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Aminopeptidase Activities Interact Asymmetrically between Brain, Plasma and Systolic Blood Pressure in Hypertensive Rats Unilaterally Depleted of Dopamine

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Abstract: Brain dopamine, in relation to the limbic system, is involved in cognition and emotion. These functions are asymmetrically processed. Hypertension not only alters such functions but also their asymmetric brain pattern as well as their bilateral pattern of neurovisceral integration. The central and peripheral renin-angiotensin systems, particularly the aminopeptidases involved in its enzymatic cascade, play an important role in blood pressure control. In the present study, we report how these aminopeptidases from left and right cortico-limbic locations, plasma and systolic blood pressure interact among them in spontaneously hypertensive rats (SHR) unilaterally depleted of dopamine. The study comprises left and right sham and left and right lesioned (dopamine-depleted) rats as research groups. Results revealed important differences in the bilateral behavior comparing sham left versus sham right, lesioned left versus lesioned right, and sham versus lesioned animals. Results also suggest an important role for the asymmetrical functioning of the amygdala in cardiovascular control and an asymmetrical behavior in the interaction between the medial prefrontal cortex, hippocampus and amygdala with plasma, depending on the left or right depletion of dopamine. Compared with previous results of a similar study in Wistar-Kyoto (WKY) normotensive rats, the asymmetrical behaviors differ significantly between both WKY and SHR strains.

Keywords: dopamine; limbic system; medial prefrontal cortex; hippocampus; amygdala; brain asymmetry; hypertension; asymmetrical neurovisceral integration; proteinases; aminopeptidases; renin-angiotensin system

1. Introduction

Brain dopamine maintains a close relationship with areas involved in cognitive functions and emotional control such as the medial prefrontal cortex, hippocampus and amygdala, included in the limbic system [1–5]. Such functions are processed asymmetrically [6]. In fact, the coexistence between dopamine, Parkinson's disease, altered emotional state and cerebral asymmetry is well known [7–9]. Hypertension not only alters those cognitive and emotional processes but can also modify the bilateral asymmetric model, not only of brain function per se but also of the asymmetric model of neurovisceral integration [10–13]. Among others, the renin-angiotensin-aldosterone system (RAAS) plays an essential role in controlling blood pressure [14]; in fact, common anti-

hypertensive treatments include inhibitors of the system at various levels [15] which have been also suggested for the prevention and treatment of neurodegenerative diseases such as Alzheimer's disease [16,17] or Parkinson's disease [18]. Within the RAAS, proteolytic enzymes and, in particular, some aminopeptidases, play a relevant role [19]. With this research, we analyze how these aminopeptidases, measured from left and right cortico-limbic locations (medial prefrontal cortex, mPFC, hippocampus, HC, and amygdala, AM), plasma and systolic blood pressure interact among them in spontaneously hypertensive rats (SHR) unilaterally depleted of dopamine. In addition to blood pressure research, SHR is a frequent animal model used to study brain disorders such as attention deficit/hyperactivity disorder [20], Schizophrenia [21] and vascular brain disorders among many others [22]. In this context, other contributions are interesting, such as the relationship between dopamine and cognitive function [23], mitochondrial dysfunction in schizophrenia [24], or schizophrenia in the dissociative model [25].

Brain functions and bidirectional neuro-visceral integrative processes are virtually asymmetric [26] but this bilateral pattern is dynamically modified depending on physiological and pathological conditions [26], as well as depending on changing environmental factors, even going so far as to change the side of predominance [27]. However, despite extensive research on brain asymmetry, its functional significance remains largely unknown [26,28–30].

Neuropeptides and peripheral peptides take an important role in many of those processes, being the functional status of these peptides is regulated in part by proteinases [27,31]. In the brain, this regulation is mainly carried out by aminopeptidases as its most abundant proteinases [32]. This is the rationale we used to analyze the bilateral distribution of leucine-aminopeptidase in the brain, a study that led to the first description of a brain asymmetry for proteinase activity, particularly in the frontal cortex of rats [33]. Simultaneously, the activities of two classical metabolic enzymes, lactate dehydrogenase and glutamate-oxalacetate aminotransferase, were measured in the same cerebral region and no significant difference was recorded in these activities between both sides of the rat brain which may suggest a specific functional role for that proteolytic activity [34].

A clear illustrative example of the functional role exerted by proteinases can be observed in the role they play in the enzymatic cascade of the RAAS [35] (Figure 1). The proteolytic activity of an enzyme results in the inactivation of its substrate and simultaneously in the activation of the new peptide resulting from such hydrolysis, with different functions from those from which it came. Therefore, its analysis offers us a dynamic view of its function under certain experimental conditions.

Despite the initial conviction of “a neuron a neurotransmitter”, nowadays the coexistence of different types of neurotransmitters in the same neuron has become evident [36]. For example, it has been demonstrated the coexistence in dopaminergic neurons of neuropeptides such as cholecystinin and neurotensin [37], which can also interact with other neuropeptides such as angiotensins or enkephalins, all of them related to emotional processing and cardiovascular regulation [38,39]. The modification of any of these peptides may affect the function of all the others, including the bilateral interactions established between them. Dopamine plays a relevant role in emotional processing in which the cortico-limbic areas of the brain are involved. Brain dopaminergic asymmetry in physiological and pathological conditions such as Parkinson's disease is also well known. Its modification will affect the functions performed by the various neurotransmitters that interact with it [39].

There is an increase in the levels of anxiety and depression in hypertensive patients [40] and in genetically hypertensive animals [41], emotional alterations in which disruptions in neuro-visceral communication may also be involved [42]. The coexistence between Parkinson's disease and hypertension has also been discussed [43] and a connection between depression, neurochemical asymmetry and cardiovascular function has been postulated [44].

