Kv4 channel blockade reduces motor and neuropsychiatric symptoms in rodent models of Parkinson's disease

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The striatum, a major input structure of basal ganglia, integrates glutamatergic cortical and thalamic inputs to control psychomotor behaviors. Nigrostriatal dopamine (DA) neurodegeneration in Parkinson's disease causes a loss of spinal and glutamatergic synapses in the striatal medium spiny neurons (MSNs). Adaptive responses, a form of homeostatic plasticity, to these changes are caused by a decrease in a potassium Kv4 channel-dependent inactivating A-type potassium (KI_A) current that increases the intrinsic excitability of MSNs. Nevertheless, the functional outcome of these compensatory mechanisms does not allow adequate behavioral recovery *in vivo*. We thus addressed the question of whether further blockade of Kv4 activity could enhance the striatal responsiveness of MSNs to DA depletion and restore normal function *in vivo*. To test this hypothesis, we examined the effects of a selective blocker of Kv4 channels, AmmTX3, on the motor, cognitive, and emotional symptoms produced by 6-hydroxydopamine lesions of the nigrostriatal DA pathway in rats. Striatal infusion of AmmTX3 (0.2–0.4 μg) reduced motor deficits, decreased anxiety, and restored short-term social and spatial memories. These results underlie the importance of Kv4 channels as players in the homeostatic responses, and, more importantly, provide a potential target for adjunctive therapies for Parkinson's disease. *Behavioural Pharmacology* 00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The dorsal striatum is a key structure of the basal ganglia that integrates sensorimotor, cognitive, and motivational information originating from cortical, thalamic, and midbrain inputs. Ninety-five percent of striatal cells are GABAergic efferent neurons with bursting properties, the medium spiny neurons (MSNs), that project to the substantia nigra pars reticulata and the globus pallidus. The remaining striatal neurons are GABAergic or cholinergic interneurons (2–3% in rodents) that influence both the timing and the pattern of the burst firing of MSNs and modulate the plasticity to synaptic inputs and neostriatal activity. The activities of MSNs influence two networks, the direct and indirect pathways, which have opposite effects on the activity of the inhibitory output interface of the basal ganglia. The indirect pathway provides excitatory input through disinhibition, whereas the direct pathway provides inhibitory input at this interface. Depending on the type of dopaminergic stimulated receptors, dopamine (DA) inversely modulates the glutamatergic corticostriatal synapses responsible for MSN bursting and, in particular, the potassium channel currents controlling spiking (Gerfen and Surmeier, 2011). Transient A-type potassium channels (KI_A) reduce the frequency of action potentials, limit back-propagations of action potential (bAPs), and regulate their timing into the MSN dendrites (Surmeier *et al.*, 1989; Nisenbaum and Wilson, 1995; Hoffman *et al.*, 1997; Häusser *et al.*, 2000; Cai *et al.*, 2004; Pawlak and Kerr, 2008). The somatodendritic KI_A currents of MSNs are generated by the activation of Kv4 channels and are found to be related to the expression of Kv4.2 subunits (Serôdio and Rudy, 1998; Tkatch *et al.*, 2000; Birnbaum *et al.*, 2004; Falk *et al.*, 2006).

Kv4 channels are widely expressed in the striatum, and DA modulates their activity (Day *et al.*, 2008). Striatal DA depletion increases MSN intrinsic excitability, as a manifestation of homeostatic plasticity to compensate for the loss of excitatory glutamatergic synapses (Day *et al.*, 2006, 2008; Marder and Goaillard, 2006; Azdad *et al.*, 2009). This intrinsic response is characterized by a decrease in Kv4 channel-dependent KI_A current in dendrites, which induces an enhancement of bAPs that, as a result, allows an increase in the Ca^{2+} dendritic signal. This compensatory mechanism, reported under experimental conditions mimicking Parkinson's disease (PD) *in vitro*, points to a crucial role of Kv4 channel activity in this process (Day *et al.*, 2008).

Because these Kv4 channels play a major role in this phenomenon, a further decrease in Kv4 channel activity should enhance striatal MSN responsiveness to DA

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depletion. To test this hypothesis, we examined the effects of AmmTX3, a synthetized specific blocker of Kv4 channels, on the behavioral deficits caused by 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic pathway in rats. AmmTX3 is a member of the α-KTX15 family of scorpion toxins, extracted from the venom of the scorpion *Androctonus mauretanicus*, that blocks Kv4 channel complexes in neurons with high affinity (Vacher *et al.*, 2002; Vacher and Martin-Eauclaire, 2004; Maffie *et al.*, 2013). The distribution of binding sites for BmTX3, another Kv4 blocker that shares the same target in the rat brain, overlaps with the expression of Kv4 channels. A high density of Kv4 channels was found in the basal ganglia, in particular in the striatum, which is affected in PD (Vacher *et al.*, 2006).

We found that AmmTX3 reduced Parkinsonian-like motor symptoms induced by nigrostriatal unilateral 6-OHDA lesions in a rat model of PD. AmmTX3 also reversed cognitive (short-term social recognition, spatial and nonspatial memory) and emotional (anxiety) deficits produced by partial bilateral DA depletion of the nigrostriatal dopaminergic pathway.

Methods

Subjects

Male Wistar rats (280–300 g; Charles River Laboratories, L'Arbresle, France) were housed with free access to food and water in a temperature-controlled room (24°C) on a 12 : 12 h dark–light cycle (lights on at 07:00 h). For the social recognition test, juvenile male Wistar rats (3 weeks) were also used. All efforts were made to minimize the number of animals used and to maintain them in good general health, in accordance with the European Communities Council Directive (2010/63/UE).

