Dose and Behavioral Context Dependent Inhibition of Movement and Basal Ganglia Neural Activity by Δ^{-9}-Tetrahydrocannabinol During Spontaneous and Treadmill Locomotion Tasks in Rats

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KEY WORDS locomotion; basal ganglia; rats; limb movement; electrophysiology; Δ^{-9}-THC

ABSTRACT The effects of Δ^{-9}-tetrahydrocannabinole (Δ^{-9}-THC) on locomotor activities and related basal ganglia neural responses were investigated in rats. A multiple-channel, single unit recording method was used to record neuronal activity in the dorsal lateral striatum, the globus pallidus, the subthalamic nucleus, and the substantia nigra pars reticulata simultaneously during spontaneous movement and treadmill locomotion. Δ^{-9}-THC treatment (0.05–2.0 mg/kg, i.p.) dose-dependently decreased spontaneous motor activity and altered walking patterns in treadmill locomotion in that stance time was increased and step number was decreased. In parallel with the behavioral effects, Δ^{-9}-THC treatment inhibited neural activity across all four basal ganglia areas recorded during both motor tests. Further, this inhibition of basal ganglia neural activity was behavioral context-dependent. Greater inhibition was found during resting than during walking periods in the treadmill locomotion test. Δ^{-9}-THC treatment also changed firing patterns in the striatum and globus pallidus. More neurons in these regions discharged in an oscillatory pattern during treadmill walking with Δ^{-9}-THC, and the oscillatory frequency was similar to that of the step cycle. Synchronized firing patterns were found in few basal ganglia neurons in the control condition (~1%). Synchronized firing patterns increased during the treadmill resting phase after Δ^{-9}-THC treatment, but still represented a very small proportion of the total neural population (1.9%). The drug treatment did not change neural responses to the tone cue proceeding treadmill locomotion. This study demonstrates dose-dependent inhibitory effects of cannabinoid injection on motor activity. This effect may be related to the behavioral context-dependent inhibition observed in the basal ganglia system where CB1 receptors are densely distributed. **Synapse 55:1–16, 2005.**

INTRODUCTION

Marijuana (*Cananbis sativa*) is one of the oldest known psychoactive substances (Maugh, 1982) and remains a widely used drug. Δ^{-9}-tetrahydrocannabinole (Δ^{-9}-THC), the major psychoactive ingredient of marijuana (Harris et al., 1977), has been the focus of much research to determine the mechanism of drug action in the central nervous system. To date, two subtypes of cannabinoid receptors, CB1 and CB2, have been identified (Howlett et al., 1990; Matsuda, 1990; Munro, 1993), although CNS responses to cannabinoids are mediated exclusively by CB1 receptors (Felder and Glass, 1998; Pertwee, 1995). Cannabinoid receptors in the brain are densely distributed in the basal ganglia

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and the cerebellum, among other regions (Herkenham et al., 1990; Moldrich and Wenger, 2000; Tsou et al., 1998). Both of these regions are closely associated with the regulation and coordination of motor activity (Herkenham et al., 1990, 1991). In the striatum (STR), CB1-immunoreactivity is observed not only in medium spiny neurons, but also in small fiber bundles that become larger as they approach the globus pallidus (GP). Cannabinoid receptors in the substantia nigra pars reticulata (SNr), the entopeduncular nucleus, and the GP appear to be localized primarily on striatal terminals, since cannabinoid receptor binding in these regions is significantly reduced following striatal lesions (Herkenham et al., 1991). The abundant presence of CB1 receptors provides an anatomical substrate for the action of cannabinoids in these regions. Numerous reports have indicated that cannabinoids modulate the function of the striatonigral (Miller and Walker, 1995; Sanudo-Pena et al., 1996), striatopallidal (Miller and Walker, 1996), and subthalamonigral (Sanudo-Pena et al., 1997) pathways.

Cannabinoids exert complex effects on motor functions. In rodents, cannabinoids inhibit motor activity and produce catalepsy (Grunfeld et al., 1969; Holtzman et al., 1969). Biphasic effects of cannabinoid treatment on motor activity have been observed in which low doses of cannabinoid increased and high doses decreased spontaneous movement (Carlini et al., 1970; Davis et al., 1972). However, a recent study showed a reversed dose–response relationship with motor activity such that a low dose inhibited and a moderate dose increased motor activity (Sanudo-Pena et al., 2000). These contradictory results may be due to the different species and cannabinoid compounds used in the experiments, as well as to the differing experimental protocols.

CB1 receptors in the basal ganglia system may be involved in pathophysiological processes of a variety of movement disorders, and drugs manipulating CB1 receptors have potential therapeutic value. For example, CB1 antagonists enhanced the anti-parkinsonian action of D2 agonist (Di Marzo et al., 2000) and the CB1 antagonist could reduce levodopa-induced dyskinesia (Fox et al., 2002). Other studies suggested that cannabinoids might have therapeutic benefits for Tourette's syndrome and dystonia (Consroe et al., 1986; Muller-Vahl et al., 1999). To date, electrophysiological study of cannabinoid effects on basal ganglia neural activity has been performed only on anesthetized animals. Miller and Walker (1996, 1998) reported that pallidal cannabinoid receptors mediated inhibition of spontaneous activity in the GP. Cannabinoid treatment increased neural firing rates in the SNr neurons (Miller and Walker 1995). The behavioral significance of these neural activity changes, however, is not clear.

A combination of behavioral tests with electrophysiological analysis is a unique approach to understanding the effect of cannabinoids on motor functions. In the present study, we employed a multiple-channel, single unit recording technique to study cannabinoid-induced basal ganglia neural activity and locomotion changes simultaneously. This method allows us to further examine the motor effect of different doses of cannabinoid and to determine behavioral context-dependent neural responses in multiple basal ganglia regions. The goal of this study is to gain a new insight into the basal ganglia neural mechanism underlying the motor effect of cannabinoid.

MATERIALS AND METHODS

Animals

Eleven adult male Sprague-Dawley rats weighing 350–400 g were used in the experiment. Animals were housed individually under a reversed dark/light cycle (lights off from 0700 to 1900) for 7 days before surgery. Animals were treated in accordance with the U.S. Public Health Service *Guide for the Care and Use of Laboratory Animals*. The experiments were approved by the Institute Animal Care and Use Committee of Wake Forest University, Health Sciences.

Surgical procedures

Rats were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.). An array of eight stainless steel Teflon-insulated microwires (50 μm diameter, NB Labs, Denison, TX, and Biographics, Inc., Winston-Salem, NC), soldered to connecting pins on a headstage, were stereotaxically lowered bilaterally into dorsal lateral striatum (STR), globus pallidus (GP), subthalamic nucleus (STN), and substantia nigra pars reticulata (SNr). The stereotaxic coordinates used to target these structures were: 0.5 mm anterior to bregma (A), 3.5 mm lateral (L) to the midline, and 3.7 mm ventral (V) to the surface of cortex for the STR; –1.0 mm A, 3.2 mm L, and 6.0 mm V for the GP; –3.5 mm A, 2.5 mm L, and 7.3 mm V for the STN; and –5.4 mm A, 2.0 mm L, and 7.8 mm V for the SNr, according to the atlas of Paxinos and Watson (1986). In addition, four ground wires were positioned about 2 mm ventral to the cortical surface. The headstage was secured onto the cranium with dental acrylic and with skull screws serving as anchors. Animals received enrofloxacin (2.5 mg/kg i.m.) before surgery to prevent infection. Animals were housed individually and allowed to recover from surgery for at least 10 days before being subjected to the experiment.

Behavioral tests

Spontaneous motor activity

Spontaneous motor activity was measured in a 33 × 33 cm plastic behavioral chamber over a 60-min experimental session. Six infrared emitters, mounted 10 cm apart and 3.5 cm above the floor, were used to detect

the rat's movement (Shi et al., 2004). Ventilation and computer fans provided background masking noise and experiments were performed in dim light in the morning (2 h into the dark cycle). The rat was placed in the behavioral chamber and a headset for 64 electrodes was gently connected via a lightweight cable to a motor-assisted 80 channel commutator to record neural activity in the basal ganglia regions. The neural and motor activities were recorded over a 60-min session. The number of beam breaks detected by the infrared sensors served as the measure of spontaneous movement. The number of beam breaks for each infrared sensor was inspected to detect any large numbers of repetitive beam breaks that can be due to artifacts related to breathing, whisking, or grooming. Spontaneous motor activity was defined as spontaneous moving from one infrared sensor to another. Video records (33 ms resolution) were used to monitor rat motor activity and to detect the repetitive beam breaks due to breathing and whisking.

Before the start of the spontaneous motor activity experiment, each rat was habituated daily to the open field apparatus for a week. Rats were placed in the behavior chamber for 1 h each day and data were collected after consistent numbers of beam breaks (less than 15% difference in beam counts) were obtained from three sequential sessions. Daily experimental sessions began about 2 h into the animals' dark cycle and lasted about 3 h. Rats received a 0.3 ml saline injection (i.p.) 20 min before each control session to control for the influence of stress caused by injection. The final session with only a saline injection served as the control session. Incremental doses of Δ^{-9}-THC were then injected 20 min before each of the experimental sessions (0.05 mg, 0.1 mg, 0.5 mg, 1 mg, and 2 mg/kg i.p.). The doses of Δ^{-9}-THC were given in order of increasing concentration.

To reduce tolerance development during Δ^{-9}-THC treatment, at least two drug-free sessions were included between successive Δ^{-9}-THC sessions. The effects of cannabinoid treatment on locomotion and neural activity was assessed in comparison with the control sessions. Different doses of Δ^{-9}-THC were tested to create dose–response curves for spontaneous motor activity.