Recently, we have described an asymmetric interaction of aminopeptidase activities between cortico-limbic areas, such as the prefrontal cortex, amygdala and hippocampus, of the left and right hemisphere, between these left or right brain areas and the same plasmatic activities and finally, between the left or right brain areas and systolic blood pressure (SBP) levels, following unilateral left or right dopamine depletions of the nigrostriatal system, in normotensive WKY rats as well as in their corresponding left or right sham controls [39]. Therefore, since emotional processing differs in hypertension and the bilateral neuro-visceral connection may be modified, it is necessary to analyze what happens in hypertensive conditions. In the present study, we report how the same brain locations, plasma and SBP interact in spontaneously hypertensive rats (SHR) unilaterally depleted of dopamine. The study comprises left and right sham and left and right dopamine-depleted (lesioned) rats as research groups. A global comparative analysis between both WKY and SHR strains of rats is also performed.

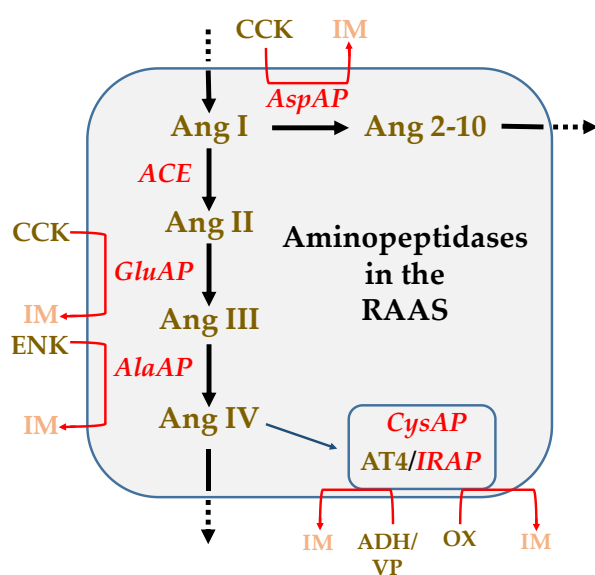


Figure 1. Schematic representation of a part of the renin-angiotensin-aldosterone system (RAAS) in which the aminopeptidase activities determined in the present work are involved. Angiotensin I (Ang I), produced by the action of renin on angiotensinogen, is hydrolyzed by aspartate aminopeptidase (AspAP) to produce Ang 2–10. AspAP can also metabolize cholecystokinin (CCK) to an inactive metabolite (IM). Moreover, Ang I, due to the action of the angiotensin-converting enzyme (ACE), produces Ang II, which is hydrolyzed to Ang III through the action of glutamate aminopeptidase (GluAP), which can also act on CCK to produce an IM. Ang III, through alanine aminopeptidase (AlaAP), will give rise to Ang IV but can also inactivate enkephalin (ENK). Ang IV binds to the AT₄ receptor that has been identified as the insulin-regulated aminopeptidase (IRAP), also known as CysAP, oxytocinase (which inactivates oxytocin, OX), or vasopressinase (which inactivates vasopressin, VP or antidiuretic hormone, ADH) [35].

2. Materials and Methods

2.1. Experimental Groups

For the present research, a total of 40 male hypertensive rats (spontaneously hypertensive rats (SHR) from Charles River Laboratories, Barcelona, Spain). They were randomly separated into 4 groups of 10 animals each. Depending on the left or right brain hemisphere that we selected, the groups were called: sham left (SL), sham right (SR), lesioned left (LL) and lesioned right (LR). The left or right sham animals were obtained by the stereotaxic injection of saline into the left or right striatum. The animals in which the left or right depletion of dopamine was carried out (lesioned) were achieved by the stereotaxic injection of the neurotoxin 6-hydroxidopamine (6-OHDA) into the left or right striatum [39].

The animals correctly left or right depleted of dopamine demonstrated, four weeks after 6-OHDA injections, an outstanding turning behavior towards the hemisphere having less dopamine content [45]. When the animals recovered from surgery, systolic blood pressure (SBP) was measured by the plethysmographic method as previously reported in detail [46] (Figure 2). The rats were then sacrificed on the same day and blood samples as well as samples from the left or right medial prefrontal cortex (mPFC), left or right hippocampus (HC) and left or right amygdala (AM) were obtained from each group and frozen until assayed. All the experiments and the care of animals were performed in agreement with the European Communities Council Directive 86/609/EEC. Approved by the bioethic committee of the University of Jaén on November 20, 2006 with number 140.

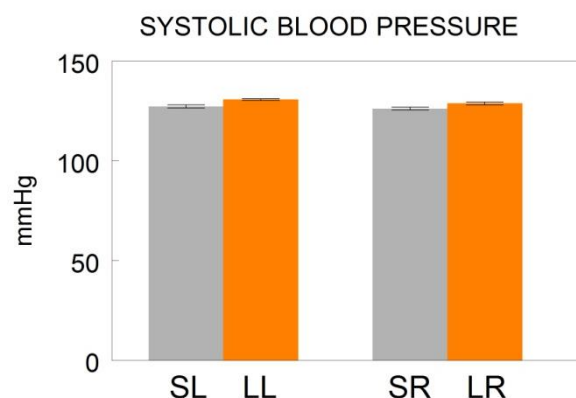


Figure 2. Mean \pm SEM levels of systolic blood pressure values expressed in mmHg after saline injection into the left (sham left; SL; $n = 10$) or right (sham right; SR; $n = 10$) striatum or after 6-hydroxydopamine injection into the left (lesioned left; LL; $n = 10$) or right (lesioned right; LR; $n = 10$) striatum of spontaneously hypertensive adult male rats. *** indicates a significant difference of $p < 0.001$ between sham versus lesioned animals in the same side. +++ indicates a significant difference of $p < 0.001$ between sham left versus sham right or between lesioned left versus lesioned right animals. Modified from [46].

