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Asymmetrical response of aminopeptidase A in the medial prefrontal cortex and striatum of 6-OHDA-unilaterally-lesioned Wistar Kyoto and spontaneously hypertensive rats

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ABSTRACT

Aminopeptidase A is responsible for the hydrolysis of angiotensin II and cholecystokinin. By measuring its activity we obtain a reflection of the functional status of its endogenous substrates. Dopamine coexists with these neuropeptides in striatum and prefrontal cortex. If the content of any of them is altered, the others and the functions they are involved in would also be affected. Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) are rat models with different motor behavior and mood. We hypothesized that aminopeptidase A activity could be modified in WKY or SHR affecting the brain dopamine. The results may provide new insights for the understanding of dopamine-related disorders such as schizophrenia, depression or Parkinson's disease. To analyze the influence of unilateral depletions of dopamine on the *intra*- and *inter*-hemispheric behavior of aminopeptidase A in striatum and prefrontal cortex of WKY and SHR, aminopeptidase A activity was measured fluorometrically, using an arylamide derivative as substrate, in the left and right sides of striatum and prefrontal cortex of WKY and SHR treated with saline (control groups) or following left or right intrastriatal injections of 6-hydroxydopamine (lesioned groups). Differential asymmetrical *intra*- and *inter*-hemispheric behaviors of aminopeptidase A were observed, depending on the lesioned hemisphere, the region and the strain analyzed. Results also demonstrated differential *intra* and *inter*-hemispheric correlations between striatum and prefrontal cortex and between both regions and motor behavior depending on the side of lesion. The changes mostly involved the left hemisphere. The functions in which the aminopeptidase A activity is involved could be modified depending on whether the dopamine depletion occurs on the left or right hemisphere.

1. Introduction

Aminopeptidase A (EC 3.4.11.7, glutamyl aminopeptidase) is responsible for the hydrolysis of angiotensin II (Ang II) and cholecystokinin (CCK) (Migaud et al., 1996; Rozenfeld et al., 2004). The measurement of its enzymatic activity constitutes an approach to the analysis of the functional status of its endogenous substrates. Dopamine coexists and/or interacts with these neuropeptides, CCK (Smolnik et al., 2002) or Ang II (Jenkins et al., 1996; Labandeira-Garcia et al., 2011), in neurons of the nigrostriatal system and corticolimbic structures such as prefrontal cortex. Their functional connectivity is critical for motor behavior (Felger et al., 2016) and cognition (Antzoulatos and Miller, 2014; Emmons et al., 2017). It is expected that, if the content of any of them is altered, the other coexisting factors, including the enzyme responsible of their metabolism, as well as the functions in which they are involved would also be affected.

Although some evidences support that morphologic and functional brain asymmetries parallel with neurochemical lateralization (Toga and

Abbreviations: 6-OHDA, 6-hydroxydopamine; Ang II, angiotensin II; CCK, cholecystokinin; LL, lesioned left; LLL, lesioned left, left side; LLR, lesioned left, right side; LR, lesioned right; LRL, lesioned right, left side; PD, Parkinson's disease; PF, prefrontal cortex; SHR, spontaneously hypertensive rats; SL, sham left; SLL, sham left, left side; SLR, sham left, right side; SR, sham right; SRL, sham right, left side; SRR, sham right, right side; ST, striatum; TL, turning left; TR, turning right; WKY, Wistar Kyoto

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Thompson, 2003; Ramírez et al., 2004; Gazzaniga, 2015; Banegas et al., 2017; Prieto et al., 2019), the link between these three aspects is not yet clearly understood and direct relationships are being required. It has been suggested that unbalanced basal brain asymmetries (reducing, increasing or changing the side of predominance) might lead or take part to neuropathological deviations of brain functions such as schizophrenia, depression or Parkinson's disease (PD). However, it is not clear whether these changes are part of the etiology or are the consequence of these disorders (Ramírez et al., 2004). One of the investigations that have expanded the attempt to relate a neurochemical asymmetry with a lateralized function has been the study of the dopamine content in the striatum in relationship with the circling behavior that the rats exhibit both spontaneously and after drug induction: the animals with unilateral injections of 6-hydroxydopamine (6-OHDA) into the substantia nigra display a circling behavior contralateral to the side containing the higher content of dopamine, i.e. ipsilateral to the side of the neurochemical lesion with 6-OHDA (Shapiro et al., 1986).

Motor and cognitive disorders such as schizophrenia, depression or Parkinson's disease, in humans (Farina et al., 1990; Pioli et al., 2008) and animal models (Sullivan et al., 2014; Pioli et al., 2008), exhibit changes in their bilateral content of dopamine as characteristic features. Also, unilateral motor symptoms arising at early stages particularly characterize Parkinson's disease (Marinus and Van Hilten, 2015). Therefore, due to their possible involvement in some brain disorders such as schizophrenia (Fuxe et al., 1995), PD (Taylor et al., 1992) or anxiety (Rotzinger and Vaccarino, 2003), it is of great interest to study the functional consequence of the coexistence of dopamine, CCK and other neuropeptides such as Ang II in the brain.

Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) clearly differ in motor and cognitive behaviors as well as in their brain dopamine content (Fan et al., 2012). In addition, unilateral 6-OHDA lesioned rats demonstrate not only hyperactivity and ipsilateral rotational behavior (Fan et al., 2012) but also cognitive alterations (Carvalho et al., 2013). Therefore, modification of the brain dopaminergic system and deviations of the basal bilateral brain behavior are connected in disorders such as PD, schizophrenia or anxiety in which the neuropeptides coexisting with dopamine may play a role in their pathogeny. WKY and SHR are two opposite rat models commonly used not only in blood pressure studies but also in other investigations involving motor and mood disorders. We hypothesized that aminopeptidase A activity, responsible for the hydrolysis and inactivation of CCK and Ang II (leading to Ang III formation), could be bilaterally modified in animals unilaterally depleted of dopamine following one-sided injections of 6-OHDA but also that such activity differs between WKY and SHR. We limited our study to the analysis of the bilateral activity of aminopeptidase A and to its possible interaction with motor behavior.