Stereotaxic surgery

Rats were anaesthetized with ketamine (5%; Virbac, France) and medetomidine (1 mg/ml; Janssen-Cilag, Issy-les-Moulineaux, France) injected subcutaneously (0.33 ml/kg). They were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, California, USA) with the incisor bar positioned 3.3 mm below the interaural line. 6-OHDA hydrobromide (Tocris Bioscience, Bristol, UK) or vehicle (0.9% NaCl in 0.1% ascorbic acid) solution was infused unilaterally into the substantia nigra, pars compacta (SNc) (8 μg/4 μl; AP –5.2, L + 2.1 and DV –7.6 mm from bregma; Paxinos and Watson, 2007) or bilaterally into the dorsomedial striatum (12 μg/3 μl/side; AP + 1.0, L +/- − 3.0, and DV − 5.5 mm from bregma) as described previously (Chen *et al.*, 2014). Sham rats received the vehicle solution only. 6-OHDA or vehicle solution was injected at a flow rate (0.33 μl/min) controlled by a micropump (CMA/100; CMA Micro-dialysis, Stockholm, Sweden) using a 10 μl Hamilton microsyringe connected by a Tygon catheter (0.25 mm internal diameter) fitted to the 30-G stainless steel injector needles. The toxin was allowed to diffuse for 3 min. For AmmTX3 injection, an intracerebral cannula was implanted unilaterally in the striatum at the coordinates AP + 1.0, L +/− 3.0, and DV − 5.5 mm from bregma, ipsilaterally to the 6-OHDA lesion, during the same surgery. In bilateral partial 6-OHDA-lesioned rats, two cannulae were implanted 1000 μm posterior to the 6-OHDA injection sites. All animals were allowed to recover for 2 weeks.

Drug treatment

AmmTX3 (0.2–0.8 μg dissolved in 0.9% NaCl; Proteogenix, Schiltigheim, France) was injected into the striatum through the implanted cannula. AmmTX3 was injected immediately before each test, except in the social recognition task (immediately before the second exposure). The animals were allocated to different groups [vehicle-sham, AmmTX3-sham (0.2 or 0.4 μg), vehicle-lesioned, AmmTX3-lesioned (0.2 or 0.4 μg)]. For locomotor and exploratory behaviors, an additional dose of AmmTX3 (0.8 μg) was tested. For motor tasks, the unilateral 6-OHDA and sham groups were tested once, first in the cylinder test then in the rotameter. For emotional and cognitive tasks, each bilateral 6-OHDA group was tested once in the elevated plus maze, social and spatial memory, every 4 days, in a counterbalanced manner, between postlesion days 14 and 25 (Chen *et al.*, 2014). For each task, all experimental groups were tested on a given day. In each group, behavioural performance in a given task was similar whatever the time of testing, ruling out a potential influnce of drug treatment (data not shown).

Behavioral procedures

All behavioral experiments were carried out in dim light (8 lm), video-recorded (Viewpoint Inc., Lyon, France), and scored later by an experimenter blind to treatment.

Motor tasks

Locomotor and exploratory activities

Locomotor activity was first monitored in individual activity chambers (34 cm × 23 cm × 18 cm) housed within a sound-attenuating cubicle and under homogenous light illumination (Imetronic, Pessac, France). Each chamber was equipped with four infrared photobeams located 2.5 cm (10 at the rear) above the floor level of the chamber. The number of front and back movements was recorded in 10-min bins over 60 min. Locomotor and exploratory activities were then recorded in a square open field (100 × 100 cm) with 30-cm-high white plastic walls, whose surface was divided into two equalized areas: external and internal zones. Each rat was placed in the center of the open field and the motor activity was measured in terms of the total distance covered in the entire maze during a 6-min test.

Cylinder test

The degree of forepaw asymmetry induced by a unilateral 6-OHDA lesion was assessed by placing the rats in a transparent Plexiglas cylinder (20 cm diameter, 30 cm height). The scores were expressed as a percentage of the total number of wall-contacts for 10 min.

Apomorphine-induced circling

Unilateral 6-OHDA-lesioned rats were tested in automated rotameter cylinders (TSE, Bad Homburg, Germany). The turning behavior (defined as a full 360° rotation of the body axes) was measured after intraperitoneal injection of apomorphine hydrochloride (0.1 mg/kg; Sigma-Aldrich, St-Quentin Fallavier, France). The total number of net rotations (contralateral − ipsilateral rotations) was recorded for 60 min.

Emotional and cognitive tasks

Anxiety

The elevated plus maze was used to assess anxiety-related behavior over a 5-min period. Each rat was placed in the central area of a plus maze with two open arms and two enclosed arms (50 × 10 cm, height: 30 cm), raised to a height of 70 cm above the floor. The percentage of time spent in the open versus closed arms was used as an index of anxiety level. Motor activity was measured in terms of the total distance covered in the entire maze.

Social memory

Short-term social memory was evaluated with the social memory test. Adult male rats spend a great amount of time investigating novel juveniles. In contrast, rats re-exposed to the same juvenile 30 min after an initial exposure show little investigatory behavior. All rats were habituated for 60 min to the testing room and the juveniles were housed in individual cages for 30 min before the experiment. The adult rats were then allowed to habituate for 10 min to the square open field (50 × 50 cm) containing a small cage with stainless steel railings (10 × 10 cm) at its center. After placing the juvenile in the small cage, the mean time spent by the animals in contact with the juvenile was recorded for 5 min. The adult rat was then removed from the open field, kept in an individual cage for a 30-min delay period, and then re-exposed to the same juvenile for 5 min. The difference in the mean time spent in contact with the juvenile between the first and the second presentations was used as an index of the level of recognition.

Spatial memory

Spatial memory was assessed with the object recognition test. Animals were submitted to five consecutive 6-min sessions (S), interspaced by 3-min intervals, in a square open field (100 × 100 cm) with 30-cm-high white plastic walls. During S1, the rats explored and acclimatized to the empty open field, which enabled recording of baseline locomotor activity and prevented neophobic reactions. In S2–S4, four objects were positioned in the open field (habituation phase). During the spatial-test session (S5), the object configuration was modified by displacing two objects. Object exploration was measured in terms of the mean time spent by the animals in contact with the different objects. The subjects' reaction to a spatial change was measured by the difference in time spent exploring the displaced and nondisplaced objects between S4 and S5 (spatial exploration index).

