly indirect and modulated via the function of other neurotransmitters, such as GABA and glutamate. Indeed, it is well documented that cannabinoids affect extracellular levels of GABA in hippocampus (Katona et al., 1999), prefrontal cortex (Pistis et al., 2002), amygdala (Katona et al., 2001), and glutamate in cerebral (Ferraro et al., 2001) and prefrontal cortex (Pistis et al., 2002) and striatum (Polissidis et al., 2013). In the striatum, CB1Rs are localized presynaptically on GABAergic and glutamatergic terminals (Matsuda et al., 1993) and postsynaptically in the somata, dendrites, and axon terminals of striatal medium spiny neurons (Fitzgerald et al., 2012). Activation of CB1R inhibits GABAergic neurotransmission in the globus pallidus (Pertwee et al., 1988), NAc (Manzoni and Bockaert, 2001), SN (Wallmichrath and Szabo, 2002), and VTA (Szabo et al., 2002) via inhibition of adenylate cyclase (Pertwee, 2006).

Striatal medium spiny projection neurons (MSNs) expressing D1 dopamine receptors (D1DRs) form the direct pathway, while neurons expressing D2 receptors (D2DRs) form the indirect pathway. Activation of D1DR leads to stimulation of adenylate cyclase (Blandini et al., 2000) and cAMP formation (Gingrich and Caron, 1993) and, in turn, to activation of the direct pathway (van der Stelt and Di Marzo, 2003) while activation of D2DR inhibits adenylate cyclase (Blandini et al., 2000) and cAMP formation, leading to inhibition of striatal MSNs that project to the nuclei of the indirect pathway.

Several studies suggest an interaction between CB1R and D1/D2 dopamine receptors (D1DR/D2DR) at the cellular level and coupling to the same effector systems (Hermann et al., 2002). Simultaneous activation of both CB1R and D2DR leads to enhanced activation of adenylyl cyclase resulting in activation of striatal neurons of the indirect pathway, which in turn activates neurons of the subthalamic nuclei, resulting in hypomotility (Glass and Felder, 1997; Kearn et al., 2005; Martín et al., 2008). Moreover, simultaneous stimulation of CB1R and D1DR leads to a net decrease in adenylyl cyclase, which in turn reduces the inhibitory activity of direct striatal projection neurons. This inhibition increases the activity of nigral neurons, resulting in a decreased motor response (Martín et al., 2008).

The present study was designed to correlate the behavioral effects of chronic WIN55,212-2 with changes in neurochemical indices. In this context and based on the key role of dopamine and GABA in the mesostriatal dopaminergic system, we investigated the effects of systemic acute and chronic administration of low and high doses of WIN55,212-2, a nonselective CB1R agonist, on motor activity patterns and characterized neurochemical alterations of dopaminergic and GABAergic systems. WIN55,212-2 has higher efficacy than other CB1R agonists (Kearn et al., 1999) and has been well characterized in behavioral and neurochemical studies (Manzoni and Bockaert, 2001; Wallmichrath and Szabo, 2002; Castañé et al., 2004; Vlachou et al., 2008; Moranta et al., 2009; Mavrikaki et al., 2010; Polissidis et al., 2013). We examined the dopamine transporter (DAT), dopamine, and GABA receptors in the striatum, NAc, SN, and VTA of WIN55,212-2-treated and vehicle-treated rats. In vitro binding and in situ hybridization experiments were used to evaluate binding and mRNA levels of the aforementioned receptors and transporters.

Methods

Animals

Male Sprague-Dawley rats (n=40) weighing 250 to 300 g (postnatal days 60 to 70) were used. The animals were housed in groups of 2 or 3 under a 12-h-light/-dark cycle with free access to food and water. Experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

WIN55,212-2 Treatment

WIN55,212-2 (Tocris, Westwoods Bus. Park) was dissolved in a vehicle solution containing 5% dimethylsulfoxide, 5% cremophor EL in 0.9% NaCl, and injected intraperitoneally (i.p.) at a volume of 3 mL/kg of body weight. Experimental animals were divided into 4 groups (n=10 per group) receiving a single daily i.p. injection of either vehicle or WIN55,212-2 (vehicle, 0.1, 0.3, and 1 mg/kg) for 20 days. Control animals (n=10) received i.p. the corresponding vehicle solution in the same injection volume. All rats were tested for locomotor activity and used for neurochemical studies (n=10/group). Animals were euthanized by rapid decapitation 1.5 hours after the last injection.

Locomotor Activity

Spontaneous motor activity was measured using an activity recording system (Model 7445, Ugo Basile) consisting of an animal cage and an electronic unit incorporating a counter and a printer. The rectangular animal cage (56×56×30 cm) had transparent sides and lid to allow observation. The cage floor had horizontal and vertical infrared sensors. The counter summed up the photocell disruptions, and a printer displayed the results at preset intervals. In our studies, a summation of photocell

disruptions of ambulatory distance and rearing for each 5-minute interval period during the 1-hour observation period was registered. Behavioral testing was performed one the first, 10th, and 20th day of the drug treatment between 8:00 AM and 4:00 PM, 10 minutes after drug administration. The postinjection time was selected taking into account that the behavioral effects lasted for approximately 1 hour.

Brain Sectioning for Neurochemical Studies

Rats were euthanized by rapid decapitation. Brains were isolated and quickly frozen in 2-methyl-butane. Coronal sections, 14 µm thick, were cut in a cryostat Leica (CM1850), thaw-mounted on gelatin-chromalum–coated glass slides (for autoradiography studies) or poly-L-lysine–coated slides (for in situ hybridization studies), dried at room temperature (RT), and stored at –75°C until experiments were performed.

Receptor Binding Autoradiography

DAT binding was assayed according to Dickinson et al. (1999) using 5 nM [^{3}H]-WIN35428 (S.A. 87 Ci/mmol; PerkinElmer Life Sciences, Belgium) as radioligand. Sections were allowed to air-dry at RT, were preincubated for 30 minutes in 20 mM sodium phosphate buffer (PBS), pH 7.4, at 4°C, and were then incubated for 90 minutes in buffer containing 0.32 M sucrose, pH 7.4, at 4°C in the presence of radioligand. Sections were washed for 2 × 1 minutes in 20 mM PBS, pH 7.4, at 4°C, briefly dipped in ice-cold distilled water, and air-dried. Nonspecific binding was determined in the presence of 30 µM benztropine (Sigma Aldrich, Greece).

D2- and D1-like dopamine receptors were assayed as described by Tarazi et al. (1998). Sections were preincubated at RT for 1 hour in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, pH 7.4, and incubated for 1 hour at RT in the presence of [^{3}H]raclopride (2 nM; S.A. 62.2 Ci/mmol; PerkinElmer Life Sciences, Belgium) and [^{3}H]SCH23390 (2.5 nM; S.A. 85Ci/mmol; PerkinElmer Life Sciences, Belgium) for D2- and D1-like receptors, respectively. For D1-like receptors, the incubation buffer contained 40 nM ketanserin (Tocris, UK) to block the 5-HT2 serotonin binding sites. After incubation, sections were rinsed 2 × 5 minutes in ice-cold buffer, briefly dipped in ice-cold distilled water, and air-dried. Nonspecific binding was determined with l µM cis-flupenthixol (Sigma-Aldrich, Greece) for both receptors.

GABA$_A$ receptor binding was performed according to Bristow and Martin (1988). Sections were preincubated at RT for 30 minutes in buffer containing 50 mM Tris-citrate, 100 mM MgCl$_2$, pH 7.4, air-dried, and incubated with 6.5 nM [^{3}H]-SR95531 (S.A. 55.3 Ci/mmol; PerkinElmer Life Sciences, Belgium) at 4°C for 30 minutes. Nonspecific binding was determined in the presence of 10 mM GABA (Sigma Aldrich, Greece). Sections were rinsed 3 × 5 min in ice-cold buffer, briefly dipped in ice-cold distilled water, and air-dried under a stream of cold air. The labeled sections were exposed to BioMax MR Film (Kodak) for 4 to 10 months. Tritium micro scales (Amersham, UK), calibrated as nCi/mg tissue equivalent, were exposed along with the tissue samples and used as standards.

In Situ Hybridization Histochemistry

Hybridization was carried out according to Giannakopoulou et al. (2012). Sections were air-dried at RT, fixed for 5 minutes in 4% paraformaldehyde in diethylpyrocarbonate (DEPC)-treated PBS (0.1 M, pH 7.4), rinsed in PBS, dehydrated in graded ethanol, and air-dried at RT. The oligonucleotide sequences used are shown in the Supplementary Material.