2. Materials and methods

2.1. Animals

Forty adult male WKY and forty adult male SHR (Charles River laboratories, Barcelona, Spain) were used in this study. The three-month-old rats weighed 240–260 g at the beginning of the experiments. The systolic blood pressure of both strains was verified at reception using tail-cuff plethysmography (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain) as previously described (Banegas et al., 2017). Systolic blood pressure clearly differed between strains being 126.4 ± 0.45 mm Hg (mean ± SEM) for WKY and 158.2 ± 2.9 mm Hg for SHR. Both WKY and SHR rats were arbitrarily divided in the following subgroups (n = 10 each): sham left, sham right, lesioned left and lesioned right. Rats were housed in well ventilated standard laboratory cages (5 per cage) at controlled room temperature (23–25 °C) under a standard 12/12 h light/dark schedule. Laboratory food and water were provided ad libitum. To avoid the influence of circadian or seasonal variations the experiments were conducted between April and June (northern hemisphere) in the laboratory under light conditions between 9:00 a.m. and 12:00 noon (Ramírez et al., 2004). Sham groups were treated with saline whereas the lesion of the left or right nigrostriatal dopaminergic pathway was achieved with the catecholaminergic neurotoxin 6-OHDA (Jolicoeur and Rivest, 1992). Four weeks after injection of saline or 6-OHDA, turning behavior was assessed to verify the efficacy of the treatment (see below). The animals were sacrificed under perfusion with saline and equithensin anesthesia (see below). Samples of left or right medial prefrontal cortex and striatum were obtained on the same day from each group and frozen until assays for determination of enzymatic activity and protein content. Measures in the left or right 6-OHDA-lesioned animals were compared with the corresponding results in left or right sham-operated animals in which the dopamine pathways were intact. The levels of aminopeptidase A activity were measured in each group of WKY rats and in each group of SHR. All experimental procedures, including the use and care of animals, were in accordance with the European Communities Council Directive 86/609/EEC and were approved by the bioethics committee of the University of Jaén.

2.2. Unilateral lesions with 6-hydroxydopamine

Animals were anesthetized with equithensin (2 mL/kg body weight) (42.5 g/L chloralhydrate dissolved in 19.76 mL ethanol, 9.72 g/L Nembutal®, 0.396 g/L propylenglycol and 21.3 g/L magnesium sulfate in distilled water) and placed in a stereotaxic instrument (David Kopf Instruments, Palo Alto, CA, USA). Then, 4 µL of 6-OHDA (Sigma-Aldrich, St. Louis MO, USA) (8 mg dissolved in 1 mL of cold saline with 0.02% ascorbic acid to inhibit oxidation) were injected through a Hamilton syringe into the left or right striatum (Jolicoeur and Rivest, 1992). A 2-mm burr hole was drilled just through the skull at horizontal coordinates approximating the position of the striatum, that is, AP 0 mm, L or R 3 mm and H −5 mm, according to the stereotaxic atlas of Paxinos and Watson (1998). Sham rats were handled similarly, but they received 4 µL of saline with 0.02% ascorbic acid instead of 6-OHDA (Fig. 1).

2.3. Assessment of rotational behavior

Quantification of the ipsilateral or contralateral rotational behavior of the different groups allowed to verify the efficacy of the 6-OHDA-induced lesions (Kane et al., 2011; Lang and Obeso, 2004). Four weeks after intra-striatal injection of 6-OHDA or saline, animals were given D-amphetamine sulfate (5 mg/kg s.c., Sigma-Aldrich) and then were placed in a 30-cm-diameter bowl. The number of turns that an animal made in the bowl was determined over six 10-min periods (1 h). Final values were the sum of the turns recorded in the 6 periods (Robinson et al., 1994). Sham rats underwent the same surgery and rotational test (Fig. 1).

2.4. Collection of tissue samples

Once the rotational behavior study was completed, the brains were perfused with saline through the left ventricle under equithensin anesthesia (2 mL/kg body weight). The brain was quickly removed (< 60 s) and cooled in dry ice. Left and right samples of the medial prefrontal cortex and striatum were dissected according to the stereotaxic atlas of Paxinos and Watson (1998). The selected area of the medial prefrontal cortex was between 12.70 mm and 11.70 mm anterior to the interaural line. The selected area of striatum was between 10.70 mm and 09.70 mm anterior to the interaural line. The location and dimensions of both areas were based in our previous experience

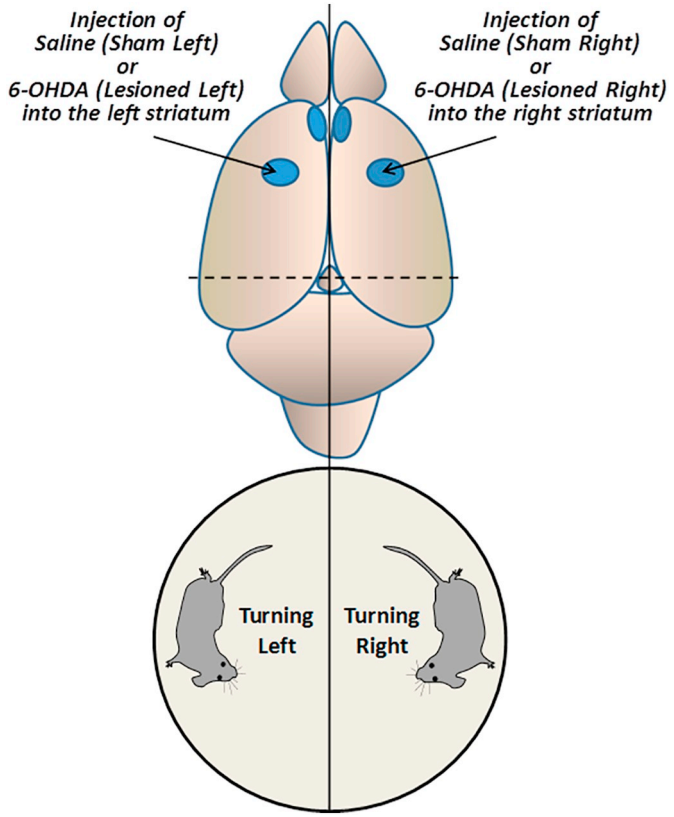

Fig. 1. Schematic representation of the experimental design. Groups (n = 10 each) of WKY and SHR were stereotaxically injected with saline into the left (sham left) or right (sham right) striatum and with 6-OHDA into the left (lesioned left) or right (lesioned right) striatum. Four weeks later, the turning behavior of each animal was quantified placing the rat in a 30-cm diameter bowl.

(Ramírez et al., 1983; Banegas et al., 2005). Tissue samples were frozen until assay.