2.2. 6-Hydroxydopamine and Saline Intrastratial Injections

The animals, previously anesthetized with equithensin (42.5 g/L chloral hydrate dissolved in 19.76 mL ethanol, 9.72 g/L Nembutal®, 0.396 g/L propylene glycol and 21.3 g/L magnesium sulfate in distilled water) (2 mL/kg body weight), were fixed to a stereotaxic (David Kopf Instruments, Palo Alto, CA, USA). In agreement with the Paxinos and Watson atlas [47], the coordinates used to inject saline or the neurotoxin 6-OHDA into the striatum were: AP 0 mm, L or R 3 mm and H-5 mm. For left or right lesioned rats, 4 μ L of 6-OHDA (8 mg dissolved in 1 mL of cold saline with 0.02% ascorbic acid to inhibit oxidation) was injected. For left or right sham rats, 4 μ L of saline with 0.02% ascorbic acid was injected.

2.3. Obtaining Brain and Plasma Samples

Four weeks after the injections of saline or 6-OHDA, to confirm that the lesions had been carried out correctly, the turning behavior of the animals was measured [46]. Then, under anesthesia, blood samples were obtained from the left ventricle. Plasma, obtained by centrifugation (10 min at 3000 \times g), was used for enzyme and protein determinations in triplicate. After obtaining the blood samples, the animals were perfused with saline (also through the left ventricle) and their brains were obtained in less than 1 min and cooled in dry ice. Subsequently, according to the Paxinos and Watson atlas [47], left and right samples of mPFC, HC and AM were obtained. The coordinates for mPFC were between 12.70 mm and 11.20 anterior to the interaural line (AIL), between 7.12 and 5.40 mm AIL for HC, and between 7.12 and 5.40 mm AIL for the AM [39].

2.4. Aminopeptidase Activities and Protein Determinations

The method for the measurement of aminopeptidases and proteins has already been described [31]. In summary, once the tissue samples were defrosted, they were homogenized (in 400 μ L of 10 mM HCl-Tris buffer, pH 7.4) and centrifuged (100,000 \times g for 30 min at 4 $^{\circ}$ C). To separate membrane proteins, the pellets were again homogenized in HCl-Tris buffer (pH 7.4) plus 1% Triton-X-100 and centrifuged (100,000 \times g, 30 min, 4 $^{\circ}$ C). In order to remove the detergent Triton-X-100, the obtained supernatants were shaken in an orbital rotor (2 h at 4 $^{\circ}$ C) together with the polymeric adsorbent Bio-Beads SM-2 (100 mg/mL). These supernatants were then used to determine, in triplicate, membrane-bound aminopeptidase activities and proteins. Aminopeptidases were measured by fluorimetry [31]. In summary, AlaAP and CysAP were measured using the aminoacyl- β -naphthylamides (aaNNap) AlaNNap and CysNNap as substrates: 10 μ L of each supernatant were incubated 30 min at 25 $^{\circ}$ C with 1 mL of the substrate solution: 2.14 mg/100 mL AlaNNap or 5.63 mg/100 mL CysNNap, 10 mg/100 mL bovine serum albumin (BSA), and 10 mg/100 mL dithiothreitol (DTT) in 50 mM of phosphate buffer, pH 7.4, for AlaAP and 50 mM HCl-Tris buffer, pH 6, for CysAP. AspAP was measured using AspNNap as substrate: 10 μ L of each supernatant was incubated for 120 min at 37 $^{\circ}$ C with 1 mL of the substrate solution (2.58 mg/100 mL AspNNap, 10 mg/100 mL BSA and 39.4 mg/100 mL MnCl₂ in 50 mmol/L HCl-Tris buffer, pH 7.4). GluAP was determined using GluNNap as substrate: 10 μ L of each supernatant was incubated during 120 min at 37 $^{\circ}$ C with 1 mL of the substrate solution (2.72 mg/100 mL GluNNap, 10 mg/100 mL BSA, 10 mg/100 mL DTT and 0.555 g/100 mL CaCl₂ in 50 mmol/L HCl-Tris, pH 7.4). In order for the enzymatic reactions to stop, 1 mL of 0.1 mol/L of acetate buffer at pH 4.2 was added. The β -naphthylamine, released by enzymatic activity, was measured, by fluorimetry, at 412 nm emission wavelengths with an excitation wavelength of 345 nm. The method allows determinations in the picomolar range. Proteins were measured by the method of Bradford [48], using BSA as a standard. Specific membrane-bound brain aminopeptidase activities were expressed as nmol of AlaNNap, CysNNap, AspNNap, or GluNNap hydrolyzed per min per mg of protein. For determinations of plasma aminopeptidase activities, the procedure was modified as follows: 25 μ L of plasma was incubated for 120 min at 37 $^{\circ}$ C with 1 mL of the substrate solutions previously indicated. Plasma aminopeptidase activities were expressed as pmol of the corresponding aaNNap hydrolyzed per min per mg of protein [46]. Fluorogenic assays were linear with respect to the time of hydrolysis and protein content.