Each probe was diluted to a concentration of 3 pmol/µL and labeled with ^{35}S-ATP (PerkinElmer Life Sciences, Belgium) at the 3' end, using the 3' terminal transferase enzyme (Roche, Germany) to a specific activity of 2 × 10^5 cpm/µL. Chromatography Sephadex G-50 columns (BioRad, Greece) were used to remove unincorporated nucleotides. Hybridization was performed in 50% formamide (vol/vol), 4 × saline-sodium citrate (SSC) buffer (1 × SSC: 0.15 M sodium chloride, 0.015 M sodium citrate), 10% dextran sulfate (wt/vol), and 10 mM dithiothreitol, with 1:100 labeled probe (0.03 pmol/µL final concentration of labeled probe). Sections were covered with 120 µL of hybridization solution and incubated for 18 hours in a humid chamber at 42°C. Nonspecific signal was determined by the addition of 100-fold excess of unlabeled probe to the hybridization solution. After hybridization, sections were washed in 1 × SSC for 20 minutes at 60°C, in 0.1 × SSC for 3 minutes at RT, dehydrated in graded ethanol, and air-dried at RT. Sections were exposed to a Kodak BioMax MR film and exposure time ranged from 2 to 8 weeks depending on probe labeling.

Quantification

Autoradiographs were scanned and optical densities were measured with MCID 7.0 software (Imaging Research Inc, St. Catharines, ON, Canada). The results are expressed in fmol/mg protein for receptor binding autoradiography and in relative optical density values for in situ hybridization. The values are expressed as mean ± standard error of the mean. The anatomical structures were defined according to the rat brain atlas (Fig. 2) (Paxinos and Watson, 2007) and analyzed at the same level. The striatal sections were divided in quadrants (dorsolateral (DL), dorsomedial (DM), ventrolateral (VL), and ventromedial (VM)). Measurements were taken for total as well as nonspecific binding signal from each animal. The specific signal was determined by subtracting nonspecific from total signal.

Statistical Analyses

For the locomotor activity studies, the significance of repeated drug effect was initially evaluated using 2-way analysis of variance (ANOVA) with repeated measures followed by 1-way ANOVA with (time) or without (drug) repeated measures and the Least Significance Difference (LSD) posthoc test as required. Statistical analysis for autoradiography and in situ hybridization assays was performed by 1-way ANOVA, followed by the Bonferroni posthoc test to identify differences in receptor binding or mRNA expression levels between WIN55,212-2-treated and vehicle-treated animals. The level of statistical significance was set at 0.05. Statistical analyses were conducted using the Statistical Package for the Social Sciences v.17.0 (SPSS, Chicago, IL).

Results

Locomotor Activity

Overall, both ambulatory activity and rearing decreased after repeated testing from day 1 to days 10 and 20 and in response to administration of vehicle and 0.1- and 0.3-mg/kg doses of WIN55,212-2 and from day 1 to day 10 in response to administration of 1.0 mg/kg of WIN55,212-2 (Figure 1A-B).

Considering the total (60-min session) ambulatory activity, 2-way ANOVA with repeated measures indicated a significant

drug effect ($F_{3,36}$=12.8, P<.001) as well as a significant time effect ($F_{2,72}$=29.08, P<.001) but not a significant interaction (drug×time, P>.05). One-way ANOVA with repeated measures per group demonstrated a significant time effect in all treatments (WIN55,212-2 0.1mg/kg: $F_{2,18}$=6.26, P<.05, WIN55,212-2 0.3mg/kg: $F_{2,18}$=20.60, P<.001 and WIN55,212-2 1mg/kg: $F_{2,18}$=13.63, P<.001) except vehicle ($F_{2,18}$=3,14, P=.067). One-way ANOVA on each day of measurement indicated significant effects between groups (day 1 $F_{3,36}$=7.54, P<.001; day 10 $F_{3,36}$=12.49, P<.001; day 20 $F_{3,36}$=7.5, P<.001). LSD posthoc test on each day indicated that WIN55,212–2 1mg/kg and WIN55,212-2 0.3mg/kg significantly differ from vehicle group (day 1 P<.05 for both doses; day 10 P<.01 and P<.001 for WIN55,212-2 0.3mg/kg and WIN55,212-2 1mg/kg, respectively; day 20 P<.01 for both doses), while WIN55,212-2 0.1mg/kg did not significantly affect total ambulatory activity (P>.05 compared to vehicle).

Considering total (60-minute session) rearing, 2-way ANOVA with repeated measures demonstrated a significant interaction of drug×time ($F_{6,72}$=3.27, P=.007). One-way ANOVA with repeated measures indicated that there was no significant time effect of WIN55,212-2 1mg/kg ($F_{2,18}$=3.45, P=.54). However, there was a significant time effect on WIN55,212-2 0.3mg/kg ($F_{2,18}$=4.39, P<.050), WIN55,212-2 0.1mg/kg ($F_{2,18}$=12.6, P<.001), and vehicle ($F_{2,18}$=7.24, P<.05) groups. One-way ANOVA on each measurement (day) demonstrated significant differences between groups (day 1: $F_{3,36}$=7.81, P<.001, day 10: $F_{3,36}$=6.83, P<.001, day 20: $F_{3,36}$= 5.20, P<.001). LSD posthoc test demonstrated that WIN55,212-2 1mg/kg had a significant effect on rearing on day 1 (P=.001) as well as on days 10 and 20 (P<.01). WIN55,212-2 0.3mg/kg significantly affected rearing only on day 1 (P<.05) but not on days 10 and 20 (P>.05). WIN55,212-2 0.1mg/kg did not significantly affect total rearing (P>.05).

Further analyses performed on the 5-minute bin (of total 60-minute session) on days 1, 10, and 20 of ambulatory activity and rearing are presented in Supplementary Material.

DAT

The levels of [³H]-WIN35428 binding in striatum and SN as well as NAc and VTA (Figures 3A-B, 5A-D) of vehicle and WIN55,212-2-treated rats are presented in Table 1. [³H]-WIN35428 specific binding decreased significantly in SN ($F_{3,36}$=20.76, P<.001) at the doses of 0.1, 0.3, and 1mg/kg of WIN55,212-2 (P<.001 at all doses) and in VTA ($F_{3,34}$=6.23, P=.002) at the same doses (WIN55,212-2 0.1mg/kg: P=.026, WIN55,212-2 0.3mg/kg: P=.049,

and WIN55,212-2 1mg/kg: P=.001). DAT binding site levels were also reduced at the dose 0.1mg/kg in NAc core ($F_{3,36}$=7.289, P<.001) and in the shell subdivision ($F_{3,36}$=8.56, P=.001). Significant decreases at the tested doses of WIN55,212-2 were also observed in DAT mRNA levels in SNpc ($F_{3,36}$=8.15, P<.001; Table 2; Figures 4A and 5E) at all doses (WIN55,212-2 0.1mg/kg: P=.042, WIN55,212-2 0.3mg/kg: P=.002, and WIN55,212-2 1mg/kg: P<.001) and in VTA ($F_{3,36}$=7.96, P<.001; Table 2; Figures 4A and 5F) at all doses as well (WIN55,212-2 0.1mg/kg: P=.002,
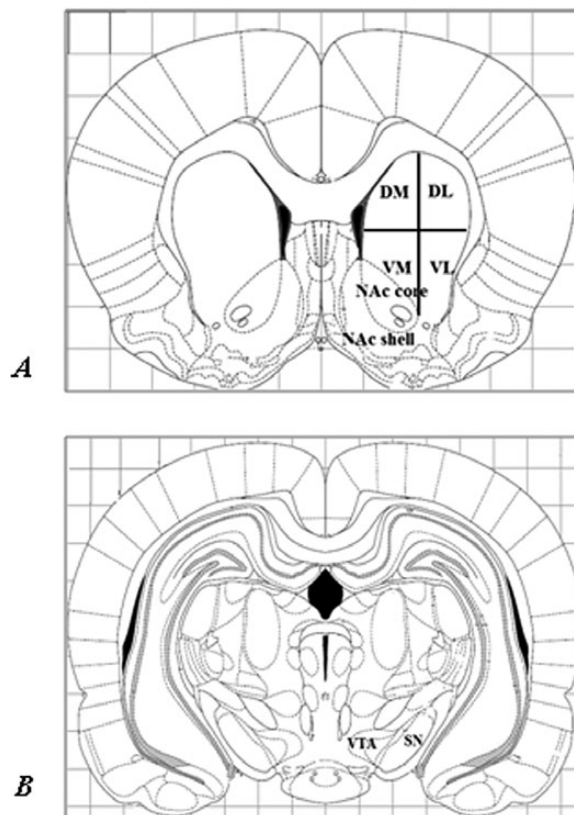