2.5. Measurement of enzymatic activity

Tissue samples were homogenized in 400 µL of 10 mM HCl-Tris buffer (pH 7.4) and ultracentrifuged at 100,000 ×*g* for 30 min at 4 °C. To solubilize membrane proteins, the pellets were re-homogenized in HCl–Tris buffer (pH 7.4) plus 1% Triton-X-100. After centrifugation (100,000 ×*g*, 30 min, 4 °C), the supernatants were shaken in an orbital rotor for 2 h at 4 °C with the polymeric adsorbent Bio-Beads SM-2 (100 mg/mL) to remove the detergent from the sample. After the bio-beads were removed, the supernatants were used to measure membrane-bound aminopeptidase activity and protein content in triplicate (Ramírez et al., 2011).

Membrane-bound aminopeptidase A activity in the selected brain regions was fluorometrically measured using as a substrate L-Glu-β-naphthylamide (L-GluNNap, Sigma-Aldrich) as previously described (Ramírez et al., 2011). The sensitivity of the method allows measurements in the pmol range. The coefficient of variation within the assays was 3.7%. However, as the enzyme undergoes some autolytic process during the assay, it may vary slightly between different assays. To avoid such problem, all enzymatic measurements (for each group) were performed on the same day. Therefore, the different groups were analyzed and compared under similar conditions. Proteins were quantified in triplicate by the method of Bradford (1976) using BSA as a standard. Specific aminopeptidase A activity was expressed as nanomoles of L-GluNNap hydrolyzed per minute per milligram of protein. Fluorogenic assays were linear with respect to the time of hydrolysis and the protein content.

2.6. Statistical analysis

In order to reach the objectives of the study, the statistical analysis was carried in three ways:

1) A descriptive analysis using count per group, mean and standard deviation for each variable.
2) A three factor ANOVA was carried out: a) The factor group with four categories (Sham Left, Sham Right, Lesioned Left and Lesioned Right). b) The factor hemisphere (with two categories: left and right) crossed with group. c) The factor rat, ten rats tested in each group, with two measures for each rat in each hemisphere. The factor group and the factor hemisphere were fixed effect factors and the factor rat was a random effect factor. The analysis was carried out by a multilevel mixed model. The interaction group by hemisphere was the first test considered and if it was significant ($p < 0.10$) then pairwise comparisons between hemispheres within groups and among groups within hemispheres were carried out using Tukey's penalization because of balanced sample sizes. If interaction was not significant, independent test among groups and between hemispheres were carried out.
3) A linear regression adjusted by group was used to study the correlation of the peripheral variable with the enzymatic activity from the right and left hemisphere.

All analyses were carried out with Stata 14.1.

3. Results

To facilitate the follow-up of the results in the figures, the hemisphere injected with saline in sham groups is colored in rose and the hemisphere injected with 6-OHDA in the lesioned ones is colored in cyan. The results are presented in Figs. 2 to 6 and in Table 1.

3.1. Turning behavior

Sham rats did not show the circling behavior that characterized left or right dopamine depletion. Mean ± SEM values of turning behavior for left (8.3 ± 11.8) or right (5.4 ± 10.7) sham WKY and for left (7.8 ± 10.6) or right (14.3 ± 11.6) sham SHR exhibited a randomly and low ipsilateral or contralateral turning behavior to the left or right intrastriatal injection of saline. In comparison to sham groups, left (252.4 ± 39.4) or right (235 ± 40.8) lesioned WKY and left (349.5 ± 93.5) or right (352.2 ± 108.7) lesioned SHR demonstrated a complete ipsilateral turning behavior (towards the side of lesion) for all animals (Fig. 2). Thus, a manifest ipsilateral rotational behavior was observed in left or right 6-OHDA lesioned WKY and SHR animals ($p < 0.001$) (Banegas et al., 2017). These results suggested an almost complete left or right hemispheric depletion of dopamine (Kane et al., 2011; Lang and Obeso, 2004).

3.2. Bilateral behavior of aminopeptidase A activity in medial prefrontal cortex and striatum

In the prefrontal cortex of WKY, there was a left predominance of aminopeptidase A in sham rats (sham left: $p < 0.05$; sham right:

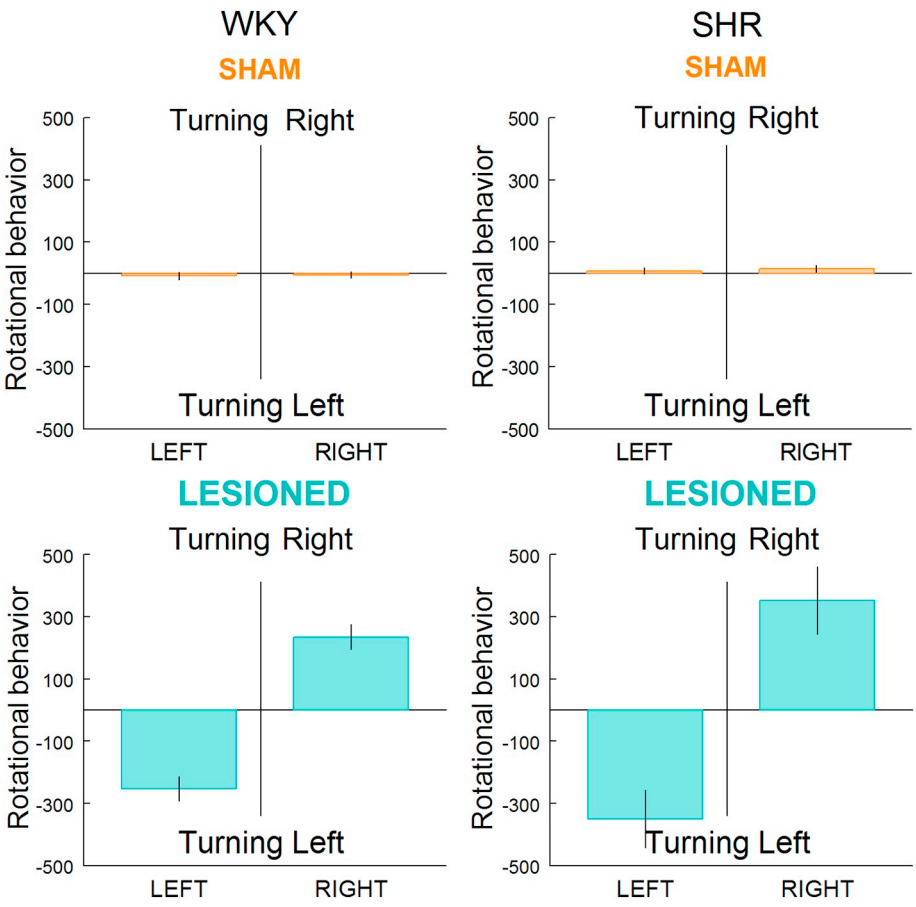

Fig. 2. Mean ± SEM levels of left (negative values) or right (positive values) turning behavior in the WKY and SHR groups after the injection of saline (Sham) or 6-OHDA (Lesioned) into the left or right striatum.