2.5. Statistical Analysis

The differences between hemispheres and groups were calculated using a two-way analysis of variance. Post hoc comparisons were performed with LSD tests. *p*-values below 0.05 were considered significant. To study the intra- and inter-hemispheric levels of correlation between neuropeptidase activities of the mPFC, HC and AM, between them and plasma aminopeptidases and between them and SBP, Pearson's coefficient of correlation was computed. Computations were performed using SPSS 13.0 and STATA 9.0 (STATA Corp., College Station, TX, USA). *p*-values below 0.05 were considered significant.

3. Results

3.1. Systolic Blood Pressure in Hypertensive Rats

While SBP levels did not vary in the left lesioned (left dopamine-depleted) animals (LL) in comparison to their sham left (SL) controls, animals with the DA-depletion of the right hemisphere (LR) significantly increased ($p < 0.001$; ***) SBP levels compared to their corresponding sham controls (SR). The right SR and LR groups showed significantly ($p < 0.001$; +++) lower SBP levels than the left SL or LL groups, respectively (Figure 2).

3.2. Aminopeptidase Activities in Brain and Plasma

In general, the data show a great variability depending on the enzymatic activity analyzed and the brain or plasmatic location (Figure 3). In plasma, a significant reduction is observed for AlaAP ($p < 0.001$), CysAP ($p < 0.001$) and AspAP ($p < 0.01$) or a tendency to decrease for GluAP in animals with left (LL) depletion of DA in comparison with their SL controls. In sharp contrast, there is a significant increase ($p < 0.001$) for GluAP, AlaAP, and AspAP or a tendency to increase for CysAP in right DA-depleted (LR) animals compared to their SR controls. At the cerebral level, the results are variable depending on the location, but in general, in most groups, less aminopeptidase activity is observed on the right side than on the left.