Figure 2. Images of the corresponding brain level from the stereotaxic atlas of the rat brain (Paxinos and Watson, 2007) with their subdivisions of (A) striatum/nucleus accumbens (NAc) and (B) substantia nigra (SN)/ventral tegmental area (VTA). Abbreviations: DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial.
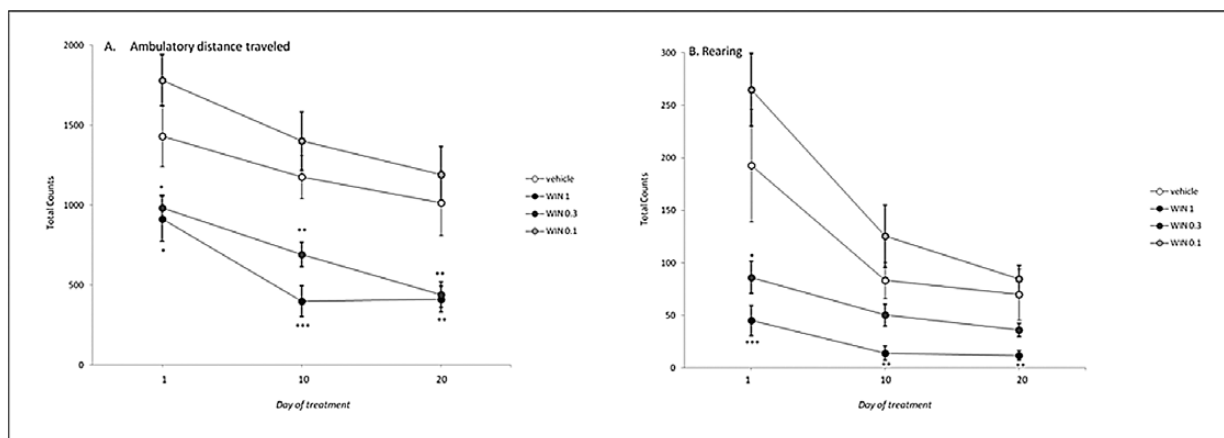

Figure 1. Effects of repeated WIN55,212-2 administration on spontaneous locomotor activity (n=10 per group). Each plot represents the photocell disruptions caused by the animal's (A) ambulatory activity and (B) rearing. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *P<.05, **P<.01, and ***P<.001.
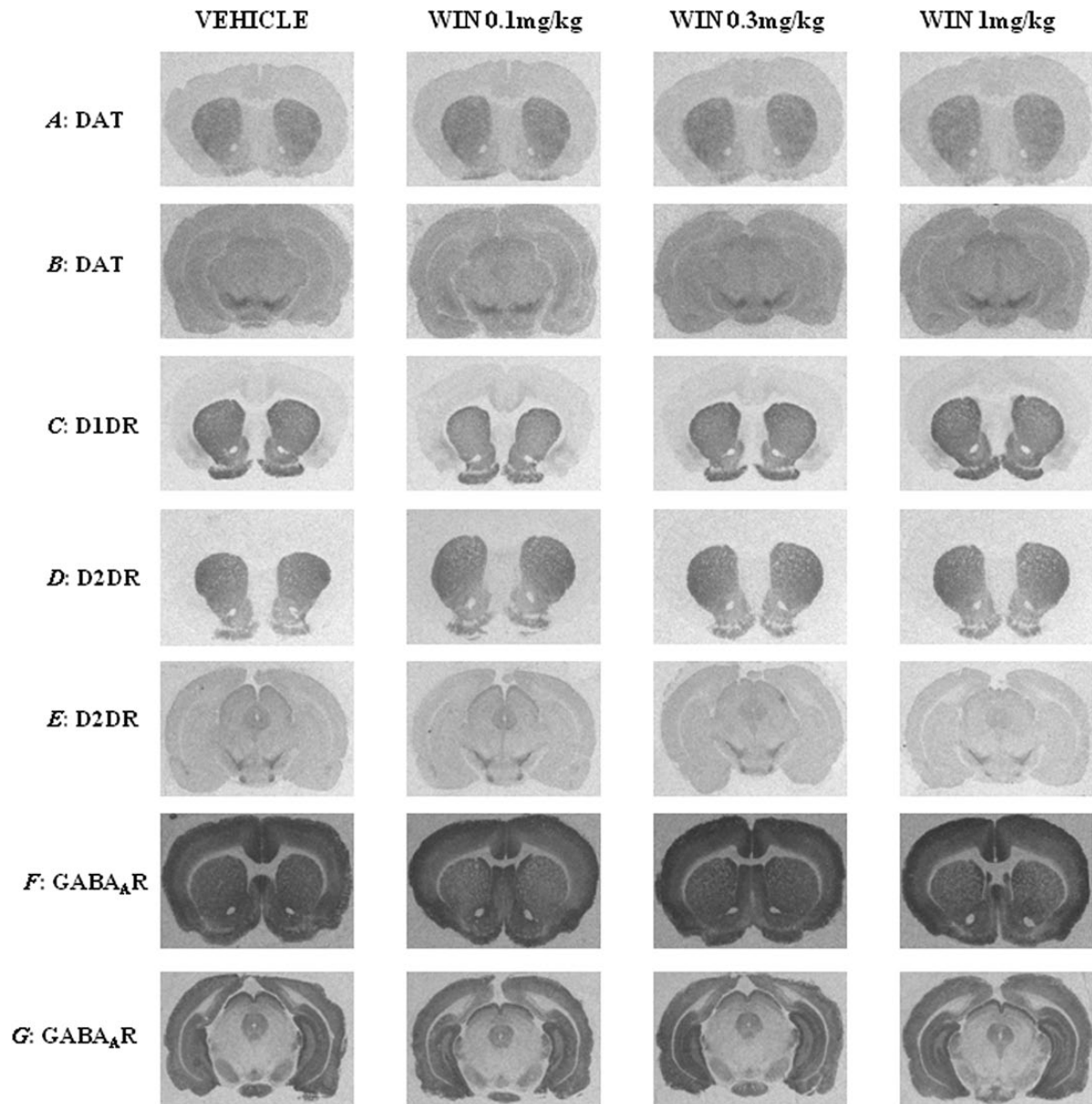
Figure 3. Autoradiographic localization of (*A*) dopamine transporter (DAT) in striatum/nucleus accumbens (NAc) and (*B*) substantia nigra (SN)/ventral tegmental area (VTA); (*C*) D1 dopamine receptor (D1DR) in striatum/NAc; (*D*) D2 dopamine receptor (D2DR) in striatum/NAc and (*E*) SN/VTA; (*F*) GABA$_A$ receptors in striatum/NAc; and (*G*) SN/VTA of different doses of WIN55,212-2.

WIN55,212-2 0.3 mg/kg: *P* =.001, and WIN55,212-2 1 mg/kg: *P*=.032). Furthermore, no significant differences between vehicle- and WIN55,212-2-treated rats were observed in DAT binding levels in the striatum (Table 1).

D1DRs

The levels of D1DR binding (Figures 3C and 6 A-B) and mRNA levels (Figures 4B and 6 C-D) in the terminal regions of the mesostriatal dopaminergic system of vehicle- and WIN55,212-2-treated rats are presented in Tables 3 and 4. Specific binding of [³H]-SCH23390 (F$_{3,34}$= 3.882, *P* =.039; Table 3) and D1DR mRNA levels (F$_{3,15}$= 13.176, *P* =.019, Table 4) increased significantly at the dose of 1 mg/kg in the core subdivision of NAc. No effects were observed at the other 2 doses.