Treadmill locomotion task

Treadmill locomotion sessions were carried out after completion of the spontaneous movement task (Shi et al., 2004). A transparent acrylic box (length 37 × width 19 × height 39 cm) was mounted on a conveyer belt driven by a motor with adjustable speed. The belt served as the floor of the chamber. Rats were trained to walk at a moderate pace on the treadmill with a constant speed (12 cm/s). The treadmill cycle consisted of a 20-sec walking phase triggered by an auditory cue and a random 10–30-sec resting period. The cue tone was presented 1.5 sec before the onset of treadmill walking and each session lasted 60 min. Control data were collected after 3–4 days of training when rats exhibited a smooth walking pattern. Rats received a 0.3 ml saline injection (i.p.) 20 min before each control session. Δ^{-9}-THC was injected (2.0 mg/kg i.p.) 20 min before the experimental sessions. Video records (33 ms resolution) were used to monitor and analyze behavior during the treadmill sessions.

Electrophysiological recording

Extracellular recordings of the four basal ganglia areas were performed at least 10 days after surgery by connecting a headstage plug and a lightweight cable between a commutator and the implanted microwire assembly. The commutator was assisted by a motor that senses the direction of the rat's movement and was free to turn as necessary, permitting unrestricted movement of the rat in the behavioral chamber. Neuroelectric signals were passed from the headset assemblies to programmable amplifiers, filters (0.5 and 5 kHz) and a multichannel spike-sorting device. As many as 62 neurons from the STR, GP, STN, and the SNr were monitored simultaneously from 64 microelectrodes. Spike activity, treadmill operation, and infrared beam breaks were recorded (1 ms resolution) and controlled with the data acquisition software Magnet (Biographics, Winston-Salem, NC). Spike train activity was analyzed offline with the PC-based programs Stranger (Biographics) and Nex (Plexon, Dallas, TX).

Drugs

Δ^{-9}-THC was obtained under ethanol (Sigma, St. Louis); 2% pluronic acid in 200 proof ethanol was added to the Δ^{-9}-THC solution at a 4:1 ratio. Saline was then dropped into the mixture until the solution turned white. The solution was then blown in the darkroom with a nitrogen stream until the ethanol was totally evaporated. Saline was then added to make the final solutions (1 mg/ml or 0.1 mg/ml when 0.05 mg/kg was needed).

Histology

At the conclusion of the final experimental session, each animal was subjected to the same anesthesia as in surgery. A positive current of 10–20 μA was passed through selected microwires for 10–20 sec to deposit iron ions. The animal was then sacrificed and perfused with 4% paraformaldehyde solution. Coronal sections (45 μm thick) were cut through the STR, GP, STN, and SNr and mounted on slides. Incubation of the mounted sections in a solution containing 5% potassium ferricyanide/10% HCl revealed iron deposits (recording sites) in the form of blue dots. Boundaries of the four brain areas were assessed with reference to the rat brain atlas of Paxinos and Watson (1986).

Data analysis

Data were processed offline with Stranger and Nex software for basic analysis and graphics. MatLab (MathWorks, Natick, MA) and SPSS (Chicago, IL) were used for advanced statistics. Data were analyzed from a single representative session from each rat.

In the spontaneous locomotion task, each session consisted of active and quiescent phases. Motor behavior was quantified by calculating the total number of beam breaks over the 1-h session, and the total number of the beam breaks were compared between control sessions and sessions with each dose of Δ^{-9}-THC. $P < 0.05$ in Student's *t*-test indicated significant comparisons.

In the treadmill locomotion test, frame-by-frame video analysis (33 ms resolution) of limb movement was conducted in both control and Δ^{-9}-THC treatment sessions. Two limb movement events were identified by video analysis: footfall, defined as the initial paw contact with the ground (soft contact), and foot off, defined as the onset of the swing phase (when the paw left the ground) (Cohen and Gans, 1975). To allow a more descriptive analysis, the step cycle was divided into two major phases, stance (paw down) and swing (paw up), defined by the onsets of footfall and foot off, respectively (West et al., 1990). All timestamps for limb movement were compiled and entered into the data file as event nodes for behavioral and electrophysiological analyses.

To identify the effects of Δ^{-9}-THC on basal ganglia neural responses, only the 2 mg/kg Δ^{-9}-THC group was chosen for electrophysiological data analysis since the most evident behavioral effect was found at this dose. In the spontaneous movement test, the session was divided into 20-sec segments and beam breaks of each segment were counted. Segments without beam breaks were counted as quiescent and segments with more than five beam breaks were counted as active segments. The mean firing rates of each neuron and the average firing rates of neurons within each region were calculated for the active phases and quiescent phases in both control and Δ^{-9}-THC treatment sessions and comparisons were made between these sessions by Student's *t*-test with a significant difference level of $P < 0.05$.

Within each treadmill session, the mean firing rates in walking and resting phases were calculated separately. A sliding-window method was used to measure and compare neural activity changes between walking and rest phases and between control and Δ^{-9}-THC treatment conditions. Counts per bin (0.1 sec bin size) of single neurons during treadmill locomotion were calculated for each trial. The results were then exported to MatLab. Neural activity was then calculated by a time window (typically 1 sec, but in some cases 250 ms for tone responses with narrow peaks) moving

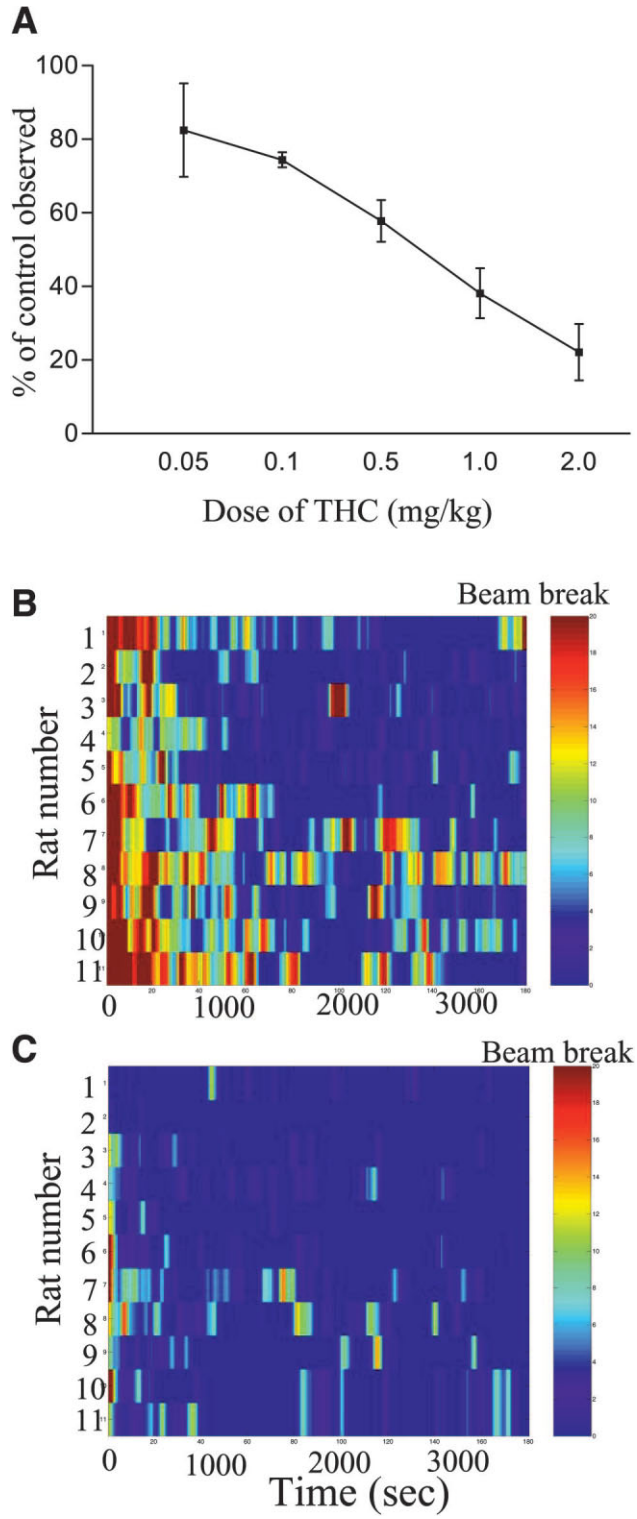

Fig. 1. Effects of Δ^{-9}-THC on spontaneous motor activity. **A:** Spontaneous motor activity was dose-dependently inhibited by 30–75% with Δ^{-9}-THC (0.05 to 2 mg/kg). **B:** Color-coded motor activity in control and (**C**) after 2 mg/kg Δ^{-9}-THC. Each line represents one rat during its 60-min experimental session. The number of photo beam breaks is color-coded (high number in red, low number in blue). A significant decrease in motor activity was found with 2 mg Δ^{-9}-THC treatment (n =11).