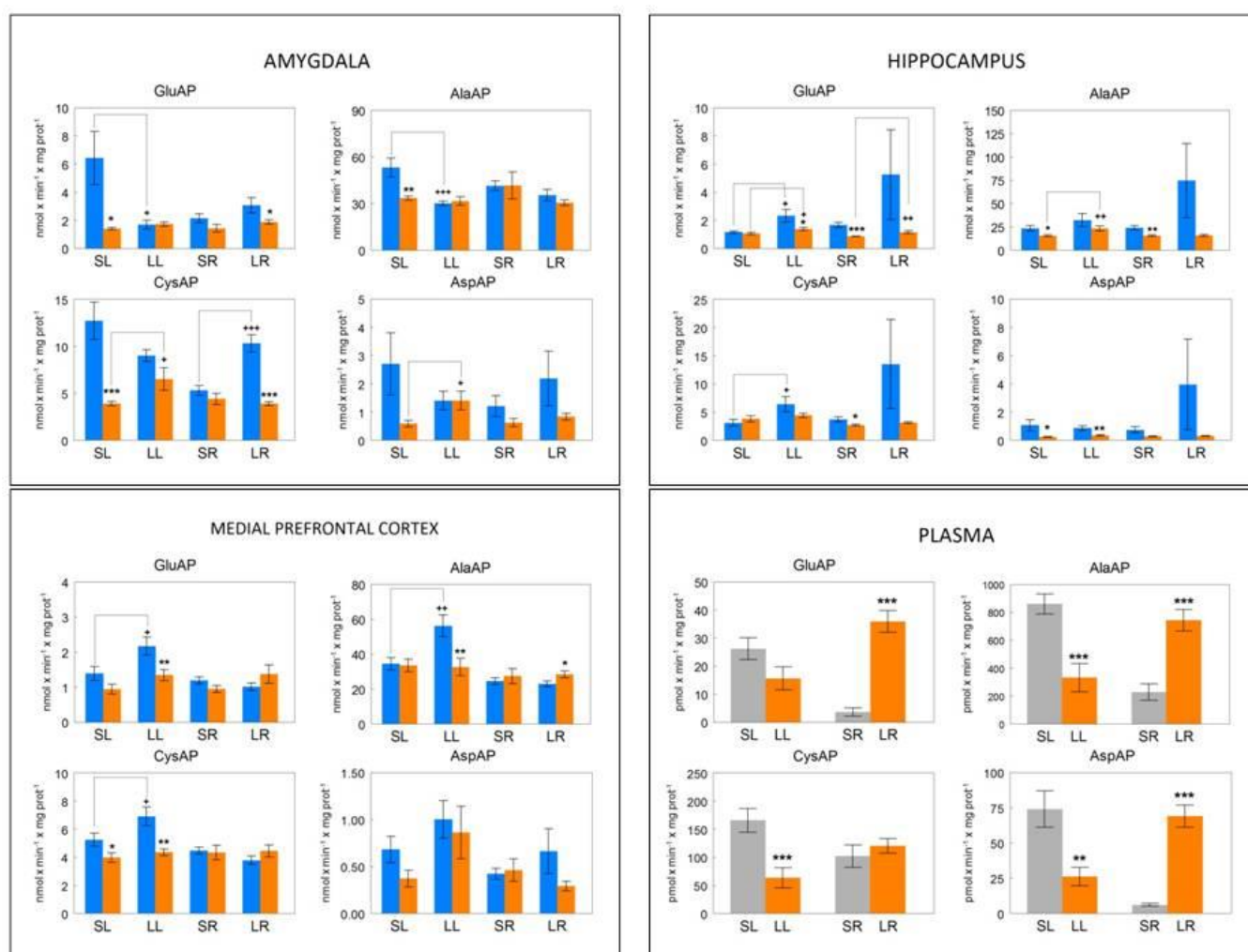


Figure 3. Glutamyl- (GluAP), alanyl- (AlaAP), cystinyl- (CysAP) and aspartyl- (AspAP) aminopeptidase activities, in their membrane-bound forms, expressed as nmol of L-Glu- β -naphthylamide, L-Ala- β -naphthylamide, L-Cys- β -naphthylamide and L-Asp- β -naphthylamide hydrolyzed per min per mg of proteins in the left (blue bars) or right (rose bars) side of the amygdala, hippocampus, medial prefrontal cortex and as pmol of the same substrates hydrolyzed per min, per mg of proteins in plasma of sham left (SL) or sham right (SR) and lesioned left (LL) or lesioned right (LR) adult male spontaneously hypertensive rats. In plasma, gray bars represent sham groups (SL, SR) and rose bars represent lesioned groups (LL, LR). Each group represents the mean \pm SEM values for 10 animals. Asterisks (*) indicate the level of statistical significance between the left and right side of the brain or between sham vs. lesioned animals in plasma. Crosses (+) indicate statistical difference on the same side, between sham vs. lesioned rats. Single ($p < 0.05$), double ($p < 0.01$) or triple symbol ($p < 0.001$). Preliminary partial data of AlaAP

from mPFC [49], CysAP from mPFC [50] and GluAP from plasma [46] and mPFC [45] were previously reported.

3.3. Sham Left Correlations in Spontaneously Hypertensive Rats

The significant correlations in the group of sham left animals are indicated in Table 1. Except for a negative correlation between the left prefrontal cortex and the left hippocampus, the rest of the intra-left hemisphere correlations were abundant and positive with a high level of significance. In contrast, the number of intra-right hemisphere correlations was much lower, although the positive ones also predominated. The inter-hemispheric correlations are divided between five negative and three positive with a moderate level of significance, involving the three brain locations analyzed. The right hemisphere showed only negative correlations with plasma aminopeptidases. The left hemisphere showed two positive and one negative correlation with plasma. No intraplasmatic correlations or correlations between brain aminopeptidases and SBP levels were observed.

Table 1. Intra- and inter-hemispheric correlations between brain aminopeptidase activities; between plasma aminopeptidase activities; between plasma aminopeptidase activities versus brain aminopeptidase activities and between aminopeptidase activities versus systolic blood pressure (SBP) in sham left spontaneously hypertensive rats (SHR).

SHAM LEFT SHR		
Intra-hemispheric		Inter-hemispheric
Sham Left (Left vs. Left)	Sham Left (Right vs. Right)	Sham Left (Left vs. Right)
LPAla vs. LPCys $r = +0.869$ $p = 0.001$	RPAla vs. RPAsp $r = +0.815$ $p = 0.004$	LPAsp vs. RPAla $r = +0.614$ $p = 0.05$
LPCys vs. LPGlu $r = +0.658$ $p = 0.03$	RPAsp vs. RPCys $r = +0.789$ $p = 0.006$	LPAsp vs. RPAsp $r = +0.699$ $p = 0.02$
LPAsp vs. RPAsp $r = +0.699$ $p = 0.02$	RACys vs. RAGlu $r = +0.872$ $p = 0.001$	LACys vs. RACys $r = -0.628$ $p = 0.05$
LAAla vs. LAGlu $r = +0.922$ $p = 0.0001$	RHAsp vs. RHCys $r = -0.838$ $p = 0.002$	LPAla vs. RAGlu $r = -0.649$ $p = 0.04$
LAAsp vs. LAGlu $r = +0.916$ $p = 0.0002$	RPAsp vs. RHAla $r = +0.637$ $p = 0.04$	LHAla vs. RPAla $r = +0.647$ $p = 0.04$
LHAla vs. LHAsp $r = +0.806$ $p = 0.004$		LHAla vs. RACys $r = -0.722$ $p = 0.01$
LHAla vs. LHCys $r = +0.744$ $p = 0.01$		LHAla vs. RAGlu $r = -0.754$ $p = 0.01$
LHAsp vs. LHGlu $r = +0.722$ $p = 0.01$		LHCys vs. RAGlu $r = -0.647$ $p = 0.04$
LPAla vs. LHCys $r = +0.683$ $p = 0.02$		
LPAsp vs. LHAla $r = +0.630$ $p = 0.05$		
LPAsp vs. LHCys $r = +0.755$ $p = 0.01$		
LPAsp vs. LHGlu $r = -0.794$ $p = 0.006$		
LPCys vs. LHCys $r = +0.662$ $p = 0.03$		
LPGlu vs. LHCys $r = +0.660$ $p = 0.03$		
Left Side vs. Plasma	Right Side vs. Plasma	Plasma vs. Plasma
LACys vs. PAla $r = -0.615$ $p = 0.05$	RAAla vs. PAsp $r = -0.649$ $p = 0.04$	NO CORRELATIONS
LHAsp vs. PIGlu $r = +0.718$ $p = 0.01$	RAGlu vs. PICys $r = -0.721$ $p = 0.01$	
LHCys vs. PICys $r = +0.678$ $p = 0.03$	RHGlu vs. PIGlu $r = -0.703$ $p = 0.02$	
Left Side vs. SBP	Right Side vs. SBP	
NO CORRELATIONS	NO CORRELATIONS	

The p -values and Pearson's correlation coefficients (r) are indicated. Negative correlations are in blue italics. Positive correlations are in red. (L) left, (R) right, (H) hippocampus, (P) medial prefrontal cortex, (A) amygdala, (Glu) GluAP, (Ala) AlaAP, (Cys) CysAP and (Asp) AspAP.