D2DRs

The levels of D2DR binding, as determined by [³H]-raclopride specific binding (Figures 3D-E and 7A-D), in the mesostriatal dopaminergic regions of vehicle- and WIN55,212-2-treated rats are presented in Table 5. Significantly decreased levels of D2DR binding were observed in the medial quadrants of striatum (DM: F$_{3,35}$=4.56, *P*=.008; VM: F$_{3,35}$=4.64, *P*=.008) at the doses of 0.1 (DM: *P*=.011 and VM: *P*=.013) and 1 mg/kg (DM: *P*=.030 and VM: *P*=.019). However, as seen in Table 6, no significant alterations were observed in D2DR mRNA levels in SN and VTA (Figures 4C, 7 E-F). Furthermore, we examined the mRNA levels of the D2S isoform (Figures 4D and 7 G-H), which corresponds to a splice variant of the D2DR, showing an expression pattern presynaptically on dopaminergic neurons of SNpc and VTA and likely represents
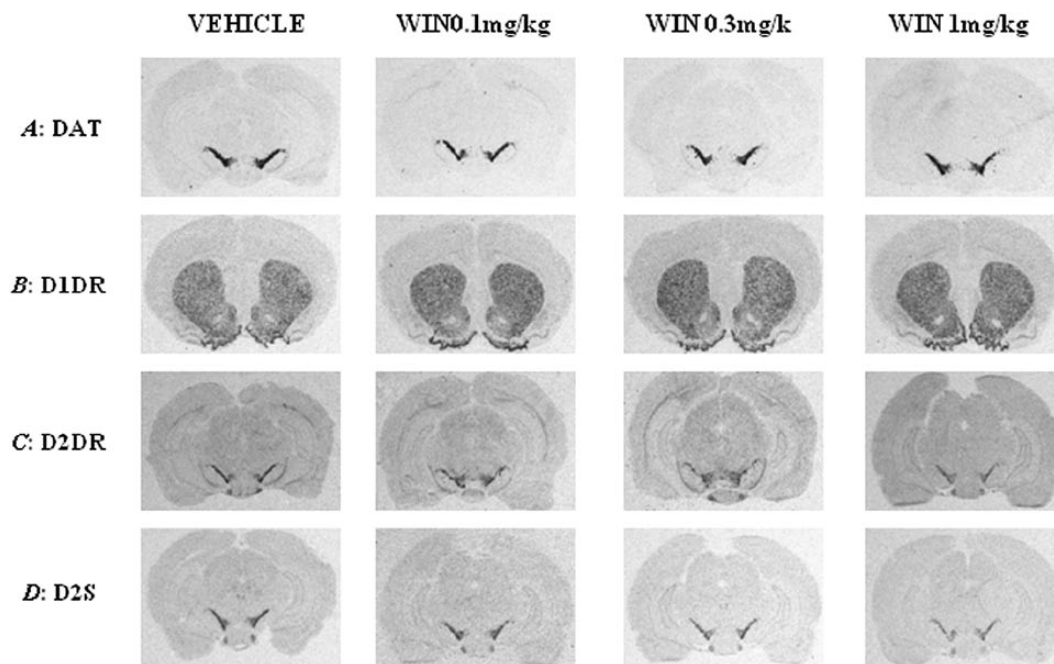
Figure 4. Representative in situ hybridization images showing the distribution of mRNA levels of (*A*) dopamine transporter (DAT) in substantia nigra (SN)/ventral tegmental area (VTA), (*B*) D1 dopamine receptor (D1DR) in striatum/nucleus accumbens (NAc), (*C*) D2 dopamine receptor (D2DR) in SN/VTA, and (*D*) D2S autoreceptors in SN/VTA of different doses of WIN55,212-2.

the dopamine autoreceptor (D2S) (Khan et al., 1998). The in situ hybridization histochemistry study for D2S mRNA revealed significant differences in mRNA levels in SNpc ($F_{3,36}$=62.74, P<.001) and VTA ($F_{3,36}$=5.93, P=.002). Particularly, statistically significant decreases were observed at all doses of WIN55,212-2 in SNpc (P<.001) as described in Table 7, while in VTA, significant decreases were observed at the higher doses (WIN55,212-2 0.3mg/kg: P=.008 and WIN55,212-2 1mg/kg: P=.003).

GABA$_A$ Receptors

The levels of GABA$_A$ binding sites were measured in striatum of WIN55,212-2 and vehicle-treated rats using [³H]-SR95531 and are presented in Table 8 and Figures 3F-G and 8. Densitometric measurements taken from striatum showed statistically significant decreases in DL ($F_{3,36}$=9.95, P<.001) and DM ($F_{3,36}$= 19.42, P<.001) quadrants at all doses tested, as well as at 0.3mg/kg in the VL subdivision ($F_{3,36}$=5.43, P=.003). More specifically, at the lowest dose of WIN55,212-2 (0.1mg/kg), the observed binding levels of GABA$_A$ receptor were significantly reduced in the DL quadrant (P=.001) and in the DM part (P<.001). Decreases were also observed with 0.3mg/kg in the DL and DM quadrants (P<.001 in both subdivisions), respectively. The same effect was also observed at the highest dose (1mg/kg) in DL (P=.003) and DM (P<.001) striatal quadrants. In the VL quadrant, GABA$_A$ receptor binding levels were significantly reduced at 0.3mg/kg (P=.005). Furthermore, a statistically significant decrease in SN ($F_{3,36}$=4.85, P=.006) was observed at 1mg/kg (P=.004).

Discussion

It is generally accepted that cannabinoid agonists induce a dose-dependent inhibition of motor activity in both humans and laboratory animals and even cause catalepsy with high doses (Gerdeman et al., 2008). In line with previous studies

(Sañudo-Peña et al., 2000; Drews et al., 2005; Rodvelt et al., 2007; Polissidis et al., 2010; 2013), our results showed that treatment with the nonselective CB1R agonist WIN55,212-2 dose-dependently suppressed ambulatory activity after single and multiple administrations.

At the neurobiological level, increases in mesostriatal dopamine neurotransmission have been associated with increased spontaneous locomotor activity after treatment with a variety of substances of abuse (Nestler, 2005; Lüscher and Malenka, 2011). However, most of the CNS actions of cannabinoids are mediated via CB1R (Gardner, 2005; Hashimotodani et al., 2007; Vlachou et al., 2008), and activation of these receptors in the striatum is associated with inhibition of motor behaviors (Giuliani et al., 2000; Darmani, 2001; Järbe et al., 2002; Schramm-Sapyta et al., 2007). Several studies have implicated that the mechanism of this CBR-induced hypomotility involves the interaction between CB1R and D1DR/D2DR dopamine receptors at the cellular level and their coupling to the same affector system (Glass and Felder, 1997; Hermann et al., 2002; Andersson et al., 2005; Kearn et al., 2005; Martín et al., 2008).

Importantly, our results indicated that chronic WIN55,212-2 administration did not induce phenomena of tolerance or sensitization of locomotor activity, although the motor-suppressant effects of the highest dose of WIN55,212-2 tested appeared to level off between days 10 and 20 of testing. Indeed, while the results of the present study demonstrated that chronic WIN55,212-2 can produce acute and sustained motor-suppressant effects, they provide no evidence that repeated administration of WIN55,212-2 leads to a behavioral sensitization similar to that observed with other drugs of abuse. Similar results have been obtained after chronic treatment with low doses of Δ⁹-THC or other CB1R agonists (Arnold et al., 1998; Muschamp and Siviy, 2002; Ellgren et al., 2004; Kolb et al., 2006; Varvel et al., 2007), although considerably higher doses of Δ⁹-THC have been reported to produce behavioral sensitization (Cadoni et al., 2001;
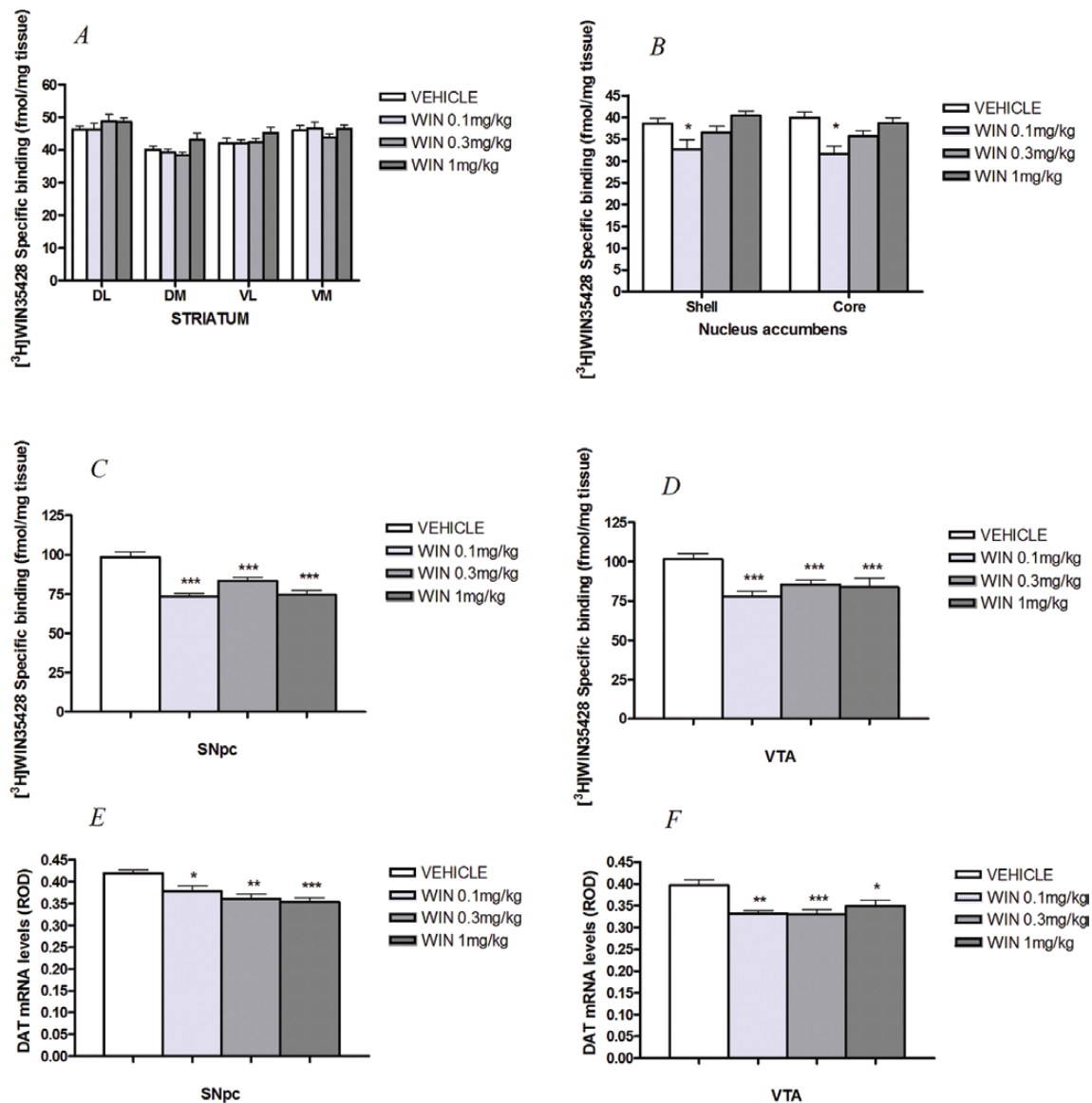
DAT binding and mRNA levels