$p < 0.001$). The left-lesioned animals reduced significantly ($p < 0.001$) the activity in the left side but not in the right one, shifting the predominance to the right side ($p < 0.05$). However, the right-lesioned rats increased ($p < 0.05$) ipsilaterally the aminopeptidase activity in the right side but decreased it ($p < 0.001$) contralaterally in the left side. In the striatum, no left vs right differences were observed in sham rats. In contrast, left ($p < 0.05$) as well as right ($p < 0.01$) lesioned rats increased aminopeptidase A activity in their left sides but not in the right ones. These results indicate a left predominance in both left- ($p < 0.01$) and right-lesioned ($p < 0.05$) WKY groups (Fig. 3, left panel).

In the prefrontal cortex of SHR, no significant differences were observed between the left and right sides of the sham groups. The left lesion increased ($p < 0.05$) the values of aminopeptidase A activity in the left side but not in the right one. This led to a left predominance ($p < 0.01$). The right lesion in SHR did not modify the enzyme activity compared to sham animals neither in the left nor in the right sides, showing no asymmetry in this group. In the striatum, no left versus right differences were observed in the sham groups. Left lesions did not modify the levels of activity in left nor in right sides. In contrast, the lesions in the right sides increased aminopeptidase A activity in both the right ($p < 0.001$) and the left ($p < 0.001$) sides in 6-OHDA treated SHR rats (Fig. 3, right panel).

Fig. 4 illustrates the enzyme activity in left versus right hemispheres whereas Fig. 5 compares the effect of sham versus lesioned rats. Fig. 4 denotes clearly the asymmetry i.e. the percentage of left or right predominance over the opposite side from mean values in the WKY and SHR groups studied. In WKY, the injection of saline into both left (sham left, 56.5%) or right (sham right, 235.7%) striatum exhibited a left predominance of the medial prefrontal cortex which is especially obvious in the sham right group. In this location, the left lesion changed the predominance towards the right side (60.4%) but the right lesion led to a virtual symmetry (3.2% of left predominance). In striatum, there was no clear left/right predominance in sham left (0.42% towards left) or sham right (13.6% towards right). However, in striatum of lesioned WKY, there was a clear left predominance independently of the side of the lesion (59.1% in left-lesioned and 49.4% in right-lesioned). In SHR, the injection of saline into both the left (sham left, 32.6%) or right side (sham right, 23.4%) showed a tendency towards left predominance in the medial prefrontal cortex. In this location, the left lesion led to a left predominance (68.2%) whereas the right one shows a tendency to a right predominance (36.2%). This data almost demonstrates an opposite behavior in prefrontal cortex between WKY and SHR after the left and right lesions. In striatum of SHR, no clear tendencies of predominance were observed in sham left (0%) or sham right (2% of right predominance) nor in lesioned left (11.5% right) or lesioned right (3.17% left).

Fig. 5 represents the effect of the lesion i.e. the percentage of increase or decrease in the left or right side of aminopeptidase A activity in the animals with a left or right lesion over the values in the left or right sham groups. In WKY, there was an opposite behavior between the medial prefrontal cortex and striatum. In the prefrontal cortex, the response was the same independently of the side of the lesion: a decrease