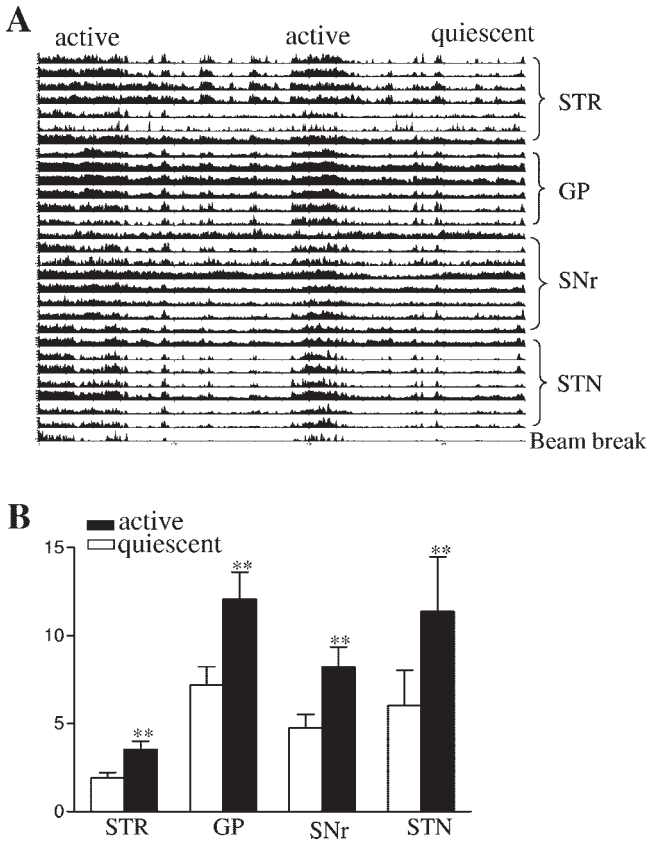

Fig. 2. Neural activity changes associated with active and quiescent phases of spontaneous motor activity. **A:** Ratemeter showing the neural activity in the basal ganglia during spontaneous motor activity. Increased neural activity was found in all four basal ganglia regions during the active phase. Increased neural activity was coincident with the high number of beam breaks labeled by ticks at the bottom line of the plot. **B:** Average firing rates in basal ganglia regions during active and quiescent phases of the spontaneous motor test. Increases in firing rates were observed in all four basal ganglia regions (mean ± SD, $^{**}P < 0.01$, Student's t-test).

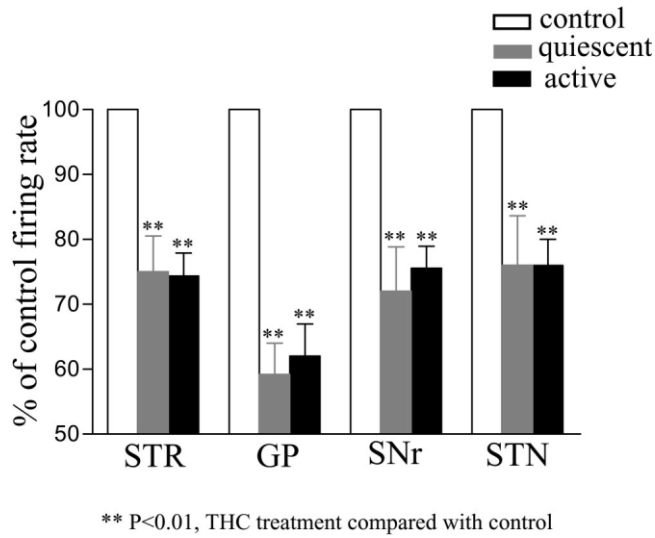

Fig. 3. Effects of Δ^{-9}-THC on basal ganglia neural activity during spontaneous motor activity. Decreases in neural firing rate were observed in all four basal ganglia regions following 2 mg/kg Δ^{-9}-THC injection. There was no difference in degree of inhibition between active and quiescent phases ($^{**}P < 0.01$, Student's t-tests. Active and quiescent phases with Δ^{-9}-THC vs. control condition).

at 0.1-sec steps across the duration of the treadmill walking phase.

The following two arbitrary criteria were used concurrently to detect significant changes in neural firing rates: 1) firing rate changes (increase or decrease) of greater than 20% during the time periods measured compared with the baseline firing rate measured 10 sec before the initiation of treadmill walking; 2) analyses of differences in at least three successive steps of the moving window reached a statistically significant level ($P < 0.05$, Student's two-tailed t-test). These measures accounted for slow and fast firing neurons to show both substantial (100%) and significant changes.

Power spectrum density analysis revealed oscillatory neural activity. This analysis was carried out during treadmill walking and resting phases in both control and Δ^{-9}-THC treatment sessions. Power spectrum density during each of these phases was computed with a 20 Hz or 10 Hz band and a 256 or 128 frequency. The formula for power spectrum computation is as follows:

$$\text{Bin size} = 1/(2 \times \text{maximum frequency})$$

$$\text{Number of bin} = 2 \times \text{number of frequency value}$$

Autocorrelograms were calculated during walking and resting phases to corroborate the results of power spectrum analysis. The number of neurons that showed oscillatory discharges was compared between control and Δ^{-9}-THC treatment groups. A chi-square test with $P < 0.05$ was considered significant.

Principal component analysis (PCA) is a statistical method for reduction and interpretation of multivariate data. PCA is essentially a method for recognizing the information that is distributed across a set of partially correlated variables into an equal number of uncorrelated principal components. Most of the variance related to significant redundant information in the population is consolidated within the first few components. Valid neurons, selected on the basis of quality of waveform and noise-free recording, were used in each brain region (with 1 sec bin size) to calculate the PCA with software from the Nex program (Plexon), and the weighted firing rate of the PCA1 in each brain region was sent to MatLab to calculate the time-based power spectrum.

Cross-correlation analysis was performed during the treadmill experiment in both control and Δ^{-9}-THC treatment conditions. One neuron was selected as the reference neuron and all other neurons recorded within that same session were defined as partner neurons for the cross correlograms. The time of occurrence of spikes from the reference neuron was set as the 0-sec time point and the partner neuron's firing within 1 sec before or after each reference neuron's spike was plot-

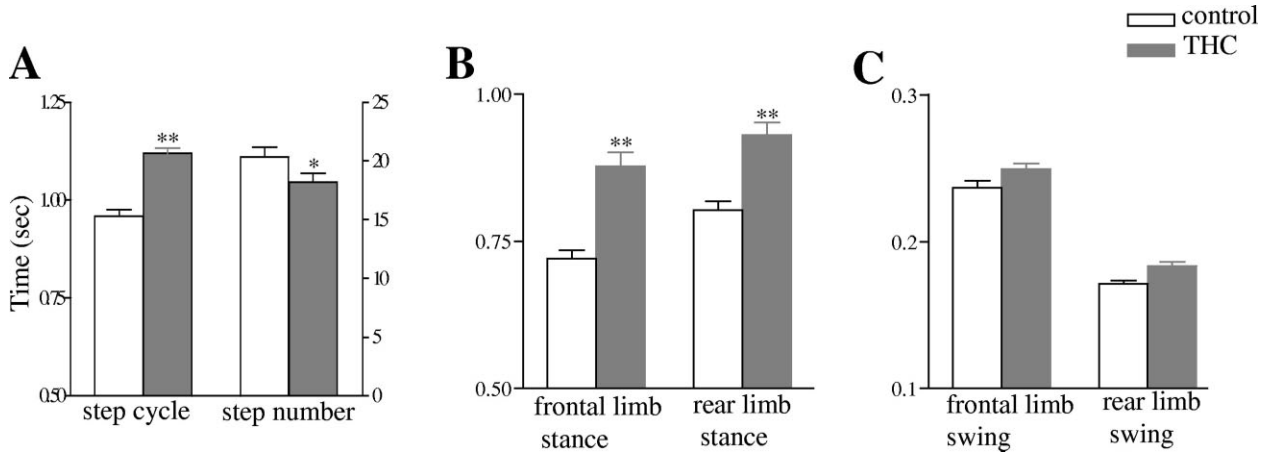

Fig. 4. Effects of Δ^{-9}-THC on stance and swing times of front and rear limbs during treadmill walking. **A:** Step cycle increased and step number decreased after Δ^{-9}-THC treatment. Both stance (**B**) and swing (**C**) times were increased with 2 mg/kg Δ^{-9}-THC, but only stance time reached significance (*$P < 0.05$, **$P < 0.01$, Student's *t*-test).

ted using a 1-ms bin size. The significance level of the cross-correlograms was tested using a 95% confidence level. Raster plots of the correlograms were examined carefully to eliminate any artifacts associated with nonneuronal noise.

RESULTS

Effects of Δ^{-9}-THC on spontaneous motor activity

When treated with the lowest dose of Δ^{-9}-THC, 0.05 mg/kg, three out of four rats tested exhibited reduced motor activity. One rat at this low dosage displayed a slight increase in motor activity. On average, there was an 18% decrease in beam breaks during the 1-h experimental session at this dose. At the 0.1 mg/kg dose and higher, Δ^{-9}-THC produced significant decreases in motor activity. The effect of Δ^{-9}-THC on spontaneous motor activity was dose-dependent, from 18% inhibition at 0.05 mg/kg to 75% at 2.0 mg/kg (Fig. 1A). Locomotor activity recovered to the pretreatment levels during the control sessions between the drug treatment sessions. The spontaneous motor activity patterns of the 11 rats in the control condition, as measured by the number of beam breaks, are depicted in Figure 1B with color-coded illustration. Rats typically displayed high levels of exploratory behavior early in the session, as demonstrated by high numbers of beam breaks within the first 10 min of the session, and then became relatively quiet. Some rats resumed high levels of activity later in the session. The spontaneous motor activity patterns of the same 11 rats treated with 2 mg/kg Δ^{-9}-THC are depicted in Figure 1C. A significant decrease in spontaneous motor activity was found at this dose of Δ^{-9}-THC.

Effects of Δ^{-9}-THC on basal ganglia neural activity during spontaneous movement test

Extracellular single neuron recording was performed in the four basal ganglia regions during spontaneous motor activity testing. A total of 99 neurons in the STR, 83 neurons in the GP, 55 neurons in the SNr, and 36 neurons in the STN were recorded from the 11 rats. In the control condition, significant increases in firing rates were observed in all four recording regions during the active movement phase. The mean firing rates of active and quiescent phases in each basal ganglia region are summarized in Figure 2B.