Lesion verification

To evaluate the extent of the lesion, binding of tritiated-mazindol to DA uptake sites in the striatum was measured on autoradiographic films, according to the procedure described by Javitch *et al.* (1985). Briefly, rats were deeply anesthetized by pentobarbital injection and killed by decapitation. Brains were rapidly frozen on powdered dry ice and stored at −80°C. Coronal brain sections (20 µm) were cut at −20°C with a Leica CM3050 S Cryostat (Leica Microsystemes SAS, Nanterre, France), mounted on Superfrost Plus slides (Roth Socheil E.u.r.l., Lauterbourg, France), and stored at −80°C. For [^{3}H]-mazindol binding, brain sections were air dried and preincubated at 4°C for 5 min in 50 mmol/l Tris buffer with 120 mmol/l NaCl and 50 mmol/l KCl (pH 7.9). Thereafter, they were incubated for 40 min at 4°C with 7 nmol/l [^{3}H]-mazindol (specific activity 27 Ci/mmol; PerkinElmer Life and Analytical Sciences, Zaventem, Belgium) in 50 mmol/l Tris buffer containing 300 mmol/l NaCl and 5 mmol/l KCl (pH 7.9), with 3 mmol/l Mdesipramine (hydrochloride; Sigma-Aldrich) added to block norepinephrine reuptake. Sections were rinsed twice for 3 min in the same incubation buffer and then for 10 s in distilled water before air drying. Autoradiographs were realized by applying the slides onto a Kodak Biomax MR film (Sigma-Aldrich) for at least 6 weeks. After exposure, the films were developed and then fixed, washed in water, and air dried. All autoradiograms were scanned. The surface of the striatum was delimitated in pixels, with reference to the method used by Paxinos and Watson (2007). The extent of bilateral striatal 6-OHDA lesions was estimated by delimiting the extent of the lesioned areas in each hemisphere as the sum of the pixels with low gray levels. As no difference was found in the surface measured on each side of the brain, the values obtained were averaged. The extent of the lesion was determined as the ratio between the lesioned and the total striatal areas, expressed as a percentage of the total striatal surface. Thereafter, the mean ± SEM was calculated for each experimental group. Cresyl-violet staining of sections was used to locate the trace of the 6-OHDA injection and cannula implantations.

Statistical analysis

In all analyses, values are presented as mean ± SEM. Effects of DA lesion and AmmTX3 treatment on behavioral performances of the different groups were tested

by means of one-way analysis of variance (ANOVA) or two-way ANOVA with 6-OHDA lesion and AmmTX3 treatment as between-subject factors. The analyses were followed by adapted post-hoc tests between groups (Tukey's test), with P less than 0.05 (Graphpad Prism6). Student's t-test was used to analyze the effects of 6-OHDA, compared with sham groups injected with vehicle.

Results

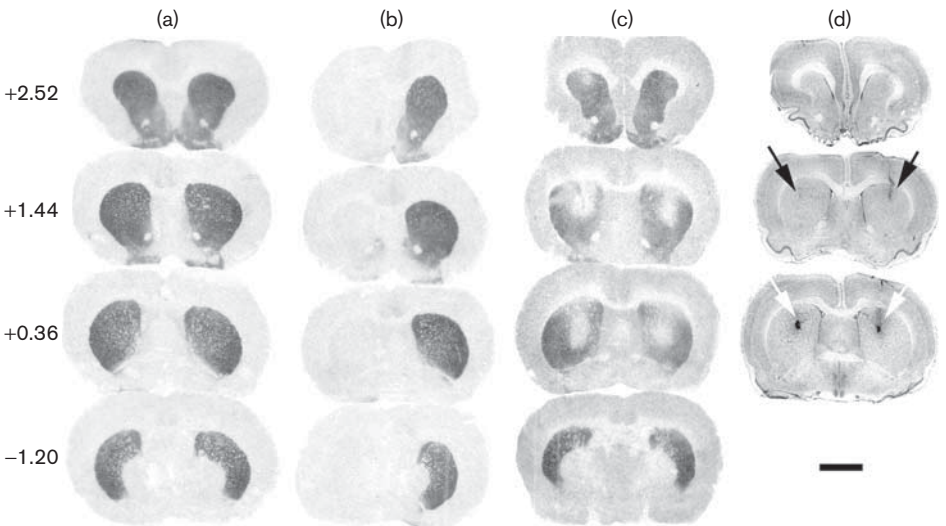
6-OHDA lesion extent

Unilateral 6-OHDA infusion in the SNc produced near-complete depletion (>90%) of DA in the ipsilateral striatum, assessed by the loss of [^{3}H]-mazindol binding in the striatum as compared with sham animals (Fig. 1a and b). Bilateral intrastriatal 6-OHDA infusions produced lesions restricted to the dorsal striatum, sparing the nucleus accumbens (Fig. 1c). The loss of [^{3}H]-mazindol labeling extended across the entire rostrocaudal level and was restricted to the medial part of the striatum in 6-OHDA-lesioned subjects. There was no difference in [^{3}H]-mazindol labeling between the four groups injected with 0.0, 0.2, 0.4, or 0.8 μg AmmTX3 in the dorsal striatum (mean decrease: 45 ± 3, 43 ± 2, 40 ± 2, 48 ± 3%, respectively; $F_{3,39} = 2.44$, NS). The partial striatal DA depletion did not induce bradykinesia or any major motor disabilities (Chen *et al.*, 2014). A representative example of 6-OHDA and AmmTX3 injection sites, spaced out on an average of 1000 μm, is shown in Fig. 1d.