3.4. Sham Right Correlations in Spontaneously Hypertensive Rats

The significant correlations in the group of sham right animals are indicated in Table 2. In contrast to the SL group, the sham right animals present a greater number of right intra-hemispheric correlations, with a higher level of significance than the left intra-hemispheric ones, but mainly, in this group, there is a marked increase in inter-hemispheric correlations largely positive and highly significant. Also noteworthy is

the greater number of correlations between the right hemisphere and plasma, compared to the low number of correlations between the left hemisphere and plasma. As was the case in SL animals, there are no correlations between brain and SBP levels and there is only a positive intra-plasma correlation.

Table 2. Intra- and inter-hemispheric correlations between brain aminopeptidase activities; between plasma aminopeptidase activities; between plasma aminopeptidase activities versus brain aminopeptidase activities and between aminopeptidase activities versus systolic blood pressure (SBP) in sham right spontaneously hypertensive rats (SHR).

SHAM RIGHT SHR		
Intra-hemispheric		Inter-hemispheric
Sham Right (Left vs. Left)	Sham Right (Right vs. Right)	Sham Right (Left vs. Right)
LAAla vs. LAGlu $r = +0.757$ $p = 0.01$	RPAla vs. RPAsp $r = +0.620$ $p = 0.05$	LAAla vs. RAAla $r = +0.638$ $p = 0.04$
LAAsp vs. LAGlu $r = +0.826$ $p = 0.003$	RPAla vs. RPCys $r = +0.889$ $p = 0.0006$	LAAsp vs. RAAla $r = +0.917$ $p = 0.0002$
LACys vs. LAGlu $r = +0.674$ $p = 0.03$	RAAla vs. RAAsp $r = +0.936$ $p = <0.0001$	LAAsp vs. RAAsp $r = +0.852$ $p = 0.001$
LHAla vs. LHCys $r = +0.702$ $p = 0.02$	RAAla vs. RACys $r = +0.920$ $p = 0.0002$	LAAsp vs. RACys $r = +0.852$ $p = 0.001$
LHCys vs. LHGlU $r = +0.658$ $p = 0.03$	RAAla vs. RAGlu $r = +0.870$ $p = 0.001$	LAAsp vs. RAGlu $r = +0.902$ $p = 0.0004$
LAAsp vs. LHAsp $r = +0.927$ $p = 0.0001$	RAAsp vs. RACys $r = +0.908$ $p = 0.0003$	LAGlu vs. RAAla $r = +0.888$ $p = 0.0006$
LAGlu vs. LHAsp $r = +0.747$ $p = 0.01$	RAAsp vs. RAGlu $r = +0.842$ $p = 0.002$	LAGlu vs. RAAsp $r = +0.830$ $p = 0.002$
<i>LPAsp vs. LHGlU $r = -0.700$ $p = 0.02$</i>	RACys vs. RAGlu $r = +0.931$ $p = <0.0001$	LAGlu vs. RACys $r = +0.762$ $p = 0.01$
<i>LPGlU vs. LACys $r = -0.780$ $p = 0.007$</i>	RPAsp vs. RHCys $r = +0.836$ $p = 0.002$	LAGlu vs. RAGlu $r = +0.780$ $p = 0.007$
	RPGlu vs. RAAla $r = +0.718$ $p = 0.01$	LHAsp vs. RAAla $r = +0.892$ $p = 0.005$
	RPGlu vs. RAAsp $r = +0.736$ $p = 0.01$	LHAsp vs. RAAsp $r = +0.850$ $p = 0.001$
	RPGlu vs. RACys $r = +0.681$ $p = 0.02$	LHAsp vs. RACys $r = +0.940$ $p = <0.0001$
		LHAsp vs. RAGlu $r = +0.907$ $p = 0.0003$
Left Side vs. Plasma	Right Side vs. Plasma	LHCys vs. RPAla $r = +0.648$ $p = 0.04$
LPAla vs. PAla $r = +0.706$ $p = 0.02$	RPGlu vs. PAla $r = +0.763$ $p = 0.01$	LHGlU vs. RPAla $r = +0.674$ $p = 0.03$
LACys vs. PIGlu $r = +0.703$ $p = 0.02$	RAAla vs. PAla $r = +0.660$ $p = 0.03$	LPGlU vs. RHGlU $r = +0.679$ $p = 0.03$
	RAAsp vs. PAla $r = +0.811$ $p = 0.004$	LAAla vs. RPGlu $r = +0.775$ $p = 0.008$
	RACys vs. PAla $r = +0.678$ $p = 0.03$	<i>LPAla vs. RHAla $r = -0.784$ $p = 0.007$</i>
	RPAsp vs. PAsp $r = +0.698$ $p = 0.02$	<i>LPAsp vs. RHAla $r = -0.719$ $p = 0.01$</i>
	RAAsp vs. PICys $r = +0.740$ $p = 0.01$	
	RACys vs. PICys $r = +0.693$ $p = 0.02$	Plasma vs. Plasma
	RAGlu vs. PICys $r = +0.700$ $p = 0.02$	PIAla vs. PICys $r = +0.842$ $p = 0.002$
Left Side vs. SBP	Right Side vs. SBP	
NO CORRELATIONS	NO CORRELATIONS	

The p -values and Pearson’s correlation coefficients (r) are indicated. Negative correlations are in blue italics. Positive correlations are in red. (L) left, (R) right, (H) hippocampus, (P) medial pre-frontal cortex, (A) amygdala, (Glu) GluAP, (Ala) AlaAP, (Cys) CysAP and (Asp) AspAP.