Figure 5. Dopamine transporter (DAT) binding site levels labeled with [³H]-WIN35428 in (A) striatum, (B) nucleus accumbens (NAc), (C) substantia nigra pars compacta (SNpc), and (D) ventral tegmental area (VTA) of chronically WIN55,212-2- and vehicle-treated rats and DAT mRNA levels in (E) SNpc and (F) VTA. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *P < .05, **P < .01, and ***P < .001, n = 9-10.

Rubino et al., 2001). Although our data did not indicate significant tolerance to the motor-suppressant effects of chronic WIN55,212-2, a study by Sim-Selley and Martin (2002) reported that chronic administration of considerably higher doses of WIN55,212-2 in mice produces tolerance to cannabinoid-mediated hypoactivity. Similar results have also been observed with higher doses of Δ⁹-THC (Rodríguez de Fonseca et al., 1994; Romero et al., 1997; Sim-Selley and Martin, 2002; Whitlow et al., 2003). Thus, it appears that behavioral tolerance or sensitization after chronic WIN55,212-2 may depend on several aspects of the experimental protocol, such as species used, dose, and route of drug administration.

The present study indicated that chronic administration of WIN55,212-2 for 20 consecutive days modulated the dopaminergic and GABAergic systems of adult rat brain. In particular, we

observed selective alterations in binding and mRNA levels of the DAT as well as dopamine and $GABA_A$ receptors in somatodendritic and terminal regions of the mesostriatal dopaminergic system (SN and striatum; VTA and NAc). Considering that only high doses of WIN55,212-2 significantly affected motor activity, the extent to which the observed neurochemical alterations are causally related to the locomotor activity findings in response to chronic administration of WIN55,212-2 is not readily apparent. It may be argued that changes in dopaminergic and GABAergic neurotransmission contribute to the sustained suppressant effects measured after high doses of WIN55,212-2.

In the present study, the use of the nonselective CB1R agonist WIN55212-2 could raise the question whether the observed alterations are attributed to CB1R or CB2R activation. CB2 cannabinoid receptors are localized primarily in immune

Table 1. DAT Binding Site Levels Labeled with [³H]-WIN35428 in Striatum, Nucleus Accumbens, SNpc, and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1 mg/kg	WIN 0.3 mg/kg	WIN 1 mg/kg
Dorsolateral striatum	46.22±1.08	46.23±1.93	48.70±2.18	46.65±1.09
	n=10	n=10	n=10	n=10
Dorsomedial striatum	40.11±1.01	39.33±0.96	38.28±1.04	43.00±2.09
	n=10	n=10	n=10	n=10
Ventrolateral striatum	41.99±1.56	42.07±0.98	42.33±1.11	45.19±1.68
	n=10	n=10	n=10	n=10
Ventromedial striatum	46.08±1.32	46.54±1.97	43.79±1.14	46.40±1.15
	n=10	n=10	n=10	n=10
Nucleus accumbens shell	38.55±1.27	32.73±2.14***	36.51±1.42	40.43±0.96
	n=10	↓15.1% n=10	n=10	n=10
Nucleus accumbens core	39.92±1.27	31.59±1.68***	35.63±1.34	38.74±1.18
	n=10	↓20.9% n=10	n=10	n=10
SNpc	98.48±3.33	73.49±1.69***	83.39±2.33***	74.79±2.47***
	n=10	↓25.4% n=10	↓15.3% n=10	↓24.05% n=10
VTA	101.47±3.61	78.10±3.28*	85.70±2.93*	83.85±5.75***
	n=9	↓23.03% n=10	↓15.5% n=10	↓17.4% n=9

Abbreviations: SNpc, substantia nigra pars compacta; VTA, ventral tegmental area. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *$P < .05$, **$P < .01$, and ***$P < .001$, n=9-10.

Table 2. DAT mRNA Levels in SNpc and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1 mg/kg	WIN 0.3 mg/kg	WIN 1 mg/kg
SNpc	0.42±0.008	0.378±0.0122*	0.361±0.010**	0.353±0.011***
		↓10%	↓14.05%	↓15.95%
VTA	0.413±0.011	0.332±0.006**	0.331±0.010***	0.349±0.013*
		↓19.6%	↓19.8%	↓15.5%

Abbreviations: SNpc, substantia nigra pars compacta; VTA, ventral tegmentall area. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *$P < .05$, **$P < .01$, and ***$P < .001$, n=9-10.

cells both in the periphery (Griffin et al., 2000) and brain microglia (Maresz et al., 2007), indicating that they are activated after brain damage or injury. In addition, they are expressed in neurons, including the striatum and midbrain (Gong et al., 2006), but the extent and level of expression remain controversial (Atwood and Mackie, 2010). However, most of the CNS actions of cannabinoids appear to be mediated via CB1R (Gardner, 2005; Hashimotodani et al., 2007; Vlachou et al., 2008). Systemic administration of CB1R agonists such as ⁹Δ-THC and WIN55,212-2 suppress motor activity, especially at higher doses (Darmani, 2001; Polissidis et al., 2013), and this effect is reversed by CB1R specific antagonists.

It is well known that DAT is localized to plasma membranes of axon terminals as well as dendrites of SNpc dopaminergic neurons (Nirenberg et al., 1996) and plays a role in reuptake of dopamine into dendrites and axon terminals (Cheramy et al., 1981). It has been reported that endogenous or exogenous (eg, WIN55,212-2) cannabinoids inhibit DAT activity in vitro (Chen et al., 2003; Steffens and Feuerstein, 2004), while other studies do not show any effect (Cheer et al., 2004; Köfalvi et al., 2005).

In the present study, we observed reduced DAT mRNA and binding levels in VTA and SNpc at all doses of WIN55,212-2, while DAT binding levels were not altered in striatum but were reduced in both NAc shell and core only at the lowest dose. These results suggest that the expression of DAT decreased at both the protein and mRNA levels in mesolimbic and nigrostriatal dopaminergic neurons and this effect is not dose dependent, with the exception of the NAc. It is interesting to point out that the effect of chronic administration of cannabinoids on DAT binding levels is evident only at the somatodendritic level of dopamine neurons and not at their axonal terminals. These brain regional differences may be a consequence of differential effect of CB1R density and function, as it is known that repeated treatment with cannabinoids (including WIN55,212-2) induces downregulation of the cannabinoid receptor (Oviedo et al., 1993; Breivogel et al., 1999; Sim-Selley, 2003; Tanda and Goldberg, 2003; Sim-Selley et al., 2006; Wu et al., 2008). In support of this notion, Moranta et al. (2009) reported brain regional differences in the synthesis of brain monoamines after chronic treatment with WIN55,212-2.