Effects of intrastriatal AmmTX3 on locomotor and exploratory behaviors

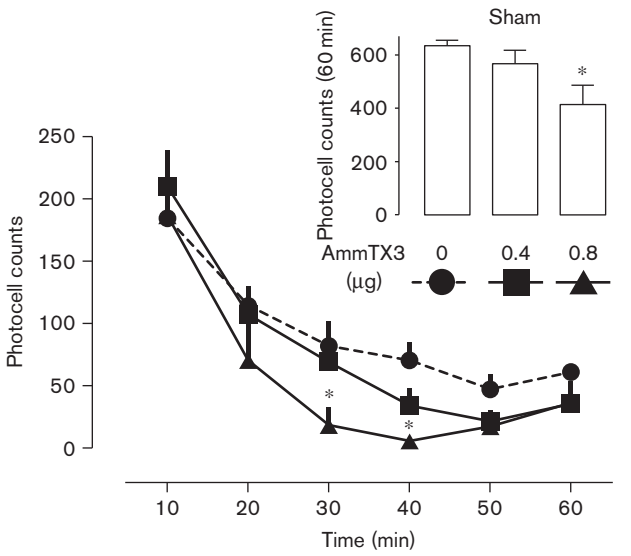
The effects of bilateral administration of AmmTX3 (0, 0.4, 0.8 μg) on locomotor activity of sham animals were evaluated in photocell cages for 60 min (in 10-min blocks; Fig. 2a). The group × time ANOVA revealed significant main effects of group ($F_{2,14} = 5.36$, $P < 0.05$) and time ($F_{5,70} = 31.33$, $P < 0.001$) but no significant interaction ($F_{10,70} = 0.66$, NS). AmmTX3 at a 0.4 μg dose had no effect on locomotor activity, evaluated in terms of front and back photocell beam breaks, whereas at a 0.8 μg dose, AmmTX3 significantly decreased locomotion at the 30 and 40 min postinjection time points (Tukey's test, $P < 0.05$). At this AmmTX3 dose, the overall locomotor response for the 60-min test was significantly different from that in vehicle-injected rats (Fig. 2a, inset). The exploratory and locomotor behaviors were further evaluated in an open field for sham and 6-OHDA-lesioned groups (Table 1) treated with AmmTX3 (0.2, 0.4, 0.8 μg) or NaCl. Two-way ANOVA revealed a significant effect of AmmTX3 treatment ($F_{3,68} = 8.82$, $P < 0.01$) but no significant lesion effect of the group × treatment interaction. Tukey's test showed that AmmTX3 at the highest dose tested (0.8 μg) only reduced the distance covered ($P < 0.05$). The exploratory behavior, evaluated in terms of the time spent in the external and internal zones, was affected neither by AmmTX3 injection, regardless of the dose, nor by the lesion ($F_{3,68} \leq 0.78$, NS, data not shown). Because of the transient decrease in locomotion, the 0.8 μg dose of AmmTX3 was not used in subsequent behavioral tests.

Fig. 1



Extent of 6-OHDA lesions. (a–c) [^{3}H]-mazindol binding to dopamine uptake sites at the level of the striatum. The numbers indicate the rostrocaudal coordinates (mm) from bregma (Paxinos and Watson, 2007). Examples of [^{3}H]-mazindol binding autoradiography in (a) a representative sham rat, (b) in a unilateral 6-OHDA-lesioned rat, and (c) in a bilateral 6-OHDA-lesioned rat. (d) Cresyl-violet staining: example of sections at the same stereotaxic coordinates. Dark arrows indicate the 6-OHDA injection sites. White arrows indicate the sites of the cannula implantations. Scale bar: 3 mm.
6-OHDA, 6-hydroxydopamine.

Fig. 2



Effects of intrastriatal AmmTX3 injection on locomotor and exploratory activities in photocell cages. Time course of beam break recordings of sham rats treated with vehicle ($n=5$) and with AmmTX3 at 0.4 μg ($n=7$) or 0.8 μg ($n=5$) doses during 60 min. (Inset) Total beam breaks over the 60-min test. Values are shown as mean ± SEM.*$P<0.05$, in comparison with the vehicle-sham group.

Table 1 Spontaneous locomotor activity measured in an open field

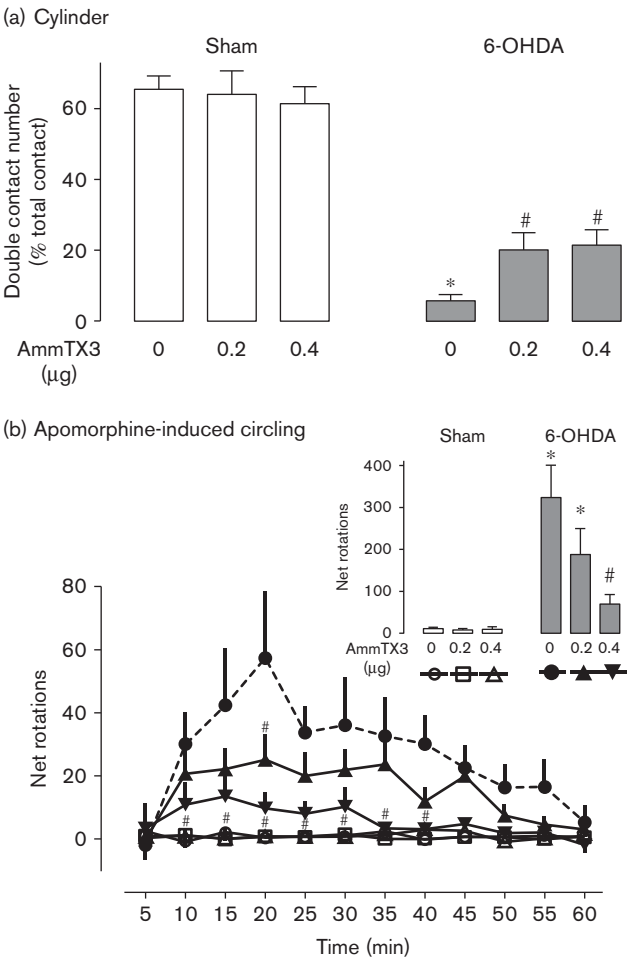
AmmTX3 (μg)	Sham	6-OHDA
0	2942 ± 183	2938 ± 187
0.2	3169 ± 173	2731 ± 245
0.4	3056 ± 312	2809 ± 112
0.8	2017 ± 334	1991 ± 166*

AmmTX3 (0.8 μg) decreased the total covered distance measured in cm (mean ± SEM).
Two-way ANOVA, Tukey's post-hoc test: *$P<0.05$ in comparison with AmmTX3-sham group.
Sham groups [AmmTX3 0 μg ($n=10$), 0.2 μg ($n=8$), 0.4 μg ($n=9$), 0.8 μg ($n=9$)], and 6-OHDA groups [AmmTX3 0 μg ($n=10$), 0.2 μg ($n=10$), 0.4 μg ($n=10$), 0.8 μg ($n=10$)].