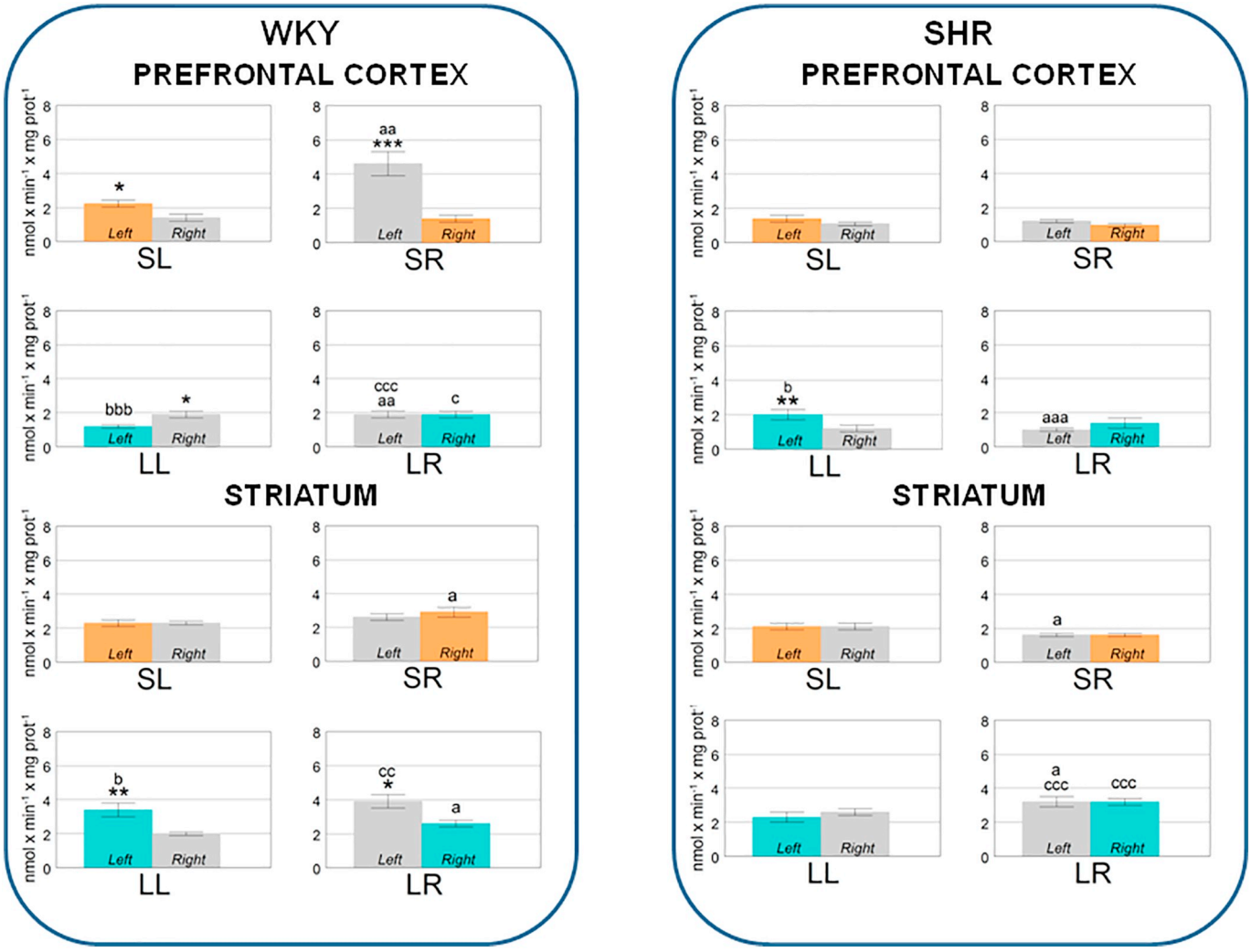

Fig. 3. Influence of left (lesioned left, LL) or right (lesioned right, LR) intrastriatal injections of 6-OHDA on the left and right levels of aminopeptidase A in the prefrontal cortex and striatum of WKY (left panel) and SHR (right panel) in comparison with rats to whom saline, instead of 6-OHDA, was injected into the left (sham left, SL) or right (sham right, SR) striatum. The side injected with 6-OHDA in cyan, the injected with saline in rose. (*) left vs right comparisons (paired data). (a) SL vs SR or LL vs LR comparisons (same side, unpaired data). (b) SL vs LL (same side, unpaired data). (c) SR vs LR (same side, unpaired data). Single asterisk or letter ($p < 0.05$); double asterisk or letter ($p < 0.01$); triple asterisk or letter ($p < 0.001$).

in the left side (88.3% in left-lesioned and 139.9% in right-lesioned) and an increase in the right side (33.3% in left-lesioned and 35.6% in right-lesioned). In contrast, the response was the reverse in the striatum: an increase in the left side (37.4% in left-lesioned and 51.7% in right-lesioned) and a decrease in the right side (15.2% in left-lesioned and 11.8% in right-lesioned). In SHR there was no clear tendency in the pattern of behavior. In the prefrontal cortex, the left lesion led to an increase (41.6%) in the left side but also an increase in the right one (11.7%). The right lesion produced a decrease (19.3%) in the left side but an increase (40.9%) in the right one. In the striatum, the left lesion produced an increase in both the left (10.3%) and right (23.1%) sides. Interestingly, the right lesion led to a marked increase in the left (100.9%) and right (90.9%) sides.

3.3. Correlational study

The *intra-* and *inter*-hemispheric significant correlations between striatum, medial prefrontal cortex and turning behavior are indicated in Table 1 and represented in Fig. 6. In WKY rats, the group of sham left demonstrated a significant contralateral negative correlation ($r = -0.711$, $p = 0.02$) between the right striatum and the left prefrontal cortex and a positive ipsilateral one between the right striatum and the right prefrontal cortex ($r = +0.711$, $p = 0.02$). The group of sham left in SHR did not show any significant correlations. The group of sham right in WKY revealed (in contrast to sham left) a significant positive contralateral correlation ($r = +0.725$, $p = 0.01$) between the left striatum and the right prefrontal cortex. No significant correlations were observed in the group of sham right of SHR.

Most correlations were revealed in both WKY and SHR groups with lesion in the left hemisphere. In WKY rats, the group with left lesions demonstrated a significant positive ipsilateral correlation between the left striatum and the left prefrontal cortex ($r = +0.710$, $p = 0.02$), a negative contralateral one between the left striatum and the right prefrontal cortex ($r = -0.792$, $p = 0.006$) and also a contralateral negative correlation between the left and the right prefrontal cortex ($r = -0.746$, $p = 0.01$). In SHR, the group with left lesions revealed a significant ipsilateral negative correlation between the left striatum and the left prefrontal cortex ($r = -0.785$, $p = 0.007$), an ipsilateral