In vivo electrophysiological studies have shown that cannabinoid agonists increase cell firing of the dopaminergic neurons located in SNpc and VTA (French et al., 1997; Melis et al., 2000; Wu and French, 2000). The increased activity of SNpc neurons is in agreement with in vivo microdialysis experiments showing enhanced dopamine release in the striatum after cannabinoid agonist treatment (Tanda et al., 1997; Solinas et al., 2008; Moranta et al., 2009; Polissidis et al., 2010, 2013). Taking into account the above findings and our results of decreased binding and mRNA levels of DAT in SN and VTA, we could suggest that chronic activation of cannabinoid receptors may lead to decreased dopamine uptake by the dendrites of mesolimbic and nigrostriatal dopaminergic neurons, but not by the nigrostriatal terminals. Furthermore, the increased extracellular levels of dopamine in the striatum could be attributed to saturation of DAT (Oleson and Cheer, 2012; Tye et al., 2013) and/or to decreased inhibitory control over dopamine release due to stimulation of D2S (see below).
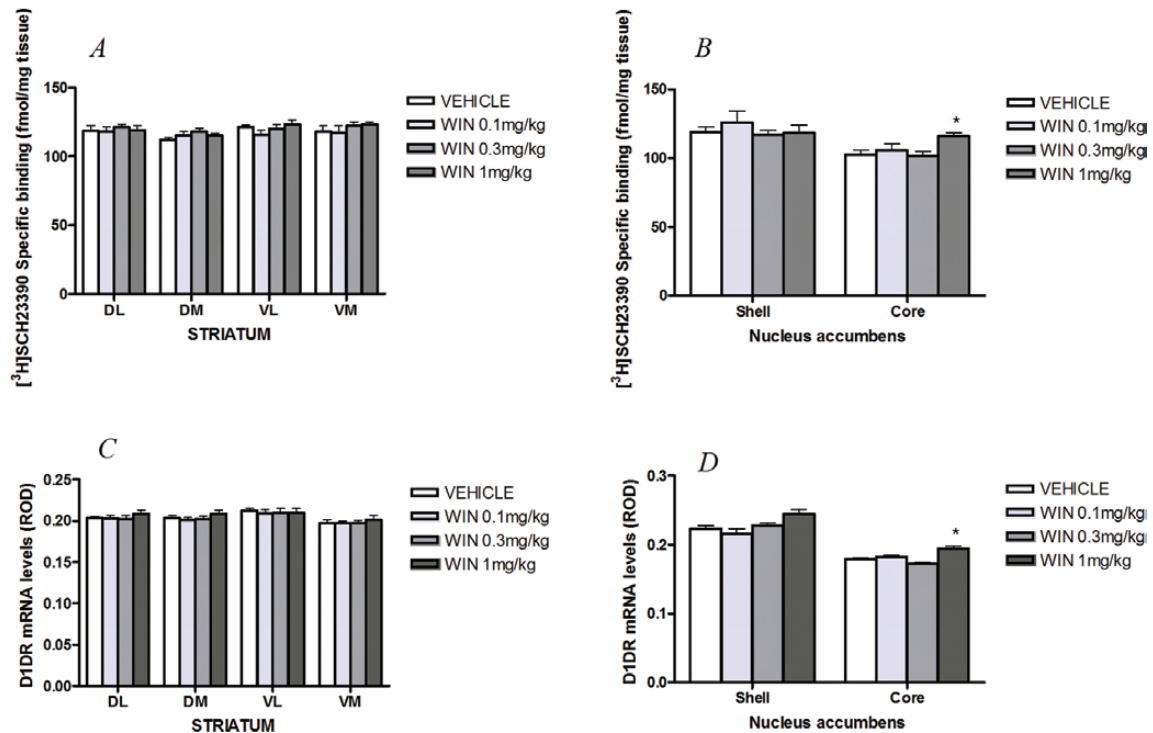
D1DR binding and mRNA levels

Figure 6. D1 dopamine receptor (D1DR) binding site levels labeled with [³H]-SCH23390 in (A) striatum and (B) nucleus accumbens (NAc) and D1DR mRNA levels in (C) striatum and (D) NAc of chronically WIN55,212-2- and vehicle-treated rats. The asterisk (*) denotes a statistically significant effect of WIN55,212-2 compared to the vehicle group: *P<.05; n=9-10 for receptor autoradiography and n=5 per group for in situ hybridization.

Table 3. DRD1 Binding Site Levels Labeled with [³H]-SCH23390 in Striatum, Nucleus Accumbens, SNpc, and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1mg/kg	WIN0.3mg/kg	WIN1mg/kg
Dorsolateral striatum	118.45±3.82 n=10	112.27±1.71 n=10	121.61±1.22 n=10	117.88±4.28 n=10
Dorsomedial striatum	118.19±3.31 n=10	115.59±2.26 n=10	115.92±3.10 n=10	117.36±4.76 n=10
Ventrolateral striatum	121.50±1.87 n=10	117.91±2.76 n=10	120.15±2.82 n=10	122.18±3.12 n=10
Ventromedial striatum	119.36±3.10 n=10	115.33±1.45 n=10	123.24±3.13 n=10	123.32±1.83 n=10
Nucleus accumbens shell	118.79±3.87 n=10	125.80±8.26 n=9	116.71±3.56 n=10	118.05±5.72 n=10
Nucleus accumbens core	102.47±3.47 n=10	105.88±4.49 n=9	101.72±2.91 n=10	116.24±2.15* ↑13.44% n=9

The asterisk (*) denotes a statistically significant effect of WIN55,212-2 compared to the vehicle group: P<.05; n=9-10.

Table 4. DRD1 mRNA Levels in Striatum and Nucleus Accumbens of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1mg/kg	WIN 0.3mg/kg	WIN 1mg/kg
Dorsolateral striatum	0.20±0.0014	0.202±0.0035	0.202±0.0042	0.208±0.0043
Dorsomedial striatum	0.203±0.0031	0.201±0.0032	0.201±0.0037	0.20872±0.0043
Ventrolateral striatum	0.21±0.0025	0.209±0.0043	0.209±0.0054	0.209±0.0047
Ventromedial striatum	0.197±0.0039	0.197±0.0027	0.197±0.0032	0.201±0.0046
Nucleus accumbens shell	0.22±0.004	0.215±0.007	0.22±0.004	0.24±0.006
Nucleus accumbens core	0.18±0.0018	0.18±0.0031	0.172±0.002	0.194±0.003** ↑7.8%

The asterisk (*) signify a statistically significant effect compared to the vehicle group: **P<.01; n=5 per group.
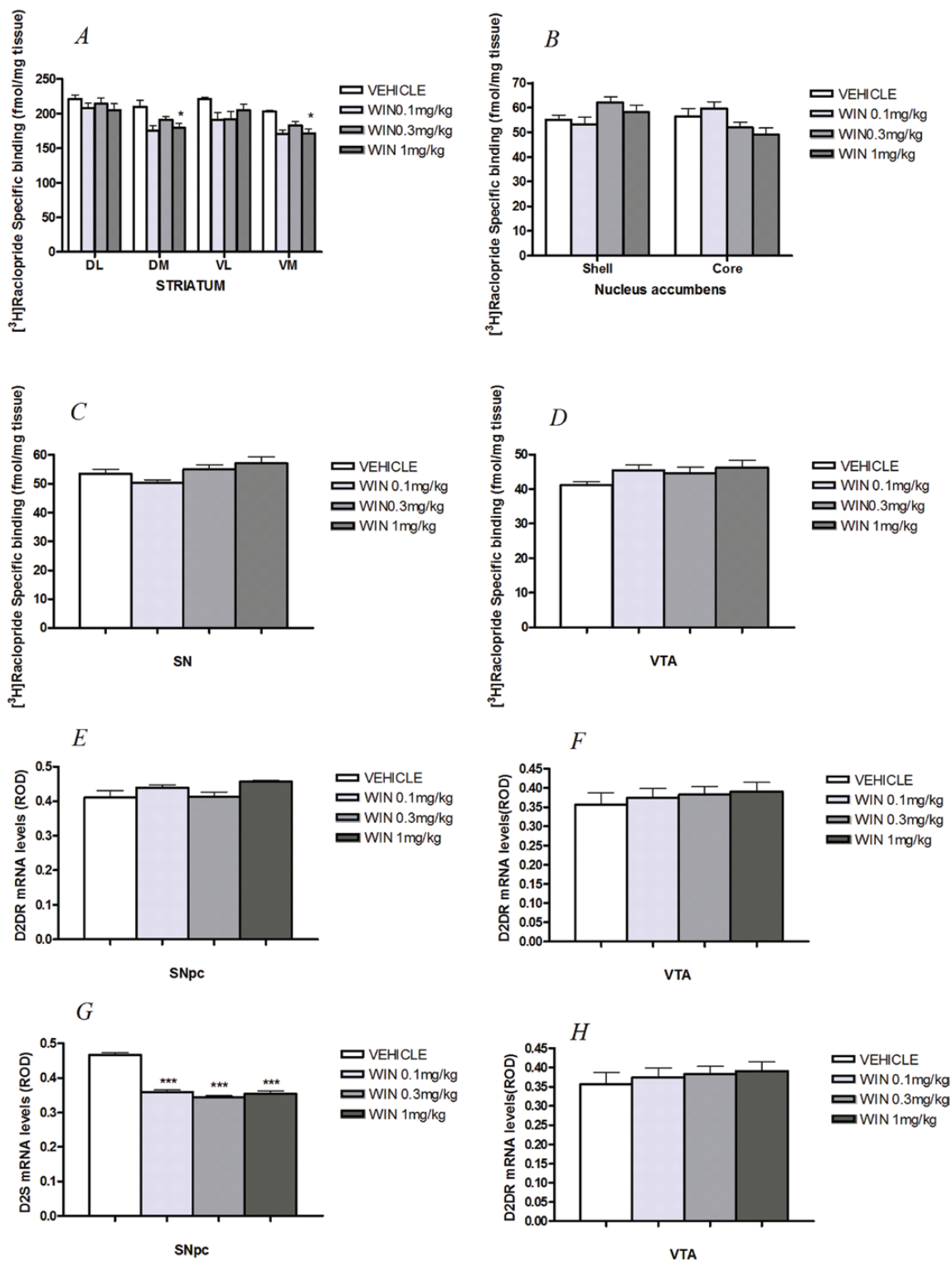
D2DR binding and mRNA levels