Kv4 channel blockade by AmmTX3 reduces Parkinsonian motor symptoms

Unilateral 6-OHDA-lesioned rats were tested for forelimb asymmetry using the cylinder test. Sham animals explored the cylinder with their forelimbs, making over 60% of double contacts on the cylinder wall (Fig. 3a). In contrast, vehicle-injected 6-OHDA-lesioned rats showed drastically reduced double contacts (Student's *t*-test, $P<0.01$). Following AmmTX3 treatment at 0.2 and 0.4 μg doses, the forelimb use pattern did not vary in sham rats ($F_{2.25}\leq 0.32$, NS). AmmTX3, at the same doses, reduced the deficits in 6-OHDA-lesioned rats in comparison with vehicle-injected 6-OHDA rats ($F_{2.27}\geq 4.35$, $P<0.05$). The total number of contacts did not vary between groups.

Fig. 3



Effects of AmmTX3 on the motor deficits induced by unilateral 6-OHDA lesions. (a) Cylinder test. Double forepaw contacts were recorded in six groups of rats: vehicle-sham ($n=10$), AmmTX3-sham [0.2 ($n=8$) or 0.4 μg ($n=10$)], vehicle-6-OHDA ($n=10$), and AmmTX3-6-OHDA [0.2 ($n=10$) or 0.4 μg ($n=10$)]. ANOVA, *$P<0.05$ in comparison with the vehicle-sham group, $^{\#}P<0.05$ in comparison with the vehicle-6-OHDA group. (b) Apomorphine-induced circling. Six groups of rats were tested: vehicle-sham ($n=10$), AmmTX3-sham [0.2 μg ($n=9$) or 0.4 μg ($n=9$)], vehicle-6-OHDA ($n=8$), and AmmTX3-6-OHDA [0.2 μg ($n=10$) or 0.4 μg ($n=9$)]. Time course of rotational asymmetry following apomorphine injection. AmmTX3 reduced apomorphine-induced net rotations (contralateral − ipsilateral turns) in 6-OHDA-lesioned rats. ANOVA, $^{\#}P<0.05$ compared with the vehicle-6-OHDA group. (Inset) Total net rotations over the 60-min test. Values are shown as mean ± SEM. ANOVA, $^{\#}P<0.05$ compared with the vehicle-6-OHDA group. ANOVA, analysis of variance; 6-OHDA, 6-hydroxydopamine.

Apomorphine-induced rotational asymmetry was then tested in the same groups of rats. Two-way ANOVA revealed a significant effect of AmmTX3 treatment ($F_{2.49}=4.94$, $P<0.01$), lesion ($F_{1.49}=31.63$, $P<0.01$), and the group × treatment interaction ($F_{2.49}=4.84$, $P<0.02$) on the overall asymmetry response during the 60-min test. AmmTX3 dose-dependently reduced apomorphine-induced rotations during the 60-min test ($F_{2,24}=4.57$, $P<0.05$) in 6-OHDA-lesioned rats and had

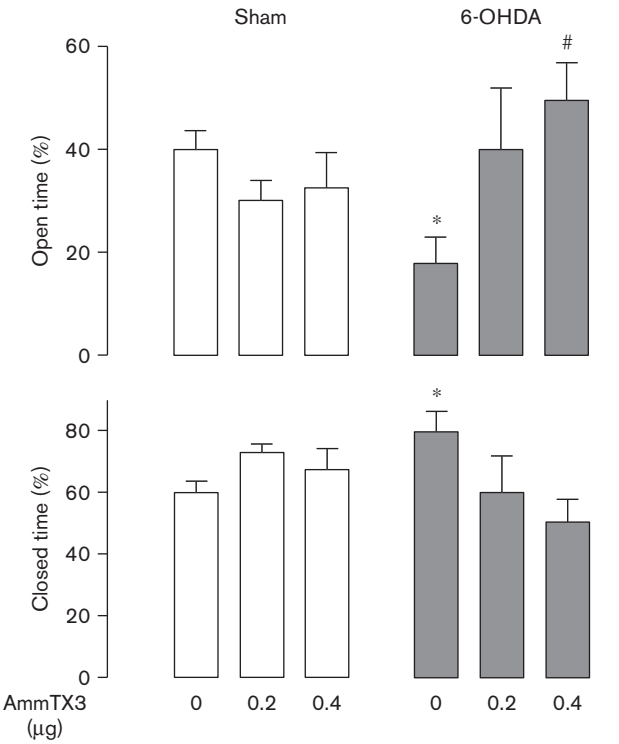
no effect on sham animals ($P_{2.25} = 0.08$, NS; Fig. 3b, inset). This effect reached significance at the 0.4 μg dose of AmmTX3 (Tukey's test, $P < 0.05$). A two-way analysis of variance with repeated measures on the apomorphine-induced rotations revealed a significant group × time interaction ($F_{55,539} = 2.07$, $P < 0.01$), and significant effects of group ($F_{5,49} = 10.19$, $P < 0.01$) and time ($F_{11,539} = 6.26$, $P < 0.01$). A significant reduction in the apomorphine stimulant effect was produced by AmmTX3 at 0.4 μg (Tukey's test, $P < 0.05$; Fig. 3b). At this dose of AmmTX3, the rotational behavior of lesioned rats was similar to that of sham rats (Tukey's test, NS).

Kv4 channel blockade by AmmTX3 reduces emotional and cognitive symptoms

Anxiety-like behavior

In the elevated plus maze test, indexing anxiety-like behavior, bilateral 6-OHDA lesions decreased the time spent in open arms and increased the time spent in closed arms, as shown in Fig. 4 (Student's t, $P < 0.01$). Administration of AmmTX3, at 0, 0.2, and 0.4 μg doses, did not significantly modify the overall percentage of time spent in the open and closed arms in sham animals ($F_{2,24} \leq 1.98$, NS). In contrast, in 6-OHDA-treated rats, AmmTX3 increased the time spent in open arms ($F_{2.21} = 3.54$, $P < 0.05$) and tended to decrease the time spent in closed arms ($F_{2.21} = 2.17$, NS), when compared with vehicle. Tukey's test indicated a significant effect at the 0.4 μg dose ($P < 0.05$). Because there was no significant difference between groups in the distance covered in the maze ($F_{5,45} = 0.64$, NS), the anxiolytic action of AmmTX3 was not produced by a change in exploratory behavior (Table 2).