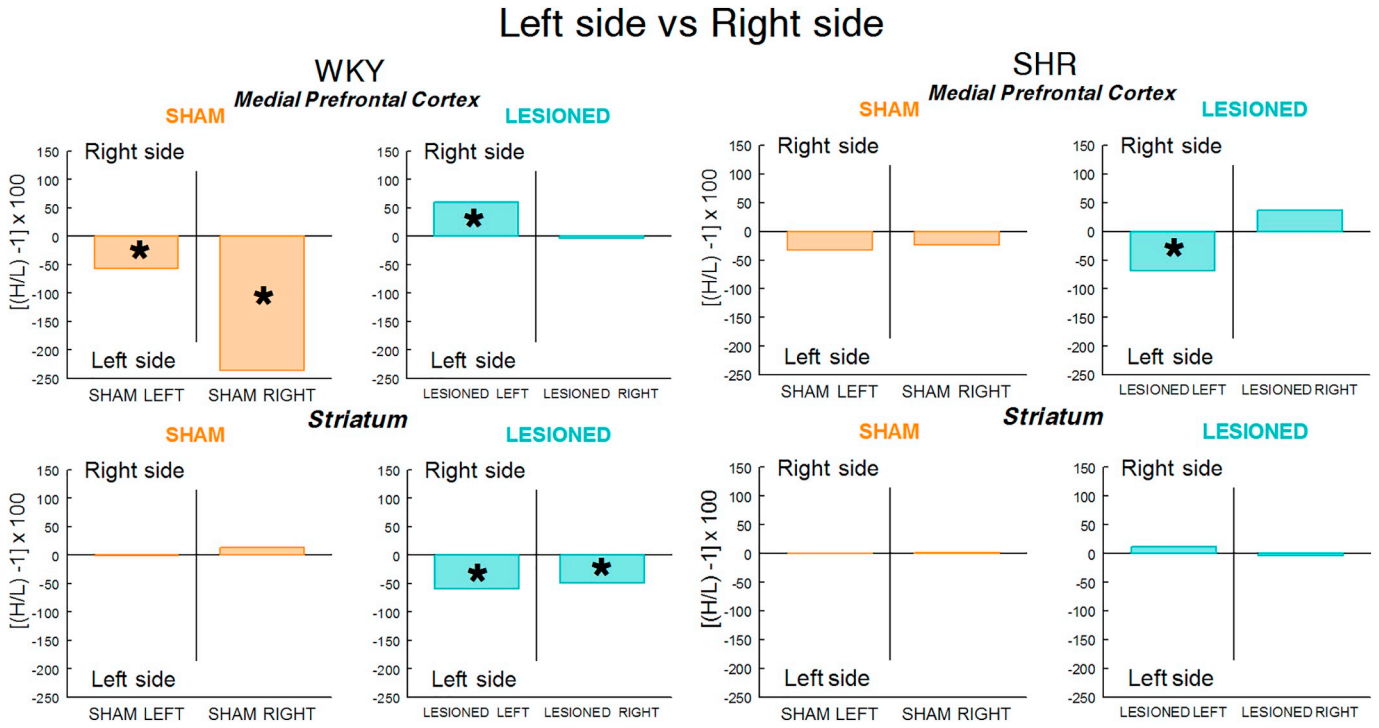

Fig. 4. Percentage of left or right predominance over the opposite side from mean values in the WKY and SHR groups studied. H/L denotes the higher value/lower value. Negative or positive values indicate the percentage of left or right predominance respectively. * indicates the group in which a left or right significant difference is reached.

positive one between the left striatum and the values of the turning behavior towards the left side ($r = +0.790$, $p = 0.006$) and also an ipsilateral negative significant correlation between the left prefrontal cortex and values of turning behavior towards the left side ($r = -0.811$, $p = 0.004$). Finally, the group of WKY animals with right side lesion demonstrated, in contrast to the group with the left lesion, a significant negative contralateral correlation between the left striatum and the values of the turning behavior (that this group manifested) towards the right side ($r = -0.675$, $p = 0.03$). However, in SHR, this lesioned right group did not show any significant correlation.

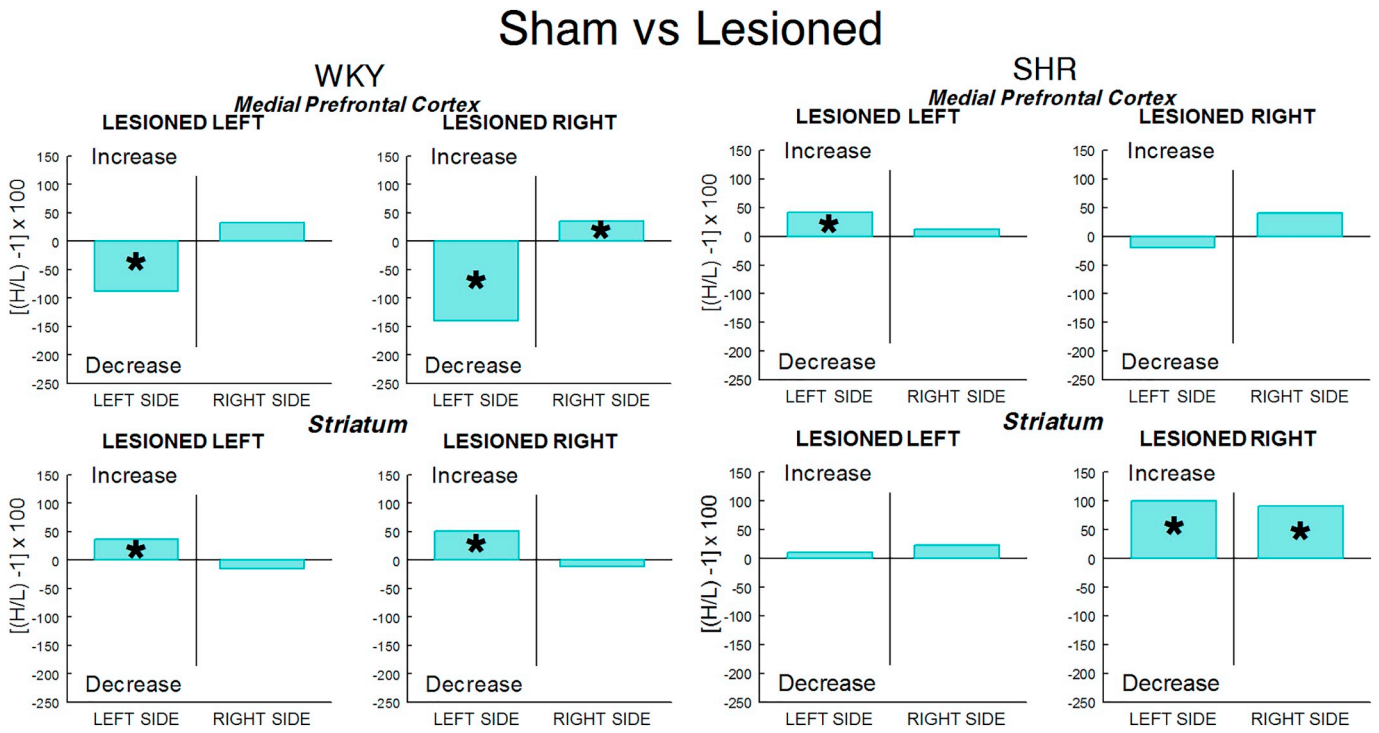

Fig. 5. Percentage of increase or decrease in the left or right side, over the values of the left or right sham groups, in the animals with a left or right lesion. H/L denotes the higher value/lower value. Negative or positive values indicate the percentage of increase or decrease over sham values respectively. * indicates the group and side in which the change reached a statistical significance.