Figure 7. D2 dopamine receptor (D2DR) binding site levels labeled with [³H]-raclopride in (A) striatum, (B) nucleus accumbens (NAc), (C) substantia nigra (SN) and (D) ventral tegmental area (VTA), D2DR receptor mRNA levels in (E) substantia nigra pars compacta (SNpc) and (F) VTA and D2 autoreceptor (D2S) mRNA levels in (G) SNpc and (H) VTA of chronically WIN55,212- and vehicle-treated rats. The asterisk (*) denotes a statistically significant effect of WIN55,212-2 compared to the vehicle group: *P < .05, **P < .01, and ***P < .001, n = 9-10.

Table 5. DRD2 Binding Site Levels Labeled with [³H]-raclopride in Striatum, Nucleus Accumbens, SN, and VTA of Chronically WIN55,212- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1 mg/kg	WIN 0.3 mg/kg	WIN 1 mg/kg
Dorsolateral striatum	221.31 ± 5.89	207.98 ± 7.35	214.27 ± 8.47	204.46 ± 6.88
	n = 9	n = 10	n = 10	n = 10
Dorsomedial striatum	210.02 ± 8.87	175.06 ± 7.29*	191.20 ± 4.75	178.97 ± 7.68*
	n = 9	↓16.65% n = 10	n = 10	↓14.8% n = 10
Ventrolateral striatum	221.08 ± 2.87	191.01 ± 7.40	191.72 ± 5.34	205.10 ± 6.84
	n = 9	n = 10	n = 10	n = 10
Ventromedial striatum	203.61 ± 5.06	170.47 ± 5.87*	182.95 ± 5.44	171.93 ± 6.14*
	n = 9	↓16.3% n = 10	n = 10	↓15.56% n = 10
Nucleus accumbens shell	54.95 ± 1.87	53.21 ± 2.95	62.00 ± 2.43	58.07 ± 2.88
	n = 10	n = 9	n = 9	n = 10
Nucleus accumbens core	56.27 ± 3.26	59.64 ± 2.68	51.94 ± 2.17	48.95 ± 2.68
	n = 9	n = 10	n = 10	n = 10
SN	53.53 ± 1.63	50.42 ± 0.89	55.05 ± 1.55	57.09 ± 2.18
	n = 10	n = 10	n = 10	n = 10
VTA	41.28 ± 0.83	45.37 ± 1.61	44.66 ± 1.74	46.17 ± 2.07
	n = 10	n = 10	n = 10	n = 10

Abbreviations: SN, substantia nigra; VTA, ventral tegmental area. The asterisk (*) denotes a statistically significant effect of WIN55,212-2 compared to the vehicle group: *$P < .05$; n = 9-10.

Table 6. DRD2 Receptor mRNA Levels in SNpc and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1 mg/kg	WIN 0.3 mg/kg	WIN 1 mg/kg
SNpc	0.411 ± 0.021	0.439 ± 0.008	0.413 ± 0.014	0.456 ± 0.0052
	n = 9	n = 10	n = 10	n = 10
VTA	0.356 ± 0.031	0.375 ± 0.024	0.382 ± 0.021	0.391 ± 0.024
	n = 9	n = 10	n = 10	n = 10

Abbreviations: SNpc, substantia nigra pars compacta; VTA, ventral tegmental area; n = 9-10.

Table 7. D2S mRNA Levels in SNpc and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1mg/kg	WIN0.3mg/kg	WIN1mg/kg
SNpc	0.46 ± 0.007	0.359 ± 0.005***	0.343 ± 0.006***	0.353 ± 0.009***
		↓21.96%	↓25.43%	↓23.26%
VTA	0.406 ± 0.022	0.36 ± 0.008	0.344 ± 0.005**	0.338 ± 0.005**
			↓15.27%	↓16.75%

Abbreviations: SNpc, substantia nigra pars compacta; VTA, ventral tegmental area. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *$P < .05$, **$P < .01$, and ***$P < .001$; n = 10 per group.

D2DRs exist in 2 isoforms (D2S, D2L) generated by alternative splicing of the same gene (Giros et al., 1989). D2Ss are localized presynaptically on both the somatodendritic and terminal regions of midbrain dopaminergic neurons (Khan et al., 1998). Activation of these receptors in mesencephalic dopaminergic neurons elicits hyperpolarization and decreases firing rate (Lacey et al., 1987; Mercuri et al., 1989, 1997; Centonze et al., 2002) while inhibiting dopamine release in their somatodendritic (Cragg and Greenfield, 1997) and terminal mesostriatal regions (Starke et al., 1989; Cragg and Greenfield, 1997; Usiello et al., 2000). Thus, dopamine release is under the inhibitory control of D2DRs (Benoit-Marand et al., 2001). In the present study, the mRNA levels of the short isoform of D2 receptor (D2S), which corresponds to the presynaptic D2DR, were reduced in SNpc and VTA after chronic administration of the CB1R agonist, WIN55,212-2. Using [³H] raclopride, which labels D2DR binding sites but does not distinguish between presynaptic and postsynaptic localization of D2DR, we were unable to detect any changes of the D2DR at the binding site level in SN and VTA. However, decreased D2DR binding levels were found in the striatum of WIN-treated rats. This change could be correlated to the decreased mRNA expression of D2S observed in SNpc and may reflect a reduction in striatal D2S. However, further immunohistochemical studies using an antibody specific for the D2S are required to verify this change. Our finding is in contrast to the results of Ginovart et al. (2012), who showed increased presynaptic $D_{2/3}$ autoreceptor binding levels after chronic Δ⁹-THC administration, but are in agreement with the results of Bossong et al. (2009), who showed reduced [¹⁴C] raclopride binding in human striatum after Δ⁹-THC inhalation.

Overall, we could suggest that the somatodendritic regions of the mesostriatal dopaminergic system, the SN and VTA, seem to be more affected than the projection regions, the striatum and NAc, by chronic administration of WIN55,212-2. Decreased mRNA levels of D2S and DAT may lead to increased activity of SNpc and VTA neurons and enhanced release of dopamine at their somatodendritic and terminal fields. This finding may be

Table 8. GABA$_A$ Receptor Binding Site Levels Labeled with [³H]-SR955321 in Striatum, Nucleus Accumbens, SN, and VTA of Chronically WIN55,212-2- and Vehicle-Treated Rats

Brain Region	Vehicle	WIN 0.1mg/kg	WIN0.3mg/kg	WIN1mg/kg
Dorsolateral striatum	82.81±2.08 n=10	72.44±0.99*** ↓12.5% n=10	71.41±1.68*** ↓13.8% n=10	73.83±1.67** ↓10.84% n=10
Dorsomedial striatum	90.81±1.84 n=10	79.05±1.29*** ↓12.9% n=10	78.73±1.23*** ↓13.3% n=10	77.22±1.25*** ↓14.96% n=10
Ventrolateral striatum	88.98±1.40 n=10	87.16±1.05 n=10	80.14±0.96** ↓9.9% n=10	83.25±2.75 n=10
Ventromedial striatum	100.21±1.59 n=10	97.21±2.89 n=10	95.29±1.62 n=10	103.26±1.07 n=10
Nucleus accumbens shell	111.90±2.49 n=10	111.15±1.89 n=10	109.23±2.5 n=10	121.13±2.44 n=10
Nucleus accumbens core	116.24±1.72 n=10	112.59±3.35 n=10	115.06±3.64 n=10	124.19±2.91 n=10
SN	84.13±1.26 n=10	77.22±3.13 n=10	80.05±3.32 n=10	70.39±2.25** ↓16.33% n=10
VTA	60.95±3.00 n=9	55.24±1.66 n=10	55.51±1.08 n=10	57.62±2.71 n=10