Fig. 4



Effects of AmmTX3 on anxiety-like behavior in the elevated plus maze. Percentage of exploration time in the open (open time) and closed (closed time) arms of the six groups: vehicle-sham ($n = 10$), AmmTX3-sham [0.2 μg ($n = 8$) or 0.4 μg ($n = 9$)], vehicle-6-OHDA ($n = 8$), AmmTX3-6-OHDA [0.2 μg ($n = 8$) or 0.4 μg ($n = 8$)]. Values are shown as mean ± SEM. ANOVA, $^{*}P < 0.05$, in comparison with the vehicle-sham group; $^{\#}P < 0.05$, in comparison with the vehicle-6-OHDA group. ANOVA, analysis of variance; 6-OHDA, 6-hydroxydopamine.

Cognitive deficits

To examine short-term recognition memory in bilateral 6-OHDA-lesioned animals, we first tested the ability of adult rats to recognize a juvenile rat after a second exposure after a 30-min interval. The sham groups clearly identified the juvenile, as found by a positive duration index (first presentation duration − second presentation duration). In contrast, 6-OHDA-lesioned rats treated with vehicle showed a weak negative duration index, showing that these rats no longer recognized the juvenile (Fig. 5). AmmTX3, at 0, 0.2 and 0.4 μg doses, did not modify the duration index evaluated in sham groups regardless of the dose ($F_{2.22} = 0.20$, NS). In contrast, in 6-OHDA-lesioned rats, AmmTX3, at the same doses, restored the recognition of the juvenile ($F_{2.21} = 4.15$, $P < 0.05$). This effect was significant at the 0.4 μg dose as no differences in the duration index were found between sham rats and lesioned rats treated with 0.4 μg of AmmTX3 (Tukey's test, $P < 0.05$), with no alteration in locomotor activity (Table 2).

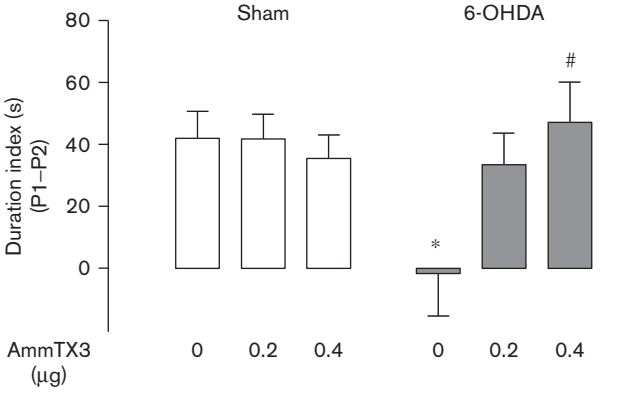
The role of AmmTX3 was further evaluated in visuo-spatial memory processes. No difference in object exploration was found between groups (sham and 6-OHDA-lesioned groups treated with 0, 0.2, and 0.4 μg of AmmTX3) during sessions 2–4 ($F_{5,51} \leq 0.96$, NS). All groups tended to decrease object exploration across sessions, as shown by the decreased duration of contact (Fig. 6a). Thus, neither 6-OHDA lesions nor AmmTX3 treatment impaired the capacity of the animals to explore the objects. During exposure to a new spatial configuration of the objects, sham groups exhibited a significantly higher exploration index for the displaced objects than for the nondisplaced objects (Student's t-test, $P < 0.05$). These rats thus discriminated and reacted to the spatial change (Fig. 6b). In contrast, 6-OHDA-lesioned animals treated with vehicle re-explored the displaced and non-displaced objects to a similar extent (Student's t-test, NS) and showed a significantly lower spatial exploration index of displaced objects compared with the vehicle-sham group (Student's t tests, $P < 0.05$). The exploration index for nondisplaced objects did not vary between 6-OHDA-lesioned and sham rats treated with vehicle. ANOVA indicated that AmmTX3 restored the spatial exploration index in 6-OHDA-lesioned rats, showing a recovery of

Table 2 Spontaneous locomotor activity measured in emotional and cognitive tests

	Sham			6-OHDA		
AmmTX3 (μg)	0	0.2	0.4	0	0.2	0.4
Elevated plus maze[a]	11201 ± 736	9582 ± 878	9588 ± 320	8823 ± 638	9161 ± 897	9088 ± 617
Social recognition[b]	5240 ± 350	5910 ± 286	5910 ± 487	5680 ± 446	6014 ± 389	5777 ± 191
Object recognition[c]	3054 ± 407	3305 ± 301	3056 ± 312	2998 ± 177	3389 ± 242	2772 ± 164

The mean covered total distance (± SEM) was measured in cm in each maze.
ANOVA, analysis of variance; 6-OHDA, 6-hydroxydopamine.
[a] $F_{5,45} = 1.37$, NS, ANOVA.
[b] $F_{5,43} = 0.59$, NS, ANOVA.
[c] $F_{5,51} = 1.11$, NS, ANOVA.

Fig. 5



Effects of AmmTX3 on short-term recognition memory in a social memory test. The index for duration of contact with the same juvenile rat corresponds to the number of contacts in the first presentation (P1) minus the number of contacts in the second presentation (P2) for the six groups: vehicle-sham ($n = 9$), AmmTX3-sham [0.2 μg ($n = 8$) or 0.4 μg ($n = 8$)], vehicle-6-OHDA ($n = 8$), AmmTX3-6-OHDA [0.2 μg ($n = 8$) or 0.4 μg ($n = 8$)]. Values are shown as mean ± SEM. ANOVA, $^{*}P < 0.05$, in comparison with the vehicle-sham group; $^{\#}P < 0.05$, in comparison with the vehicle-6-OHDA group. ANOVA, analysis of variance; 6-OHDA, 6-hydroxydopamine.