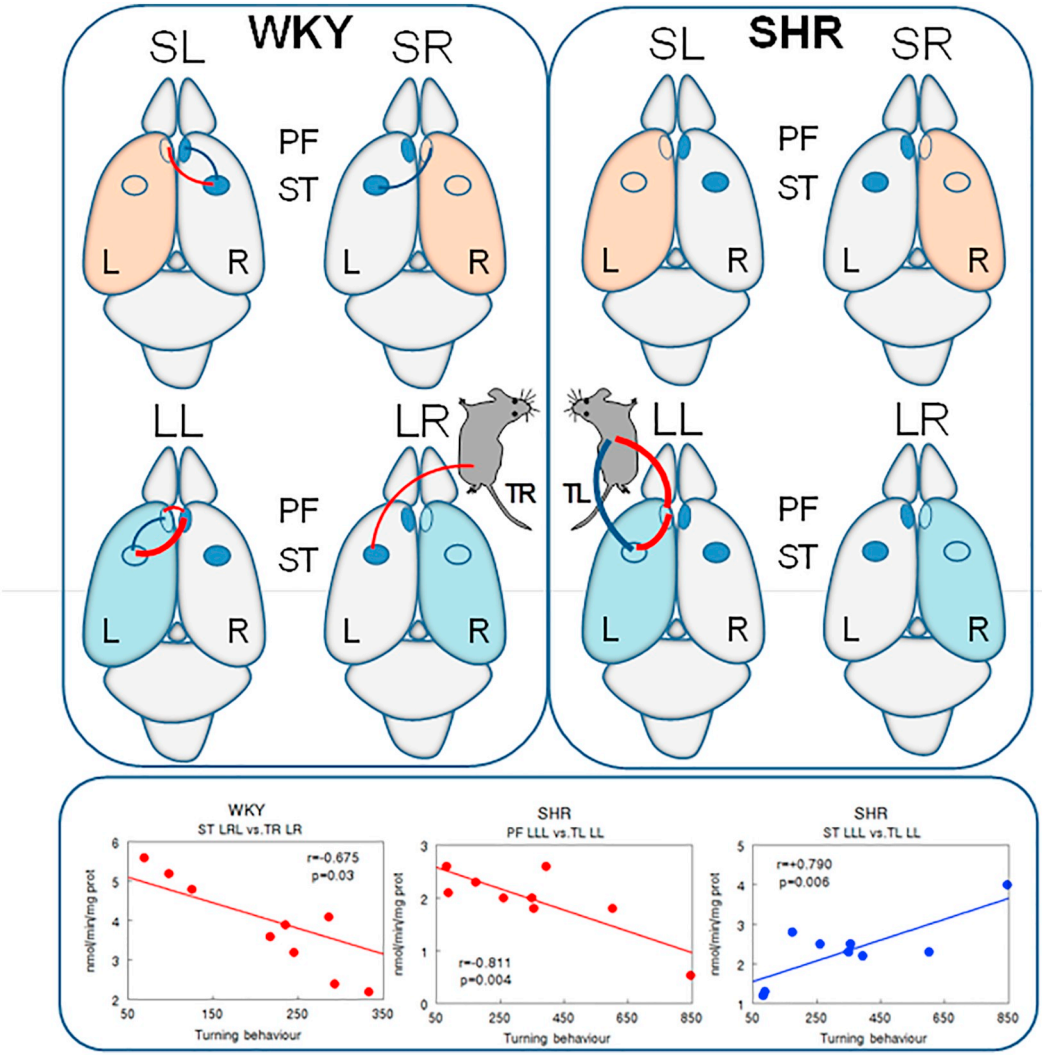

Fig. 6. Top panels: Intra-and inter-hemispheric correlations between striatum (ST) and prefrontal cortex (PF) and with turning behavior towards the right side (TR) or towards the left one (TL), in sham left (SL), sham right (SR), lesioned left (LL) and lesioned right (LR) WKY and SHR rats. The side injected with 6-OHDA in cyan, the injected with saline in rose. Positive correlations in blue and negative ones in red. The thickness of lines is proportional to the degree of significance. Bottom panel: Positive (blue) or negative (red) correlations between aminopeptidase A of the left or right side of the ST or PF in left- or right-lesioned WKY or SHR vs. turning behavior values of the same group. LRL, left side of lesioned right; LLL, left side of lesioned left. Pearson's (r) as well as p-values are indicated.

4. Discussion

The present results demonstrate: 1) A clear influence of the unilateral intrastriatal 6-OHDA injections on ipsilateral and contralateral aminopeptidase A activity which suggests an ipsi- and contralateral relationship of dopamine with this enzyme and consequently with its endogenous substrates such as CCK and/or Ang II. 2) That the *intra*- and *inter*-hemispheric response to left or right intrastriatal injections of 6-OHDA differed depending on the lesioned side, the brain region studied and the strain used. 3) That the *intra*- and *inter*-hemispheric correlations of aminopeptidase A activity between striatum and prefrontal cortex differ depending on the lesioned side and the strain. 4) That aminopeptidase A activity correlates with motor behavior values depending on the lesioned side and the strain. While there was a contralateral correlation of aminopeptidase A between striatum and the turning direction of right lesioned WKY, this correlation was in contrast ipsilateral between the enzymatic activity in striatum and prefrontal cortex with the turning direction of left lesioned SHR. 5) That the main changes and interactions imply essentially the left hemisphere.

The normal rats exhibit a spontaneous turning behavior, the levels of dopamine being higher in the contralateral striatum than the side of the turning preference (Glick, 1983). Therefore, animals unilaterally lesioned with 6-OHDA turn ipsilaterally towards the side of the lesion with lower dopamine level (Fig. 1). This turning behavior is increased after amphetamine administration that increases dopamine in the synaptic cleft (Robinson et al., 1994).

Although dopamine levels were not quantified in this work, the values for turning behavior after 6-OHDA lesions were unambiguously and systematically high enough in comparison with sham animals to be assure of a clear dopamine depletion in the lesioned groups (Kane et al., 2011; Carvalho et al., 2013).

Regarding the link between a neurochemical asymmetry and a specific function, there are several publications that support the involvement of CCK. When CCK8 was injected into the right nucleus accumbens, the horizontal activity of rats was increased when compared to animals injected into the left side. In contrast, CCK8 improved learning and memory when injected into the left but not into the right amygdala (Belcheva et al., 1994). Also, animals pretreated with CCK

Table 1
Significant correlations between striatum, prefrontal cortex and turning behavior.