In parallel with the inhibition of motor activity, Δ^{-9}-THC treatment (2 mg/kg i.p.) significantly decreased the mean firing rates in all four basal ganglia regions during both active and quiescent phases relative to the control session (Fig. 3). A similar degree of Δ^{-9}-THC-induced inhibition was found in both the active and quiescent phases in all the basal ganglia regions tested (Fig. 3).

Effects of Δ^{-9}-THC on treadmill locomotion

A regular walking pattern with smooth, active forward steps could be established after 3–4 training sessions in the control condition. Rats were mostly quiet during the 10–30-sec random resting period. Slight movement occurred occasionally during the resting period, especially in the first few trials as rats explored the new environment, but such movement was not comparable with the continuous, active walking during the treadmill walking phase.

Δ^{-9}-THC treatment (2 mg/kg i.p.) markedly altered rats' treadmill walking patterns. Drug-treated rats displayed an irregular walking pattern during the treadmill walking phase, with periods of motionlessness followed by periods of larger catching up steps. The

catching up was triggered by the tail touching the rear wall of the treadmill chamber. To quantify this irregular walking pattern, video analysis of limb movement was made for eight rats during the treadmill walking phase in both control and Δ^{-9}-THC treatment conditions. In the control condition, the average step cycle was 0.96 ± 0.33 sec. For the forelimb, the means of stance (when the paw remained on the floor) and swing (when the paw was in the air) phases were 0.72 sec and 0.23 sec, respectively. For the hindlimbs, the means for stance and swing were 0.80 sec and 0.16 sec, respectively. In the Δ^{-9}-THC treatment condition, the average step cycle increased to 1.12 ± 0.29 sec ($P < 0.01$ vs. control session, Student's two-tailed t-test, Fig. 4). Δ^{-9}-THC-treated rats needed to take larger steps with a longer step cycle to catch up with the treadmill, which moved at a constant speed. The means of forelimb stance and swing phases were increased to 0.88 sec and 0.25 sec, respectively, with Δ^{-9}-THC treatment ($P < 0.01$ vs. control session for stance time). The means of hindlimb stance and swing phases were increased to 0.93 sec and 0.18 sec, respectively ($P < 0.01$ vs. control session for stance time, Fig. 4). The number of steps decreased from 20 to 18 per trial by Δ^{-9}-THC treatment ($P < 0.05$, Fig. 4A).

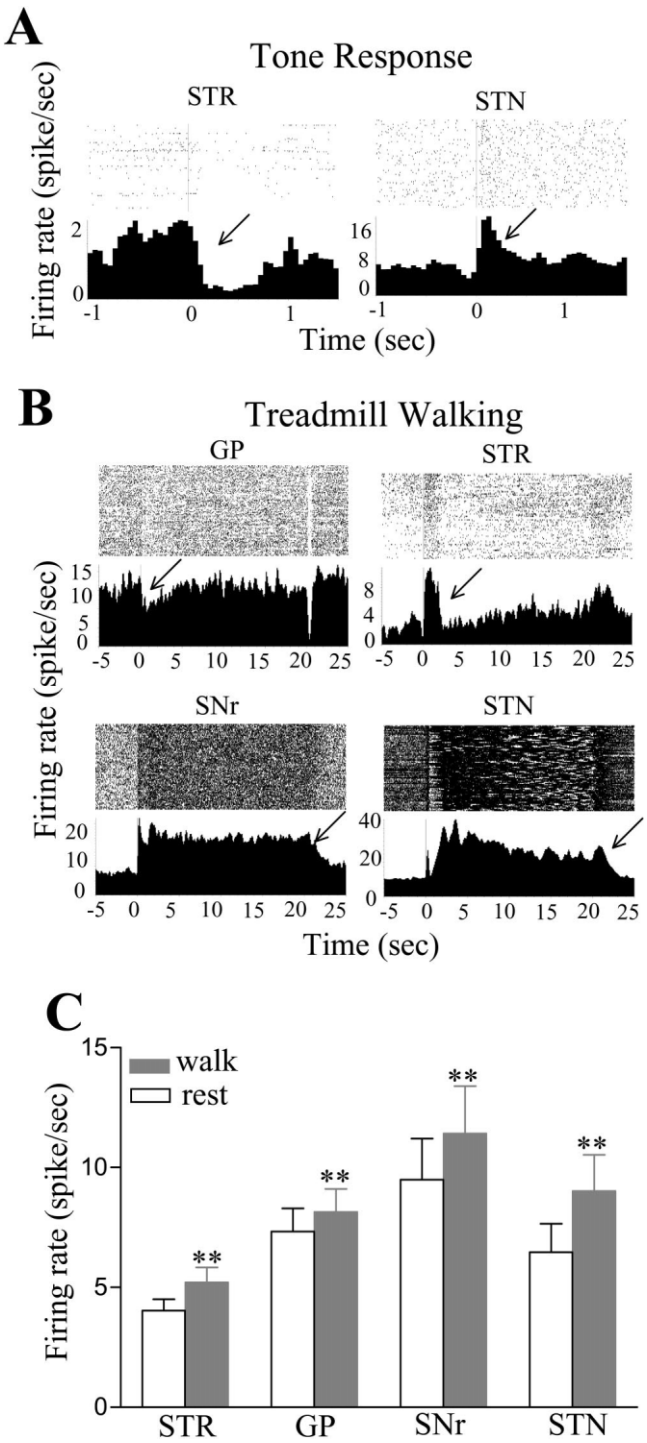

Fig. 5. Neural responses during tone presentation and treadmill locomotion. **A:** Raster and perievent histogram showing different neural responses to the cue tone (200 ms duration) presented 1.5 sec before treadmill onset. An STR neuron (left) decreased and an STN neuron (right) increased firing rate upon tone presentation (0 sec point). **B:** Different neuronal responses in the basal ganglia regions during the treadmill walking phase (from 0–20 sec timestamp). Transient responses at the start and termination of treadmill locomotion in a GP neuron (upper left). A sizable drop in spike discharge occurred immediately at the start and termination of treadmill walking (arrows). An STR neuron exhibited an initial peak of discharge and gradually increased spike activity during the treadmill walking phase (upper right). An SNr neuron increased its firing rate throughout the treadmill walking period (lower left). An STN neuron increased its firing rate with an initial peak (lower right). **C:** Average firing rates of each basal ganglia region during walking and resting phases. Mean firing rates increased in all regions during the walking phase (**$P < 0.01$ vs. resting phase, Student's t-test).

Effect of Δ^{-9}-THC on neural responses during treadmill locomotion task

A total of 291 neurons from 11 rats were recorded in the treadmill experiment (100 in STR, 88 in GP, 62 in SNr, and 41 in STN). Two major neural responses were analyzed in both the control and Δ^{-9}-THC treatment conditions. One was associated with the auditory cue and the other was observed during treadmill locomotion. Peak responses immediately following the tone cue were observed in all four basal ganglia regions (Fig. 5A). In the control condition, responses ranged from 14% of the population in the SNr to about 30% of the population in the STR. Δ^{-9}-THC treatment did not change neural responses to the tone in any of the four basal ganglia regions (Table I).

More prevalent neural responses were found during the treadmill locomotion phase. More than half of neurons in all recorded regions altered their firing rates during the treadmill walking phase in the control condition. The most robust response was observed in the STN, where 90.2% of recorded neurons altered their firing rates during treadmill walking. Table I summarizes the neuronal responses associated with tone and

TABLE I. Basal ganglia neural responses during the treadmill task in control and Δ^{-9}-THC treatment conditions

					Treadmill walking											
			Tone responding neurons		Total responding neurons		Initiation		Excitatory		Inhibitory		Inhibitory/ Excitatory	Termination	
Brain area	Total number of neurons		N	% of total	N	% of total	N	% of total	N	% of total	N	% of total	Ratio * 100	N	% of total
STR	100	control	33	33.0	74	74.0	28	28.0	61	61.0	4	4.0	6.6	9	9.0
		THC	33	33.0	87*	87.0	34	34.0	76*	76.0	6	6.0	7.9	8	8.0
GP	88	control	19	21.6	57	64.8	23	26.1	41	46.6	10	11.4	24.4	7	8.0
		THC	16	18.2	74**	84.1	23	26.1	55*	62.5	7	7.9	12.7	12	13.6
SNr	62	control	10	16.1	49	79.0	25	40.3	39	62.9	4	6.4	10.2	3	4.8
		THC	12	19.4	50	80.6	24	38.7	46	74.2	1	1.6	2.2	3	4.8
STN	41	control	13	32.0	37	90.2	25	61.0	36	87.8	1	2.4	2.8	0	0
		THC	13	32.0	38	92.7	27	65.8	38	92.7	1	2.4	2.6	4	9.8

In both control and Δ^{-9}-THC treatment conditions, more STR neurons were responsive to the tone cue preceding the start of treadmill walking, and more STN neurons responded during the treadmill walking phase. Total responding neurons refers to the number of neurons that responded at least once at different phases of treadmill walking. Initial responses refer to the transient neural responses that occurred at initiation of walking. Excitatory and inhibitory responses refer to the continuous responses during the 20-sec treadmill walking phase. Termination responses refer to the transient responses at the termination of the treadmill walking phase. There were significantly more neurons in the STR and GP responding to treadmill walking after Δ^{-9}-THC treatment (chi-square test, $P < 0.01$ for GP; $P < 0.05$ for STR), and in these two brain regions the number of neurons with excitatory responses increased after Δ^{-9}- THC treatment (chi-square test, $P < 0.05$).