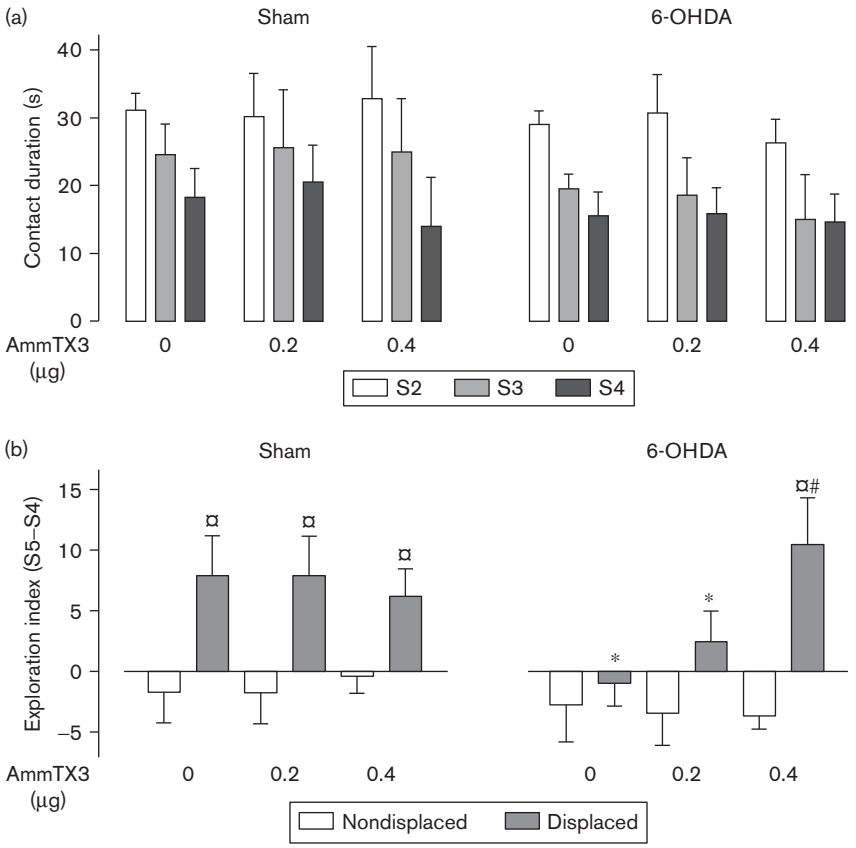
spatial discrimination ($F_{2.26} = 3.65$, $P < 0.05$). Post-hoc comparisons revealed that only 6-OHDA-lesioned rats treated with AmmTX3 at a dose of 0.4 μg explored the displaced objects more than the nondisplaced ones (Tukey's test; $P < 0.05$). The exploration index for displaced objects observed in 6-OHDA-lesioned rats treated with AmmTX3 (0.4 μg) was similar to that of sham rats ($F_{3,33} = 1.02$, NS). This effect cannot be attributed to an alteration of locomotor activity induced by DA depletion as there was no difference between groups (Table 2).

Discussion

The results of the present study show that AmmTX3, a selective Kv4 channel blocker, reduces motor symptoms after extensive unilateral 6-OHDA lesions and completely reverses anxiety, short-term social and spatial memory deficits after a partial nigrostriatal 6-OHDA lesion. The beneficial effects of AmmTX3 in lesioned rats were evident in all behavioral tests at a dose of 0.4 μg (with a clear tendency at 0.2 μg). In contrast, a dose of 0.8 μg depressed motor activity, demonstrating a subtle modulation of these potassium channels *in vivo*.

In the extensive hemiparkinsonian model of PD, the near-complete degeneration of nigrostrial neurons induces an asymmetric sensorimotor behavior impairment comparable to the neuronal loss seen in late-stage PD patients, which provokes a complex motor disorder manifested as resting tremor, muscle rigidity, and bradykinesia. Decreasing dopaminergic activity input to the so-called 'direct and indirect' pathways induces a shift in the opposite direction of their excitability. This creates an imbalance in the regulation of the motor thalamus, thus favoring suppression of movement and subsequent akinesia (Blandini *et al.*, 2000; Gerfen and Surmeier, 2011). The increase in spike generation in the MSNs of the indirect striatopallidal pathway arises, among other mechanisms, from a reduction in postsynaptic Kv4 channel activity (Day *et al.*, 2008; Azdad *et al.*, 2009). Thus, modulation of the activity of these Kv channels may represent one approach to reduce akinesia. Indeed, Kv4 channel blockade in the striatum after local injection of AmmTX3 decreased the level of both forelimb asymmetry and circling behavior. Limb-use asymmetry and apomorphine-induced circling behavior have been shown to be associated with low levels of striatal DA (Schwarting and Huston, 1996). Previous studies have shown that Kv channels modulate the concentration of neurotransmitters. Indeed, 4-AP, an organic compound that blocks mostly KI_A currents in a nonselective manner, by inhibiting different Kv subfamily members (Kv1.4, Kv3.3 and Kv4.2), increases neurotransmitter release (DA, glutamate, acetylcholine), modulates network oscillations, and enhances cortical excitation (Damsma *et al.*, 1988; Morales-Villagrán and Tapia, 1996; Miura *et al.*, 2007; Luca and Singer, 2013). Systemic administration of 4-AP attenuates apomorphine-induced rotational asymmetry in a rat PD model by re-equilibrating the balance of DA receptor sensitivity between the intact and the lesioned sides (Haghdoost-Yazdi *et al.*, 2011). Indeed, 4-AP was shown to enhance DA release, essentially by inhibiting presynaptic Kv1 and Kv3 channels (Damsma *et al.*, 1988; Morales-Villagrán and Tapia, 1996; Cepeda *et al.*, 2001; Luca and Singer, 2013). In addition,

Fig. 6



Effects of AmmTX3 on short-term visuospatial memory deficits in an object recognition test. The duration of object contacts was recorded in the six groups: vehicle-sham ($n=10$), AmmTX3-sham [0.2 μg ($n=8$) or 0.4 μg ($n=9$)], vehicle-6-OHDA ($n=10$), AmmTX3-6-OHDA [0.2 ($n=10$) or 0.4 μg ($n=9$)]. (a) Habituation phase. Duration of contact (in s) with the objects in sessions (S) 2–4. Object familiarization was evaluated in terms of the decrease in contact duration from S2 to S4. (b) Spatial recognition. The spatial exploration index is the difference in duration of contact between S5 and S4 for displaced or nondisplaced objects. ANOVA, $^{¤}P<0.05$ for the comparison between displaced and nondisplaced objects; $^{*}P<0.05$, compared with the vehicle-sham group; $^{\#}P<0.05$, in comparison with the vehicle-6-OHDA group. Values are shown as mean ± SEM. ANOVA, analysis of variance; 6-OHDA, 6-hydroxydopamine.