Abbreviations: SN, substantia nigra; VTA, ventral tegmental area. The asterisks (*) signify a statistically significant effect compared to the vehicle group: *$P < .05$, **$P < .01$, and ***$P < .001$; n=9-10.
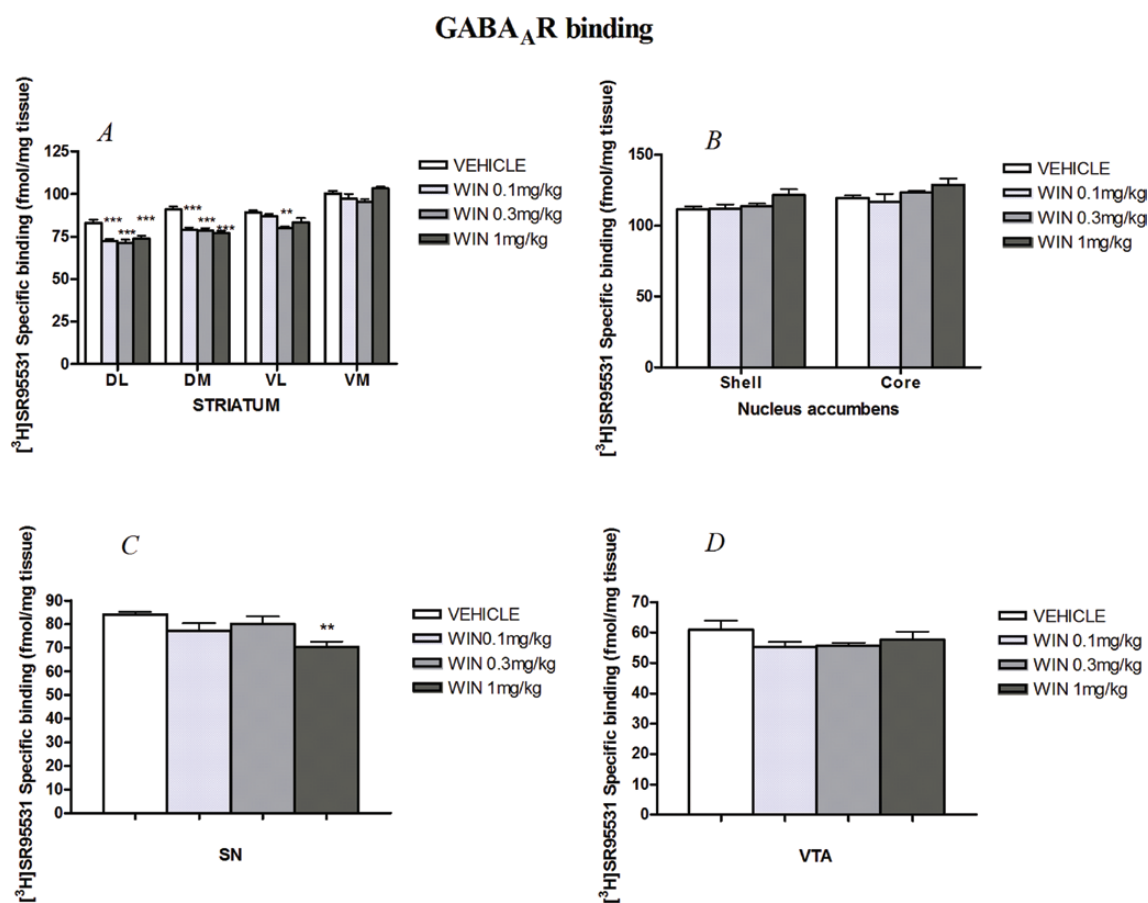

Figure 8. GABA$_A$ receptor binding site levels labeled with [³H]-SR955321 in (A) striatum, (B) nucleus accumbens (NAc), (C) substantia nigra (SN), and (D) ventral tegmental area (VTA) of chronically WIN55,212-2- and vehicle-treated rats. The asterisks (*) signify a statistically significant effect compared to the vehicle group: **$P < .01$, and ***$P < .001$, n=9-10.

attributed to the fact that chronic exposure to WIN55,212-2 can cause neuroadaptive alterations (ie, downregulation) of CB1 receptor (density and function) which is region specific (Sim-Selley and Martin, 2002).

It is generally accepted that regulation of dopamine release in striatum is mainly the consequence of alterations in dopaminergic cell firing in the SNpc and the VTA. However, several studies have revealed local regulation of DA release by other

neurotransmitters and modulators, such as glutamate. Recent studies suggest that the glutamatergic regulation of dopamine release is inhibitory (Rice et al., 2011). Taking into consideration the above and that activation of CB1R receptors on corticostriatal terminals would inhibit glutamate release, this activation may lead to increased DA release.

Our results have also shown that D1DR mRNA and binding were not altered in striatum; however, in NAc core, both mRNA and binding levels were increased only at the highest dose. This dose-dependent effect is specific for the mesolimbic pathway, and it may be related to the pronounced motor-suppressant effects of the high dose of WIN22,212-2 that persisted over the course of administration. It has been suggested that simultaneous stimulation of CB1R and D1DR reduces the inhibitory activity of direct striatal projection neurons, resulting in a decreased motor response (Martín et al., 2008).

It is well known that NAc plays a pivotal role in reward and aversive learning and learning flexibility (Graybiel, 2008). In NAc, similar to dorsal striatum, MSNs express either D1DR or D2DR along with other receptors and neuropeptides, and their distinct roles in learning have only recently been explored (Nakanishi et al., 2014). Furthermore, recent investigations suggest a differential involvement of D1-MSNs and D2-MSN cell populations in NAc in drug-related behaviors (Laviolette et al., 2008; Hikida et al., 2010; Smith et al., 2013). In particular, distinct roles of D1DR and D2DR in the core and shell of NAc have been implicated in the modulation of reward by nicotine (Laviolette et al., 2008) and in the acquisition of cocaine-related learning (Espana and Jones, 2013; Smith et al., 2013).

Seif et al. (2011) provided evidence that endocannabinoids mediate the ability of DA receptors to enhance action potential firing in NAc core neurons in vitro, requiring coactivation of D1DR and D2DR. The selective upregulation of D1DRs in NAc core observed in the present study after high doses of chronic cannabinoid administration suggest that the core vs shell and D1DR vs D2DR MSNs of NAc may respond differently to repeated cannabinoid administration, and these cell-type specific alterations in NAc core may contribute to cannabinoid-related behaviors.

It has also been shown that CB1R are localized presynaptically on GABAergic neurons in several brain regions (Matsuda et al., 1993) and their activation inhibits GABA release (French et al., 1997; Pistis et al., 2002; Szabo et al., 2002; Lupica and Riegel, 2005; Szabo and Schlicker, 2005). More precisely, in SN pars reticulata, CB1R are located on GABAergic striatonigral terminals. Furthermore, dendrites of dopaminergic neurons in SNpc extend into the SN pars reticulate, where they form synapses with CB1-containing axon terminals (Fitzgerald et al., 2012). Considering this localization of CB1R, a possible mechanism for the increased dopamine release after cannabinoid treatment may involve an indirect disinhibition of dopamine neurons (French et al., 1997; Szabo et al., 2002; Lupica and Riegel, 2005). It is thus suggested that CB1R, because of their localization, can modulate GABA release and in turn control the activity of the dopaminergic cells in the midbrain (Wu and French, 2000; Laviolette and Grace, 2006; Fernández-Ruiz et al., 2010).

Our results have indicated that GABA$_A$ receptor binding levels were reduced in the dorsal striatum at all doses and SN at the highest dose. Therefore, in addition to the reduction of GABA released from the striatonigral terminals of the direct pathway, the observed reduction of GABA$_A$ receptors in SN at least at the higher dose would increase the activity of the GABAergic cells of SNr, leading to hypomobility. Overall, these results indicate that the effects of WIN55,212-2 on motor activity could be mediated at least partially via GABA$_A$ receptors expressed in the nigrostriatal

pathway. In conclusion, our data indicate that chronic administration of the cannabinoid agonist WIN55,212-2 did not induce phenomena of tolerance or sensitization of locomotor activity. Furthermore, repeated cannabinoid administration induced neuroadaptive alterations of the dopaminergic and GABAergic systems in a region-, dose-, and neurotransmitter-dependent manner.

Acknowledgments

Statement of Interest

None.

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