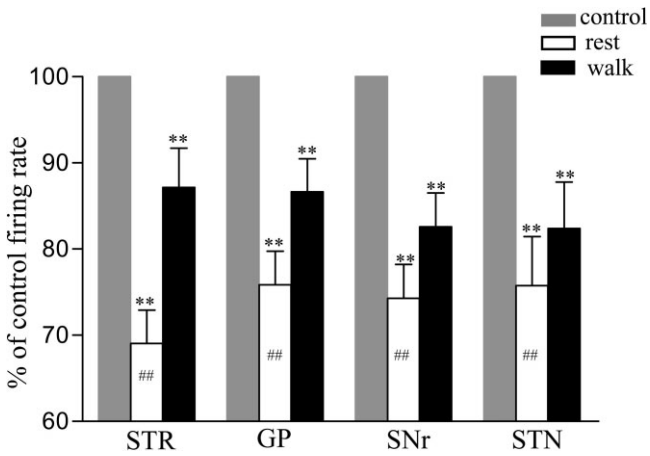

Fig. 6. Δ^{-9}-THC treatment (2.0 mg/kg) inhibited basal ganglia activity during walking and resting phases of the treadmill experiment. Δ^{-9}-THC caused a more potent inhibition during the resting than the walking period (**$P < 0.01$, Δ^{-9}-THC vs. control. ##$P < 0.01$, resting vs. walking phase with Δ^{-9}-THC).

walking in control and Δ^{-9}-THC treatment conditions. Examples of neural responses are shown in Figure 5B. The vast majority of neural responses during treadmill walking were excitatory (increased firing rate) and only a few neurons decreased their firing rates during treadmill walking. The GP had the highest number of neurons with inhibitory responses (~10% of total neurons). However, even in the GP far more neurons (nearly 50%) exhibited excitatory responses during treadmill locomotion. In general, the mean firing rate of all the basal ganglia regions increased significantly during the treadmill walking phase relative to the resting phase (Fig. 5C).

Δ^{-9}-THC treatment affected the mean firing rates of basal ganglia neurons during the treadmill experiment. Figure 6 summarizes these mean firing rate changes in each basal ganglia region during treadmill walking and resting phases. Similar to what had been found in the spontaneous motor activity test, Δ^{-9}-THC treatment generally inhibited basal ganglia neurons during both walking and resting phases. In addition, Δ^{-9}-THC elicited different degrees of inhibition during the walking vs. resting phases. A higher degree of inhibition was found during the resting than the walking phase in all four basal ganglia regions (Fig. 6), especially in the STR and GP. In these two regions, differences not only in the mean firing rate, but also in the number of inhibitory neurons were found between resting and walking phases in Δ^{-9}-THC-treated rats. Δ^{-9}-THC treatment significantly decreased the number of neurons exhibiting inhibitory neural responses during the walking phase in comparison with the resting phase in the STR and the GP (Fig. 7).

Δ^{-9}-THC treatment elicited more limb movement-related responses in the STR and GP. Most of these responses were excitatory responses (Table II). Detailed analysis of these responses revealed that a disproportional inhibition of neural activity during rest vs. walking phases by Δ^{-9}-THC may be the result of the appearance of limb movement-related neural responses during the walking phase (see below).

Effects of Δ^{-9}-THC treatment on basal ganglia neuronal activity related to limb movement

As described above, Δ^{-9}-THC caused a prolonged step cycle during treadmill locomotion. Offline video analysis was done to timestamp when the forelimb and hindlimb touched and left the floor. These data were used to create a perievent histogram to reveal the neural responses to limb movement. A total of 82 neurons in the STR, 74 neurons in the GP, 49 neurons in the SNr, and 38 neurons in the STN were analyzed to identify limb-related neural responses during treadmill walking.

Table II summarizes the limb movement-related neural responses in each basal ganglia region in both control and Δ^{-9}-THC treatment conditions. Although large num-

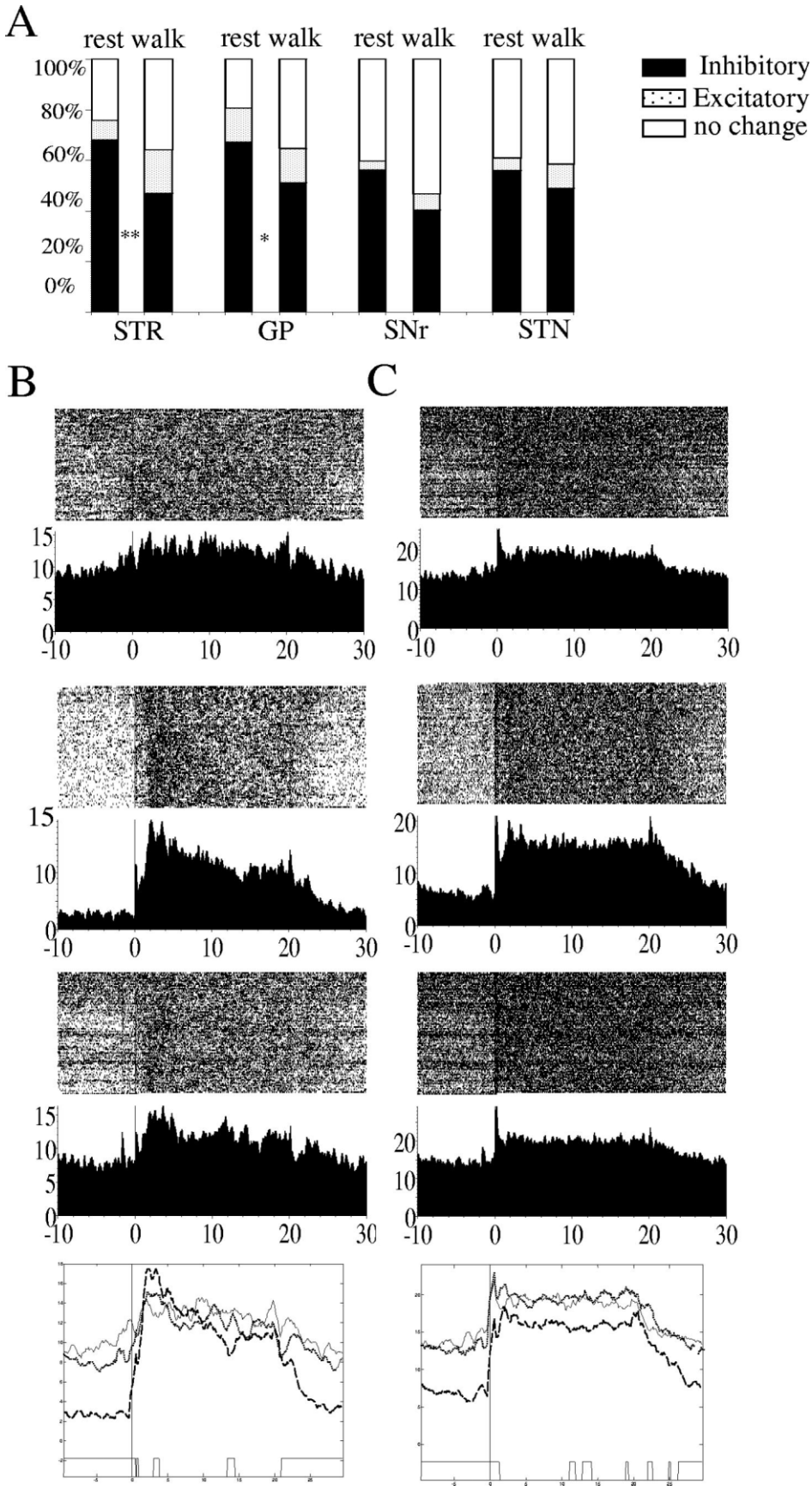

Fig. 7. Δ^{-9}-THC changed the type of neural responses during treadmill locomotion. **A:** Populations of neurons exhibited excitatory, inhibitory, or no responses during treadmill walking and resting phases. Overall, inhibitory neurons greatly outnumbered excitatory neurons. Significantly more inhibitory neurons were observed in the resting phase in the STR and GP (**$P < 0.01$, *$P < 0.05$, chi-square test). This difference in number of inhibitory neurons was due to the different action of Δ^{-9}-THC during walking vs. resting phases. **B:** An STR neuron with different responses to Δ^{-9}-THC treatment during walking and resting phases. A slightly increased firing rate was observed during the treadmill walking phase in the control condition (from 0–20 sec, top panel). Δ^{-9}-THC inhibited responses during the resting phase (–10 to 0 sec and 20–30 sec, middle panel). A similar neural response pattern appeared following Δ^{-9}-THC treatment (bottom panel). Comparison of the control (solid line), the Δ^{-9}-THC (dashed line), and the recovery after Δ^{-9}-THC sessions (dotted line) reveals a selective decrease in firing rate during the resting phase in the Δ^{-9}-THC session ($P < 0.01$, ANOVA). **C:** A similar response pattern, selective inhibition of Δ^{-9}-THC during resting phase, was observed in this GP neuron.

TABLE II. Neural responses associated with limb movement in each basal ganglia region during treadmill walking in control and Δ^{-9}-THC treatment conditions

	Brain regions							
	STR		GP		SNr		STN	
	Num	%	Num	%	Num	%	Num	%
Control	18	22.0	7	9.4	3	6.1	2	5.2
THC	30*	36.6	16*	21.6	8	16.3	4	10.5
total	82		74		49		38	

Neurons in different regions showed different response percentages to limb movement during treadmill walking in control and Δ^{-9}-THC conditions. Neurons were generally more responsive to limb movement after Δ^{-9}-THC treatment, but increases were significant only in the STR and GP (chi-square test, $P < 0.05$).