4-AP also acts on postsynaptic Kv4 channels (Tseng *et al.*, 1996; Tseng, 1999) and has been shown to normalize motor behavior by regulating the firing rate of Purkinje cells in a mouse model of spinocerebellar ataxia (type 1; Hourez *et al.*, 2011). Our results confirm these findings, as the specific blockade of Kv4 channels by AmmTX3 increases the intrinsic striatal dendritic excitability, thus reducing rotational asymmetry through postsynaptic mechanisms that remain to be demonstrated.

In PD, nonmotor symptoms such as anxiety and cognitive deficits may appear before the classical motor deficits (Dagher and Robbins, 2009; Nègre-Pagès *et al.*, 2010; Blonder and Slevin, 2011). In the present study, 6-OHDA injection in the dorsal striatum caused an average of 40% striatal DA depletion and no motor symptoms, similar to that observed in the early stages of PD. We recently demonstrated that bilateral 6-OHDA lesions restricted to the nigrostriatal DA pathway, but not to the mesolimbic system, were associated with major emotional deficits, expressed as increased levels of anxiety and impaired short-term spatial and short-term social memories (Chen *et al.*, 2014), in line with other recent studies in rodent partial PD models (Branchi *et al.*, 2008; Tadaiesky *et al.*, 2008; Drui *et al.*, 2013).

In the present study, the striatal blockade of Kv4 channels with AmmTX3 fully reversed the anxiety-like phenotype produced by similar partial 6-OHDA lesions. The same treatment with AmmTX3 in nonlesioned animals did not alter these emotional processes. Interestingly, genetic deletion of the Kv4.2 channel in mice induces various effects in anxiety-like tests (Kiselycznyk *et al.*, 2012; Lugo *et al.*, 2012). Kv4.2-knockout mice demonstrate increased freezing behavior in a pavlovian fear conditioning test, but no deficit in a light/dark exploration test, whereas opposite results are obtained in the elevated plus maze. Moreover, Kv4.2-knockout mice exhibit normal locomotion and sensorimotor gating or hyperactivity (Barnwell *et al.*, 2009; Kiselycznyk *et al.*,

2012; Lugo *et al.*, 2012). These contradictory results could be explained by different compensatory mechanisms in conditioned knockout animals compared with those undergoing acute pharmacological treatment. The same treatment with AmmTX3 prevented social recognition and visuospatial short-term memory deficits produced by the 6-OHDA lesion, while not altering the performance of sham animals. This is in line with our recent findings showing that short-term memory in a radial maze task is not affected by intracerebroventricular injection of AmmTX3 (Truchet *et al.*, 2012). In contrast, in a long-term memory task, AmmTX3 delays learning and the switch from an egocentric to an allocentric strategy in the radial maze. These impairments were associated with the transient upregulation of Kv4.2 and Kv4.3 mRNA levels in the striatum, underlying the critical involvement of Kv4 channels in this area (Truchet *et al.*, 2012). Moreover, Kv4.2-knockout mice exhibit spatial deficits in long-term memory processes in the Morris water maze (Lockridge and Yuan, 2011; Lugo *et al.*, 2012). Interestingly, in mouse models of HIV-1 encephalitis and Alzheimer's disease, two pathologies that alter learning and memory processes, KI_A current blockade by 4-AP or Tx3–1, a peptidic toxin, restored spatial memory (Keblesh *et al.*, 2009; Gomes *et al.*, 2013). Taken together, these findings highlight the involvement of Kv4 channels in memory processes and the importance of the modulation of these channels in reversing memory impairments associated with these pathological conditions.

In Parkinsonian models, DA depletion in the striatum increases the intrinsic excitability of the MSNs, associated with a loss of spines and glutamatergic synapses *in vitro* (Calabresi *et al.*, 2000; Day *et al.*, 2006, 2008; Fino *et al.*, 2007). This increase in excitability is in part due to a facilitation of bAPs in distal dendrites and spines and enhanced excitatory synaptic corticostriatal transmission (Calabresi *et al.*, 2000; Azdad *et al.*, 2009; Evans *et al.*, 2013). Under physiological conditions, the amplitude of bAPs is limited by the KI_A currents induced by the activity of Kv4 channels, which also limits the amplitude of excitatory postsynaptic potentials and accelerates the time course of their decay (Hoffman *et al.*, 1997; Hopf *et al.*, 2003; Magee and Johnston, 2005). Both actions lengthened the latency to firing the first action potential (Hoffman *et al.*, 1997; Thompson, 2007). After DA depletion, KI_A currents in MSNs are reduced, as Kv4 channels inactivate much faster (Azdad *et al.*, 2009). Therefore, an enhancement of dendritic responsiveness of these neurons could occur, leading to a high probability of firing in response to excitatory postsynaptic potentials. These intrinsic alterations, leading to a homeostatic restoration, could compensate for the reduction in glutamatergic efficiency. However, motor and nonmotor behavioral deficits are still observed in 6-OHDA animals. Here, we show that a stronger reduction in KI_A currents, by pharmacological blockade of Kv4 channels, fully eliminated the deficits induced by 6-OHDA-induced DA depletion. AmmTX3, by inhibiting KI_A currents in the remaining MSN spines, could enhance this homeostatic response.

Conclusion

The present data demonstrate that Kv4 channels play an important role in regulating cellular mechanisms underlying emotional behaviors and spatial memory mediated by the basal ganglia. The positive action of AmmTX3 on motor and nonmotor symptoms of PD may result from the enhancement of homeostatic responses to maintain, as far as possible, the neural computation within the affected neuronal network. Kv4 channels may therefore represent potential targets in the treatment of motor, emotional, and cognitive deficits in the early stages of PD.

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Conflicts of interest

There are no conflicts of interest.

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