3.5. Lesioned Left Correlations in Spontaneously Hypertensive Rats

The significant correlations in the group of animals with left DA depletion (LL) are indicated in Table 3. In this group, a high number of positive intra-hemispheric correlations in the left hippocampus are observed, with a high level of significance. Three negative correlations between the left amygdala and the left hippocampus are also observed. The right intra-hemispheric correlations are also mainly positive and a negative correlation is observed, also between the amygdala and the hippocampus. In the inter-hemispheric correlations, similar to what occurred in the SL animals, there is a remarkable number of negative correlations, comparable to the positive ones and involving the three areas analyzed. The number of correlations, all of them positive, is similar between the left hemisphere and plasma than between the right hemisphere and plasma, but in the latter, the level of significance is much higher. Unlike the two previous groups, there is a high number of highly significant intra-plasma correlations. Finally, it is noteworthy that this group of LL is the only one in which correlations between the brain and SBP levels appear, three being negative with the left hemisphere and one positive with the right hemisphere.

Table 3. Intra- and inter-hemispheric correlations between brain aminopeptidase activities; between plasma aminopeptidase activities; between plasma aminopeptidase activities versus brain aminopeptidase activities and between aminopeptidase activities versus systolic blood pressure (SBP) in lesioned left spontaneously hypertensive rats (SHR).

LESIONED LEFT SHR		
Intra-hemispheric		Inter-hemispheric
Lesioned Left (Left vs. Left)	Lesioned Left (Right vs. Right)	Lesioned Left (Left vs. Right)
LPAla vs. LPCys $r = +0.674$ $p = 0.03$	LPAla vs. LPCys $r = +0.674$ $p = 0.03$	LPAsp vs. RPLGlu $r = +0.672$ $p = 0.03$
LPCys vs. LPLGlu $r = +0.689$ $p = 0.02$	RPAsp vs. RPCys $r = +0.639$ $p = 0.04$	LPLGlu vs. RPAsp $r = -0.629$ $p = 0.05$
LHAla vs. LHAsp $r = +0.923$ $p = 0.0001$	RPAsp vs. RPLGlu $r = +0.616$ $p = 0.05$	LPLGlu vs. RPCys $r = -0.636$ $p = 0.04$
LHAla vs. LHCys $r = +0.975$ $p < 0.0001$	RPCys vs. RPLGlu $r = +0.808$ $p = 0.004$	LAAla vs. RAAla $r = +0.662$ $p = 0.03$
LHAla vs. LHGlu $r = +0.930$ $p = 0.0001$	RHAla vs. RHCys $r = +0.906$ $p = 0.0003$	LAAsp vs. RAGlu $r = -0.703$ $p = 0.02$
LHAsp vs. LHCys $r = +0.856$ $p = 0.001$	RPAla vs. RHAla $r = +0.650$ $p = 0.04$	LAGlu vs. RAAsp $r = +0.620$ $p = 0.05$
LHAsp vs. LHGlu $r = +0.946$ $p < 0.0001$	RPAsp vs. RACys $r = +0.782$ $p = 0.007$	LAGlu vs. RAGlu $r = -0.658$ $p = 0.03$
LHCys vs. LHGlu $r = +0.901$ $p = 0.0004$	RAGlu vs. RHGlu $r = -0.739$ $p = 0.01$	LPAsp vs. RAAsp $r = -0.627$ $p = 0.05$
LACys vs. LHAla $r = -0.673$ $p = 0.03$		LPCys vs. RACys $r = -0.709$ $p = 0.02$
LACys vs. LHAsp $r = -0.646$ $p = 0.04$		LPLGlu vs. RAAla $r = -0.679$ $p = 0.03$
LACys vs. LHGlu $r = -0.749$ $p = 0.01$		LPLGlu vs. RACys $r = -0.803$ $p = 0.005$
		LAAla vs. RPLGlu $r = +0.631$ $p = 0.05$
Left Side vs. Plasma	Right Side vs. Plasma	Plasma vs. Plasma
LPAsp vs. PAla $r = +0.751$ $p = 0.01$	RPAla vs. PAla $r = +0.617$ $p = 0.05$	PAla vs. PAsp $r = +0.926$ $p = 0.0001$
LPAsp vs. PAsp $r = +0.660$ $p = 0.03$	RPCys vs. PAla $r = +0.849$ $p = 0.001$	PAla vs. PCys $r = +0.892$ $p = 0.0005$
LPAsp vs. PCys $r = +0.700$ $p = 0.02$	RPCys vs. PAsp $r = +0.898$ $p = 0.0004$	PAla vs. PIGlu $r = +0.927$ $p = 0.0001$
LPAsp vs. PIGlu $r = +0.618$ $p = 0.05$	RPCys vs. PCys $r = +0.741$ $p = 0.01$	PAla vs. PIGlu $r = +0.927$ $p = 0.0001$
LAAla vs. PAsp $r = +0.670$ $p = 0.03$	RPCys vs. PIGlu $r = +0.907$ $p = 0.0003$	PAsp vs. PCys $r = +0.905$ $p = 0.0003$
LAAla vs. PCys $r = +0.670$ $p = 0.03$	RPLGlu vs. PAla $r = +0.919$ $p = 0.0002$	PAsp vs. PIGlu $r = +0.995$ $p < 0.0001$
LAAla vs. PIGlu $r = +0.672$ $p = 0.03$	RPLGlu vs. PAsp $r = +0.933$ $p < 0.0001$	PAsp vs. PIGlu $r = +0.906$ $p = 0.0003$
	RPLGlu vs. PCys $r = +0.859$ $p = 0.001$	
	RPLGlu vs. PIGlu $r = +0.936$ $p < 0.0001$	
Left Side vs. SBP	Right Side vs. SBP	
LAAsp vs. SBP $r = -0.685$ $p = 0.02$	RAGlu vs. SBP $r = +0.754$ $p = 0.01$	
LACys vs. SBP $r = -0.806$ $p = 0.004$		
LAGlu vs. SBP $r = -0.650$ $p = 0.04$		

The p -values and Pearson's correlation coefficients (r) are indicated. Negative correlations are in blue italics. Positive correlations are in red. (L) left, (R) right, (H) hippocampus, (P) medial prefrontal cortex, (A) amygdala, (Glu) GluAP, (Ala) AlaAP, (Cys) CysAP and (Asp) AspAP.