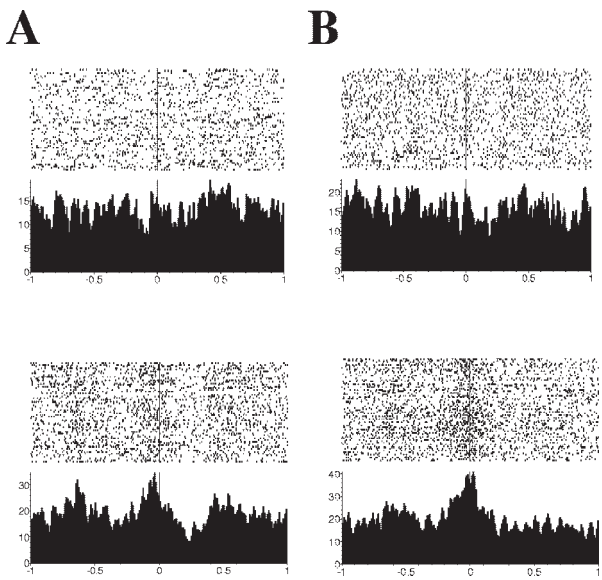

Fig. 8. Limb movement-related neural activity induced by Δ^{-9}-THC treatment. **A:** Activity of an STR neuron in relation to limb movement in control (top) and Δ^{-9}-THC treatment (bottom) conditions. The timestamp of the front paw touching the floor was used as a reference event to create this raster and perievent histogram. Neural activity did not change in control conditions. Biphasic neural responses were found. Spike activity first increased and then decreased around the event of paw contact at 0 sec. **B:** An example of limb movement-related neural responses in a GP neuron. In this case, Δ^{-9}-THC treatment induced a robust response before paw contact.

bers of treadmill locomotion-related neurons were found, there were fewer neurons with limb movement-related responses in the control condition. Δ^{-9}-THC treatment elicited more limb movement-related neural responses in all four regions (Table II, Fig. 8), but increases in limb movement-related neurons only differed significantly in the STR and GP (chi-square test, $P < 0.05$).

Oscillatory and synchronized neural activity in the basal ganglia regions during treadmill locomotion

The oscillatory firing pattern was measured by power spectrum analysis during treadmill walking and resting phases. In the control condition only a few neurons displayed oscillatory neural firing patterns during the treadmill walking phase (≤5%). Δ^{-9}-THC treatment increased the number of neurons displaying an oscillatory firing pattern in all basal ganglia regions studied, but the increase was significant only in the STR and GP (Table III, chi-square test, $P < 0.05$). Examples of the oscillatory firing pattern are shown in Figure 9. The oscillation frequency is around 1 Hz, similar to the step cycle of treadmill walking. A time-based power spectrum, using weighted firing rate generated from the principal component analysis (PCA 1), revealed that this oscillatory firing pattern occurred only during treadmill walking (not resting) in the STR and GP (Fig. 9C,D). As to the question of whether all the limb movement-related neurons oscillated during walking phase, the results showed that many limb movement-related neurons did not oscillate in the control condition during walking, whereas significantly more limb movement-related neurons exhibited oscillatory firing patterns with Δ^{-9}-THC treatment (Table IV). This dissociation between limb movement and oscillatory neural responses is depicted in Figure 10.

Synchronized activity was analyzed by the pairwise cross-correlograms method for all simultaneously recorded neurons of the basal ganglia regions. Correlated activity was found between neurons in the following two categories: 1) intraregional cross-correlations (between neurons within the same region); 2) interregional correlations (between neurons in different regions). In the control condition, among a total of 3,502 pairs of neurons from 311 neurons recorded, only 48 (1.4%) and 37 (1%) significant cross-correlations were observed between neuron pairs during treadmill walking and resting phase, respectively. No significant difference was detected between these two phases. In the Δ^{-9}-THC treatment condition, 66 (1.9%) and 63 (1.8%) significant cross-correlations were observed between neuron pairs during walking and resting phases, respectively. In general, Δ^{-9}-THC treatment resulted in more neural correlation during resting phase ($P < 0.05$ vs. control condition, chi-square test). With regard to each type of cross-correlation (there were 10 types of intrainterregional cross-correlations from four basal ganglia regions, e.g., cross-correlation between STR and GP, STN and SNr neurons, etc.), there was no significant difference between the control and Δ^{-9}-THC-treated conditions for each type of these cross-correlations (Fig. 11).

TABLE III. Effects of Δ^{-9}-THC on oscillatory firing patterns in the basal ganglia during treadmill walking

	Brain regions							
	STR		GP		SNr		STN	
	Num	%	Num	%	Num	%	Num	%
Control	5	5.0	1	1.1	3	4.8	1	2.4
THC	27	27.0**	8*	9.1	3	4.8	0	0
After THC	4	4.0	0	0	2	3.2	1	2.4
total	100		88		62		41	

Fewer neurons exhibited oscillatory firing in the control than the Δ^{-9}-THC condition in the STR and GP.
*$P < 0.05$, **$P < 0.01$, chi-square test when compared with control.

Histological localization of recording sites

Potassium ferricyanide staining revealed the recording sites as blue dots in the STR, GP, STN, and SNr. Figure 12 shows the locations of recording sites included in this report.

DISCUSSION

Cannabinoid receptors are localized on the cell bodies and neuronal processes of striatal neurons that project to the SNr and GP (Herkenham et al., 1991). They have also been found in the terminals of the subthalamo-nigral glutaminergic pathway. In situ hybridization revealed low to moderate levels of CB1 receptor mRNA in the majority of the neurons in the STN (Mailleux and Vanderhaeghen, 1992). Such abundant cannabinoid receptors in the basal ganglia may account, at least in part, for their effects on motor activity. Early studies demonstrated a biphasic effect of cannabinoids on spontaneous movement: low doses of cannabinoids caused increases in motor activity, while high doses inhibited motor activity (Abel, 1970; Davis et al., 1972; Sulcova et al., 1998). Presently, uniform and dose-dependent inhibition of spontaneous motor activity was found when animals were treated with Δ^{-9}-THC from 0.05 to 2.0 mg/kg. The lack of an excitatory effect of the low dose of Δ^{-9}-THC here may be due to the differences in animal species (rats vs. mice) or chemical components (Δ^{-9}-THC vs. anandamide) used. Our finding, however, is in line with other reports indicating a similar dose-dependent decrease in spontaneous motor activity and force-time trajectory (Carlinin et al., 1970; McLaughlin et al., 2000).

We recently developed a rat model to evaluate motor activity in which unilateral DA-lesioned rats display akinesia during treadmill walking (Chang et al., 2003). This treadmill model was used in this study to evaluate the effects of cannabinoid treatment on locomotion. The locomotor behavior of rats treated with Δ^{-9}-THC was unlike that of DA-lesioned rats. DA-lesioned rats displayed akinesia and were pushed back to the rear wall of the treadmill chamber and then struggled to catch up with the treadmill. On the other hand, Δ^{-9}-THC treatment induced an irregular movement characterized by a short period of immobility followed by large catch-up steps when the tail of the animal touched the wall of the chamber. As a result, stance time (when the paw was in contact with the floor) in Δ^{-9}-THC-treated rats was increased significantly in comparison with the control condition, when steps were smooth and regular. This is in sharp contrast with DA depletion conditions in which swing time (when the paw was in the air) is decreased (unpubl. obs.). Different limb movement patterns between dopamine depletion and Δ^{-9}-THC treatment conditions suggest that Δ^{-9}-THC treatment does not induce akinesia in treadmill locomotion. A common mechanism may account for the effects of Δ^{-9}-THC on both the spontaneous and treadmill locomotion tests. In both conditions the rat is reluctant to move, resulting in an 80% reduction in spontaneous movement and sensory triggered (tail touch), involuntary treadmill walking with altered steps.

In this and our previous studies, tight coupling of basal ganglia neural activity with motor activity was observed in control conditions (Shi et al., 2004). Significant increases in discharge rates across the basal ganglia were observed during both spontaneous and treadmill movement. This uniform increase in activity does not directly fit the classical model of basal ganglia thalamocortical pathways (Alexander et al., 1990). Rather, these findings suggest a regulatory role of the basal ganglia to provide active background for information processing in basal ganglia thalamocortical pathways (Shi et al., 2004). Basal ganglia neural responses during motor activity observed in these studies provide a control for studying drug and pathophysiological actions on movement and related basal ganglia neural responses.

Information on basal ganglia neural responses to cannabinoids in behaving animals is lacking. The present study employed a multiple-site, single-unit recording method to study the neural and behavioral responses to cannabinoid treatment in awake, freely moving rats. In line with the robust decrease in motor activity, Δ^{-9}-THC treatment at the most behaviorally effective dose (2 mg/kg) significantly reduced neural activity in all four basal ganglia regions studied. Neurons inhibited greatly outnumbered those excited by Δ^{-9}-THC (Fig. 7) and average firing rates were decreased by Δ^{-9}-THC in the four basal ganglia regions (Fig. 6). These results demonstrate pronounced cannabinoid inhibition of basal ganglia activity at a dose that inhibits motor activity.