WKY			SHR		
Correlation	r	p	Correlation	r	p
Sham left			Sham left		
St SLR WKY vs Pf SLL WKY	*−0.711*	*0.02*	No correlations		
St SLR WKY vs Pf SLR WKY	+0.771	0.02			
Sham right			Sham right		
St SRL WKY vs Pf SRR WKY	+0.725	0.01	No correlations		
Lesioned left			Lesioned left		
St LLL WKY vs Pf LLL WKY	+0.710	0.02	*St LLL SHR vs Pf LLL SHR*	*−0.785*	*0.007*
St LLL WKY vs Pf LLR WKY	*−0.792*	*0.006*	St LLL SHR vs TR LL SHR	+0.790	0.006
Pf LLL WKY vs Pf LLR WKY	*−0.746*	*0.01*	*Pf LLL SHR vs TR LL SHR*	*−0.811*	*0.004*
Lesioned right			Lesioned right		
St LRL WKY vs TR LR WKY	*−0.675*	*0.03*	No correlations		

Intra- and inter-hemispheric correlations between striatum (St) and prefrontal cortex (Pf) and with turning behavior towards the right side (TR) or towards the left one (TL) in the WKY and SHR groups studied. Negative correlations in italics and red. LL, lesioned left, LR, lesioned right, SLR (sham left, right-side), SLL (sham left, left-side), SRL (sham right, left-side), SRR (sham right, right-side), LLL (lesioned left, left-side), LLR (lesioned left, right-side), LRL (lesioned right, left-side).

before striatal lesions with 6-OHDA demonstrated a higher circling behavior than animals pretreated with vehicle (Nikolaus et al., 1997). In humans, the administration of CCK4 increased electro encephalographic asymmetries at midtemporal recording sites compared to placebo (Knott et al., 2003). Regarding Ang II, its injection (2 nmol) into the contralateral striatum produced dose-related tight rotations which are ipsilateral to the side of the lesion in rats with unilateral nigrostriatal lesions with 6-OHDA (Jenkins et al., 1995). Rotations were suppressed by the co-administration of the AT1 antagonist losartan (2 nmol), which had no effect when injected alone. Further, the pre-administration of the dopamine antagonist haloperidol (2 mg/kg i.p.) blocked the effect of Ang II supporting the hypothesis that Ang II is involved in the dopamine release in striatum through its binding to the AT1 receptors (Jenkins et al., 1995).

Although the primary lesion in PD is an unilateral degeneration of the dopaminergic pathways, it has been suggested that this degeneration could also involve other systems including the neuropeptidergic one whose bilateral behavior in PD has been poorly studied (Cubo et al., 2010; Banegas et al., 2017). It was also proposed that left dopaminergic degeneration in Parkinson's patients plays a markedly greater role than the right one in relation to non-motor symptoms. These patients would present much more severe cognitive alterations than patients with right dopaminergic degeneration (Braszko et al., 1987). This asymmetrical behavior may also be noticed in schizophrenic patients: an increase of dopamine in the left amygdala in comparison with the right one was observed using post mortem brain samples from these patients (Reynolds, 1983). It was also reported that, while normal subjects exhibited no clear turning preference, a manifest left circling preference was observed in untreated schizophrenic patients suggesting a dopaminergic asymmetry in brain areas that control motor behavior in these patients (Bracha, 1987). However, neither neuroleptic chronic treatment nor the addition of dopaminergic agonists demonstrated a significant left or right turning behavior in these schizophrenic patients (Levine et al., 1997). In contrast, the chronic administration of antidepressant in right turning rats eliminated turning biases but not in the left ones (Carlson et al., 1996). Further, the effects of left or right dopamine depletion of the prefrontal cortex on circling behavior in rats exhibiting left or right turning biases were greater in left- than in right-turning animals (Carlson et al., 1996). In addition, it was also reported that left 6-OHDA lesioned animals increased dramatically their systolic blood pressure in comparison with the right-lesioned ones (Banegas et al., 2011).

The present results demonstrating the most frequent and significant correlations (Table 1) as well as the main changes after lesions (Fig. 5) in the group of left lesioned animals could support the hypothesis of a differential *intra-* and *inter* asymmetrical behavior of the aminopeptidase targets. These results reveal the importance of the left hemisphere in disorders in which there are imbalances in the basal asymmetrical distribution of dopamine. It clearly suggests a role for aminopeptidase A and its endogenous substrates CCK and Ang II in the underlying mechanism.

Differences in the content of brain dopamine as well as cognitive and motor differences between WKY and SHR have also been reported (Fan et al., 2012). Moreover, CCK and Ang II are involved in cognitive and motor functions (Smolnik et al., 2002; Braszko et al., 1987). The present results definitely link dopamine with aminopeptidase A activity and therefore with its endogenous substrates CCK and Ang II. They also associate this enzymatic activity of the left hemisphere with motor behavior, independently of the side of lesion (but remarkably in left-lesioned rats). These observations are important for potential therapeutic strategies based on the control of aminopeptidase A activity through activators or inhibitors. It is also noteworthy to realize the opposing behavior of aminopeptidase A activity between the medial prefrontal cortex and the striatum in WKY after 6-OHDA lesions (Fig. 5). This is not the case in SHR. The meaning of such a difference remains to be elucidated. However, this could be related to observations suggesting a disrupted connection between frontal cortex and striatum in PD. Indeed, an altered caudate outflow consequence of PD would lead to deficits in cognitive functions depending on the integrity of the prefrontal cortex (Taylor et al., 1986). Furthermore, taking into account that patients with PD exhibit altered cognitive functions similar to those observed in patients with prefrontal cortex lesions, a disruption in corticostriatal circuits and a deficiency in frontal dopamine were proposed (Monchi et al., 2004). More recently, an altered brain metabolic connectivity between frontal cortex and striatum was described in early PD (Sala et al., 2017). Moreover, an asymmetric molecular

frontostriatal network breakdown with left basal ganglia was suggested in impulse-control-disorder symptoms of PD (Premi et al., 2016).

In conclusion, the results of the present research suggest that the functions in which the aminopeptidase A activity is involved could be modified depending on whether the lesion occurs on the left or right hemisphere.

Brain asymmetry is a complex phenomenon. It is not a static but a dynamic process influenced especially by diurnal rhythms, seasonal changes (Ramírez et al., 2004), light and dark conditions (Sánchez et al., 1996) or hormonal fluctuations (Rueda et al., 2016). In our study, we have limited these differential factors to left or right depletion of dopamine following left or right brain lesion. However, because there is a basal brain asymmetry with different functions for each hemisphere, we cannot expect a clear and systematic bilateral pattern of response under a specific unilateral alteration. To support the present hypothesis, cognitive tests and bilateral determinations of the susceptible endogenous substrates of aminopeptidase A should be performed in left and right 6-OHDA-lesioned WKY and SHR.

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Declaration of Competing Interest

The authors report no conflicts of interest.

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