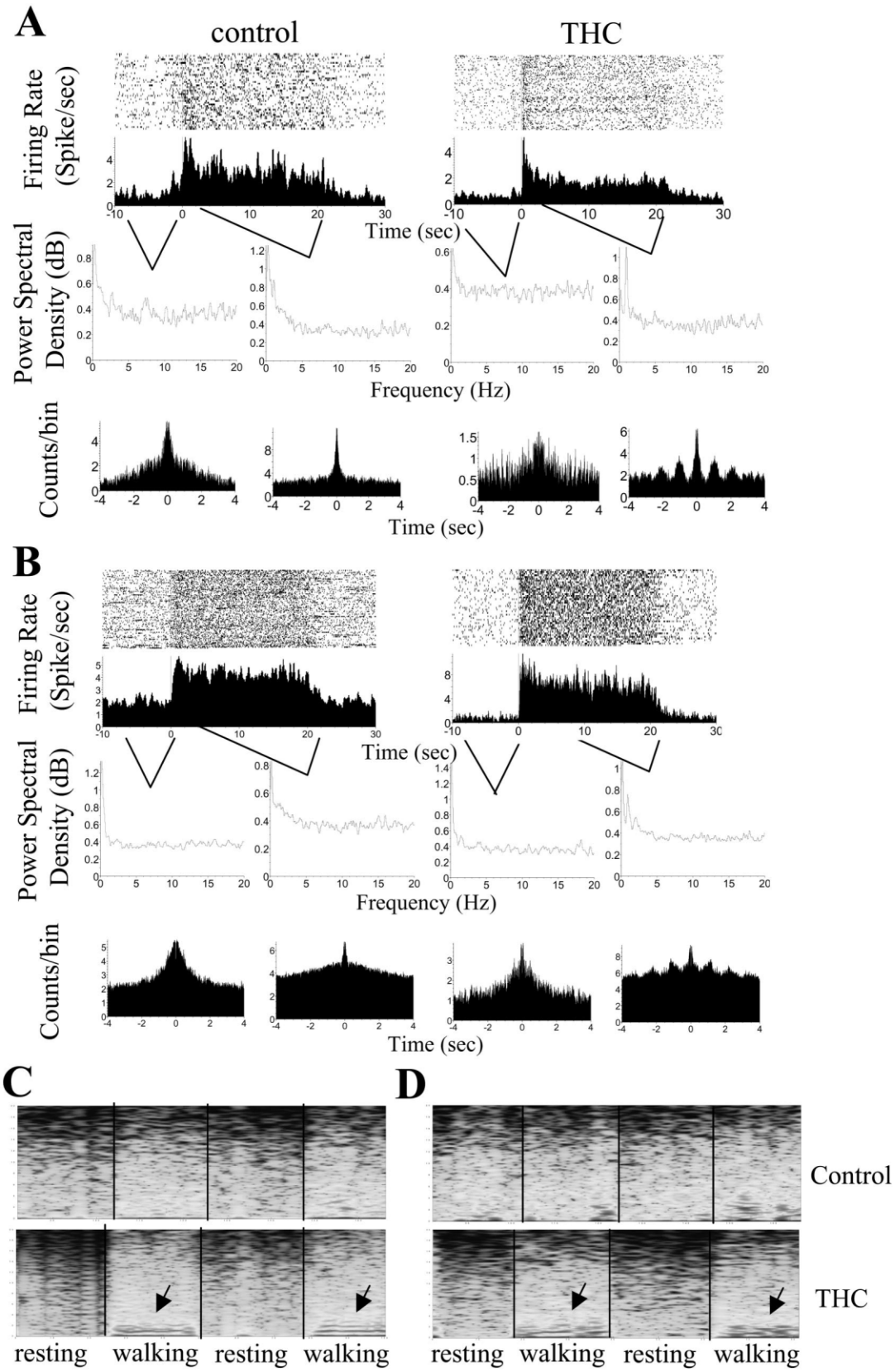

Fig. 9. Δ^{-9}-THC induced oscillatory firing patterns in the basal ganglia. **A:** Example neural responses in STR during control and Δ^{-9}-THC treatment conditions. Neural activity increased with treadmill walking during both control and Δ^{-9}-THC treatment conditions (top). Power spectrum analysis revealed a peak ~1 Hz during the treadmill walking phase in the Δ^{-9}-THC treatment condition (middle). An autocorrelogram shows multiple peaks at 1-sec intervals during treadmill walking in the Δ^{-9}-THC treatment condition (bottom). **B:** An example GP neuron displaying a similar oscillatory firing pattern during the treadmill walking phase with Δ^{-9}-THC. **C:** Time-based power spectrum analysis of ensemble neural activity in the STR. The analysis is based on the principal component 1 from six neurons simultaneously recorded in the STR during the treadmill test. A low-frequency oscillation appeared during the treadmill walking phase in the Δ^{-9}-THC treatment condition (indicated by arrows). **D:** The same analysis performed in five simultaneously recorded GP neurons revealed oscillatory firing during the treadmill on phase with Δ^{-9}-THC treatment.

TABLE IV. Relationship between limb movement-related and oscillatory neurons in control and Δ^{-9}-THC treatment conditions

	Brain regions							
	STR		GP		SNr		STN	
	limb	osc	limb	osc	limb	osc	limb	osc
Control	18	5**	12	1	3	3	2	1
THC	30	27	25	8	8	3	4	0

Δ^{-9}-THC treatment increased limb movement associated and oscillatory neuronal activity in the STR and GP. Not all limb movement responsive neurons were oscillatory. The proportion of oscillatory neurons increased significantly in the STR with Δ^{-9}-THC treatment.
**Chi-square test, $P < 0.01$ when compared with control.

The STR is an input station of the basal ganglia with both excitatory cortical and dopaminergic nigral afferents. A limited number of in vitro studies have investigated cannabinoid effects on striatal neural activity. Cannabinoids can inhibit both inhibitory GABA (Hoffman and Lupica, 2001; Szabo et al., 1998) and excitatory glutamate release (Gerdeman and Lovinger, 2001; Huang et al., 2001; Robbe et al., 2001) in the synapses of medium spiny neurons of the STR. The net outcome of these opposite effects on neural activity is unclear. It may depend on the cannabinoid dose and interaction of the STR with other brain structures in the context of ongoing behavioral activity (discussed below). The overall decrease in striatal firing rate may be due to the dominant inhibition of cortical glutamatergic input to the striatum as suggested by in vitro studies (Gerdeman and Lovinger, 2001; Huang et al., 2001; Robbe et al., 2001). Such inhibition could be the result of direct inhibition of cortical neurons or of presynaptic effects on the cortical projection terminals.

The GP (in rat) is part of an indirect pathway which receives striatal GABAergic input and sends inhibitory GABAergic projections to the STN. Abundant CB1 receptors are located in the afferent striatal terminals in the GP. Behavioral studies have indicated that the GP plays a critical role in cannabinoid regulation of motor function. Intra-GP cannabinoid injection induced ipsilateral turning in the rat (Sanudo-Pena and Walker, 1998), and bilateral injection of Δ^{-9}-THC into the GP could induce catalepsy in the rat (Pertwee and Wichens, 1991). An in vitro slice demonstration of GABA uptake blockade in the GP by Δ^{-9}-THC suggests that such effects may be mediated via effects on GABAergic transmission (Maneuf et al., 1996). Extracellular recording in anesthetized rats showed that systemic and local cannabinoid administration inhibited spontaneous firing rates of GP neurons (Miller and Walker, 1996, 1998). This finding is similar to our findings in awake, behaving rats. Cannabinoid inhibition of GP neurons may be due to accumulation of GABA in the synaptic cleft or to dampened excitatory input from the STN.

The STN is a pivotal structure in the basal ganglia and the only structure comprised of neurons with an excitatory neurotransmitter. CB1 receptors and their mRNA have been found in the STN (Mailleux and Vanderhaeghen, 1992). To our knowledge, there is no electrophysiological study of STN neural response to cannabinoids. Only an indirect study has been done to examine the effects of cannabinoids on SNr responses to STN activation. In that study, WIN55.212-2, a cannabinoid agonist, blocked SNr neural responses to bicuculline-induced STN activation (Sanudo-Pena and Walker, 1997). Another study demonstrated ipsilateral turning behavior with intra-STN cannabinoid agonist injection (Miller et al., 1998). Marked inhibition was found in the STN in our study. The mechanism of cannabinoid inhibition is unknown. It could be the result of inhibition of glutamate release from cortical inputs, or intrinsic inhibitory effects. Cannabinoids suppress inward sodium current and N-type calcium current in neuroblastoma cells, which may account for some of their inhibitory effects in the CNS (Caulfield and Brown, 1992; Mackie and Hill, 1992; Turkanis et al., 1991a,b). Furthermore, potassium A currents are activated by cannabinoids in the hippocampus (Deadwyler et al., 1993). The alteration in the activation of the A current maintains the cell near the resting membrane potential. This action of cannabinoids could counteract the effects of fast, transient, depolarizing action potentials in the hippocampus and other brain regions. Cannabinoid inhibition of STN neurons may partially account for the inhibition in its projection areas, the GP and SNr, as observed in this study.

The SNr, together with the internal GP, are the basal ganglia output stations and neural activity changes in these regions are thought to be responsible for a variety of movement disorders such as Parkinson's disease and dystonia. Previous studies have reported an increase in spontaneous neural activity in the SNr by local and systemic administration of cannabinoid agonists in anesthetized rats (Miller and Walker, 1995; Tersigni and Rosenberg, 1996). Local injection of SR 141716A, a cannabinoid antagonist, significantly decreased SNr neural activity, suggesting that endogenous cannabinoids may exert tonic regulation of striato-nigral transmission by inhibiting GABA release into the SNr (Tersigni and Rosenberg, 1996; Wallmichrath and Szebo, 2002a). In an in vitro study, Wallmichrath and Szebo (2002b) demonstrated that cannabinoid agonism decreased the amplitude of internal capsule stimulation-induced IPSPs, but did not

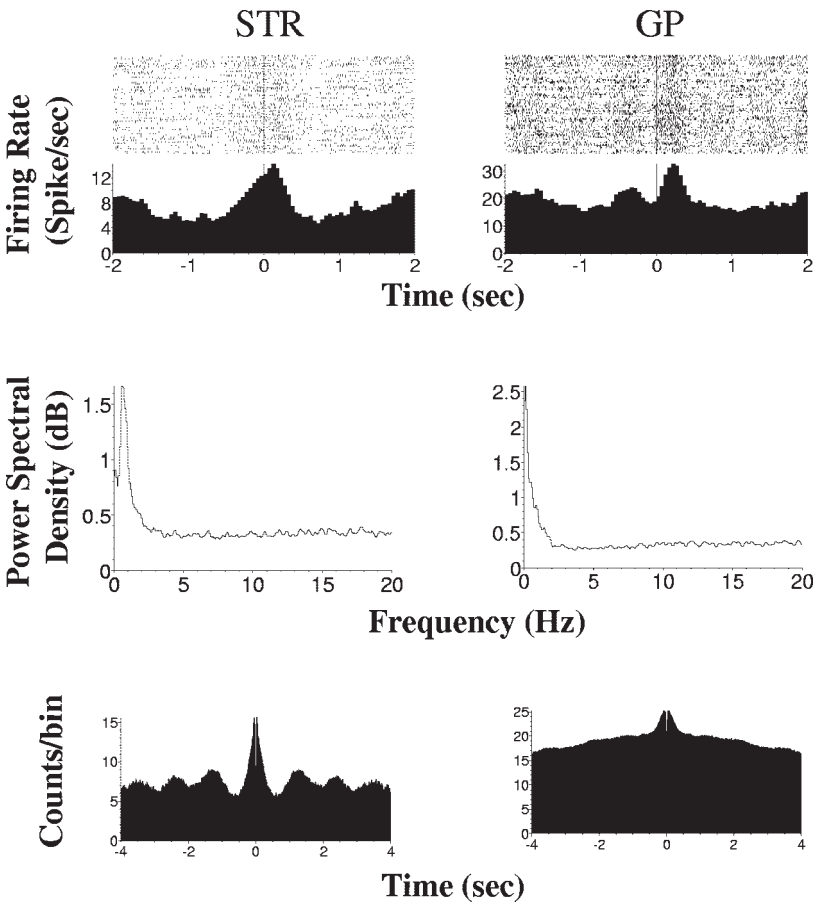

Fig. 10. Dissociation of limb movement related neural activity and oscillatory firing. An example case with similar limb movement-related neural responses exhibited with different oscillatory firing patterns is shown. An STR and GP neuron were recorded simultaneously during the treadmill test. They both exhibited robust limb movement-related activity when the front paw left the floor at time 0 (top). However, power spectrum and autocorrelogram analyses revealed that the STR neuron had an oscillatory firing pattern, whereas the GP neuron did not (middle and bottom).

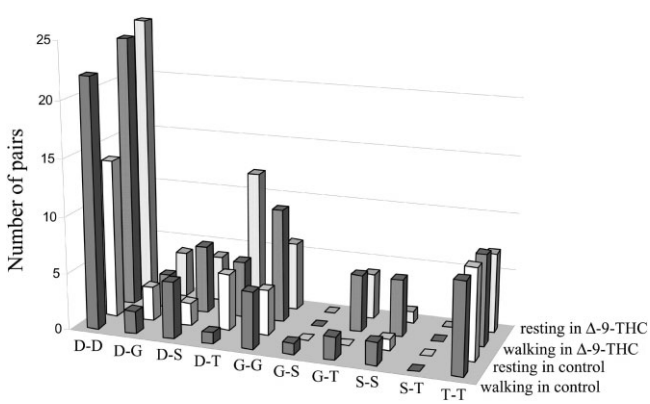

Fig. 11. Cross-correlation between basal ganglia neurons during control and Δ^{-9}-THC treatment conditions. Cross-correlograms of 3,502 pairs were analyzed. These pairs were between D (dorsal striatum), G (GP), S (SNr), and T (STN). Δ^{-9}-THC treatment increased cross-correlated neurons in the resting phase of the treadmill test. However, this increased population accounted for <2% of total population.

influence spontaneous IPSPs or the neural firing rate in the SNr. Other studies, however, found that the cannabinoid receptor agonist WIN 55.212-2 could induce hyperpolarization and inhibit spontaneous firing of SNr neurons in whole cell patch clamp recording, suggesting postsynaptic cannabinoid inhibition of SNr neurons (Chan et al., 1998). The inhibition of SNr neurons observed in the present study could be the result of such a postsynaptic inhibitory cannabinoid action or a decreased excitatory input from the STN. The implication of this nigral inhibition on motor activity is not clear. In theory, a decrease in basal ganglia output is associated with hyperkinetic conditions such as dystonia while an increase in basal ganglia GABAergic output is associated with hypokinetic conditions such as Parkinson's disease. However, experimental data vary substantially with regard to SNr neural responses. Increased, decreased and unchanged firing rates have been reported in these conditions (Hutchison et al., 2003; Rohlfs et al., 1997; Sanderson et al., 1986; Vitek, et al., 1999; Wichmann et al., 1999). It is noteworthy that in addition to neural firing rates changes, changes in firing patterns in the basal ganglia also play a critical role in regulating motor function (Brown, 2003; Raz et al., 2000).

Interestingly, the effects of cannabinoids on basal ganglia neural activity were dependent on the rats' behavioral state. Δ^{-9}-THC exerted more inhibition during the resting than the walking condition in the treadmill locomotion test. This differential degree of inhibition may be the result of behaviorally dependent activation of synaptic connections. It is possible that the motor cortex is activated during forced treadmill walking and thus increases the release of glutamate in

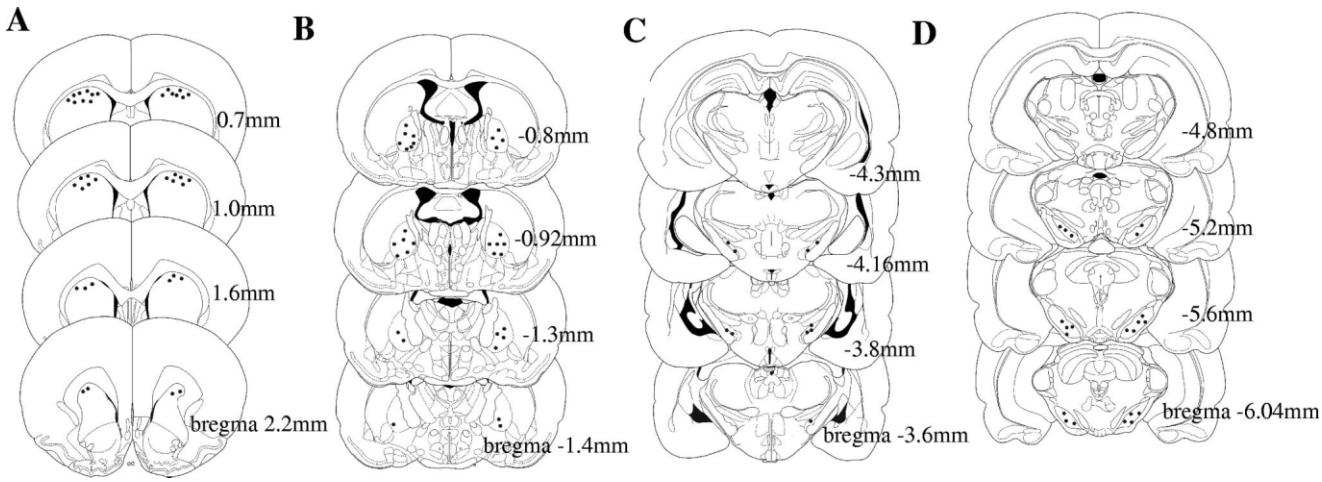

Fig. 12. Histological localization of recording electrodes included in this study. A: striatum. B: globus pallidus. C: subthalamic nucleus. D: substantia nigra pars reticulata.

cortico-striatal and cortico-subthalamic terminals which may to some extent offset the inhibitory effects of Δ^{-9}-THC. The present finding that Δ^{-9}-THC greatly increased limb movement-related neural responses in the STR, the region with direct sensorimotor cortical input, is consistent with this hypothesis.

Oscillatory firing patterns were increased by Δ^{-9}-THC treatment in the STR and GP. The frequency of oscillation is very similar to that of step circle (~1 Hz). The similar frequency and coincident increases in oscillatory and limb movement-related firing in the STR and GP strongly suggest that the oscillation is the result of increased limb movement-related neural responses. It is not clear why some limb movement-related neurons did not exhibit oscillatory firing patterns, whereas other neurons with similar limb movement-related activity in the same animal did. It may be related to the precise coding of a subset of limb movement by individual neurons. According to classical theory, normal information flow in the basal ganglia consists of parallel, separate processes (Alexander et al., 1990). Basal ganglia malfunction is thus expected to disrupt such parallel processing and cause "cross-talk" between neurons of different basal ganglia regions. A study in primates revealed an increase in synchronized basal ganglia neural activity in parkinsonian conditions (Raz et al., 2000). In the present study, a significant increase in synchronized activity among basal ganglia neurons was found after Δ^{-9}-THC treatment; however, such a small proportion of synchronized neurons (<2%) calls into question the functional significance of this increase.

Basal ganglia integrate sensorimotor processes and auditory tone-induced responses. In the present study, Δ^{-9}-THC did not affect sensory responses at the dose that significantly changed movement and motor-related neural activity. This finding suggests that cannabinoid treatment in the basal ganglia does not appreciably affect sensory processing.

In summary, electrophysiological and behavioral effects of Δ^{-9}-THC were studied in rats. Δ^{-9}-THC induced dose-dependent inhibition of spontaneous movement and altered treadmill walking patterns. Uniform inhibition was found in all the major basal ganglia regions in both spontaneous and treadmill locomotion tests. The degree of inhibition was dependent on the behavioral context. That is, a higher degree of inhibition was found during resting than walking. Limb movement-related neural firing and oscillatory firing were increased by Δ^{-9}-THC treatment. The observed broad inhibition of basal ganglia neural activity and motor functions suggests a sedative effect of cannabinoid agonism on basal ganglia neural processing and motor functions.

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