3.6. Lesioned Right Correlations in Spontaneously Hypertensive Rats

The significant correlations in the group of animals with right DA depletion (LR) are indicated in Table 4. A clear predominance is observed in the intra-left hemisphere correlations, most of them being positive with a high level of statistical significance. There is a smaller number of intra-right hemisphere correlations in which two negative correlations are also observed. Compared to the group of left-lesioned animals, the right ones present less inter-hemispheric correlations, with a similar number of negative correlations. The number of intra-plasma correlations is similar to that of the LL animals, all of them positive but with a lower level of significance. While the left prefrontal cortex exhibits one positive correlation with plasma, the right hippocampus exhibits three negative correlations with plasma. In this group, no correlations with SBP are observed.

Table 4. Intra- and inter-hemispheric correlations between brain aminopeptidase activities; between plasma aminopeptidase activities; between plasma aminopeptidase activities versus brain aminopeptidase activities and between aminopeptidase activities versus systolic blood pressure (SBP) in lesioned right spontaneously hypertensive rats (SHR).

LESIONED RIGHT SHR		
Intra-hemispheric		Inter-hemispheric
Lesioned Right (Left vs. Left)	Lesioned Right (Right vs. Right)	Lesioned Right (Left vs. Right)
LPAla vs. LPAsp $r = +0.769$ $p = 0.009$	RHAsp vs. RHGlu $r = +0.744$ $p = 0.01$	LPCys vs. RPLGlu $r = +0.627$ $p = 0.05$
LPAla vs. LPCys $r = +0.624$ $p = 0.05$	RHCys vs. RHGlu $r = +0.679$ $p = 0.03$	LPLGlu vs. RAGlu $r = +0.635$ $p = 0.04$

LPAsp vs. LPCys $r = +0.773$ $p = 0.008$
 LAAla vs. LAAsp $r = +0.759$ $p = 0.01$
 LHAla vs. LHAsp $r = +0.970$ $p = <0.0001$
 LHAla vs. LHCys $r = +0.977$ $p = <0.0001$
 LHAla vs. LHGlu $r = +0.989$ $p = <0.0001$
 LHAsp vs. LHCys $r = +0.997$ $p = <0.0001$
 LHAsp vs. LHGlu $r = +0.992$ $p = <0.0001$
 LHCys vs. LHGlu $r = +0.993$ $p = <0.0001$
 LAGlu vs. LHAla $r = +0.619$ $p = 0.05$
 LAGlu vs. LHAsp $r = +0.646$ $p = 0.04$
 LAGlu vs. LHCys $r = +0.630$ $p = 0.05$

Left Side vs. Plasma

LPGlu vs. PIGlu $r = +0.677$ $p = 0.03$

Left Side vs. SBP

NO CORRELATIONS

RPAla vs. RAAsp $r = +0.724$ $p = 0.01$
 RPCys vs. RACys $r = -0.683$ $p = 0.02$
 RPGlu vs. RHAsp $r = -0.790$ $p = 0.006$
 RAAsp vs. RHCys $r = +0.698$ $p = 0.02$
 RAGlu vs. RHAsp $r = +0.653$ $p = 0.02$

Right Side vs. Plasma

RHAsp vs. PAla $r = -0.638$ $p = 0.04$

RHAsp vs. PICys $r = -0.677$ $p = 0.03$

RHCys vs. PICys $r = -0.663$ $p = 0.03$

Right Side vs. SBP

NO CORRELATIONS

LHAla vs. RAGlu $r = -0.624$ $p = 0.05$
 LHAsp vs. RAAla $r = -0.636$ $p = 0.04$
 LHGlu vs. RAAla $r = -0.626$ $p = 0.05$
 LHGlu vs. RAGlu $r = -0.615$ $p = 0.05$

Plasma vs. Plasma

PAla vs. PICys $r = +0.938$ $p = <0.0001$

PAla vs. PIGlu $r = +0.734$ $p = 0.01$

PAsp vs. PICys $r = +0.744$ $p = 0.01$

PAsp vs. PIGlu $r = +0.743$ $p = 0.01$

PICys vs. PIGlu $r = +0.780$ $p = 0.007$

The p -values and Pearson's correlation coefficients (r) are indicated. Negative correlations are in blue italics. Positive correlations are in red. (L) left, (R) right, (H) hippocampus, (P) medial prefrontal cortex, (A) amygdala, (Glu) GluAP, (Ala) AlaAP, (Cys) CysAP and (Asp) AspAP.

3.7. Comparisons between Groups of Spontaneously Hypertensive Rats

In Figure 4 we observe that the comparison of the total number of significant correlations between SL vs. LL (A) is opposite to the comparison between SR vs. LR (B). In the comparison between LL vs. LR (C), a greater number of Intra-Left than Intra-Right correlations is observed in both LL and LR, and a greater number of inter-hemispheric correlations in LL than in LR (C). In the correlations between plasma and brain in the left groups SL and LL (D) there are more Plasma-Left and Plasma Right correlations in LL than in SL (D). This pattern is opposite to that observed in the right group's SR and LR (E). When comparing LL vs. LR (F) there is a higher number of Intra-Left and Intra-Right correlations in LL than in LR (F). With systolic blood pressure (SBP), significant correlations are only observed in the LL group (G,H,I).

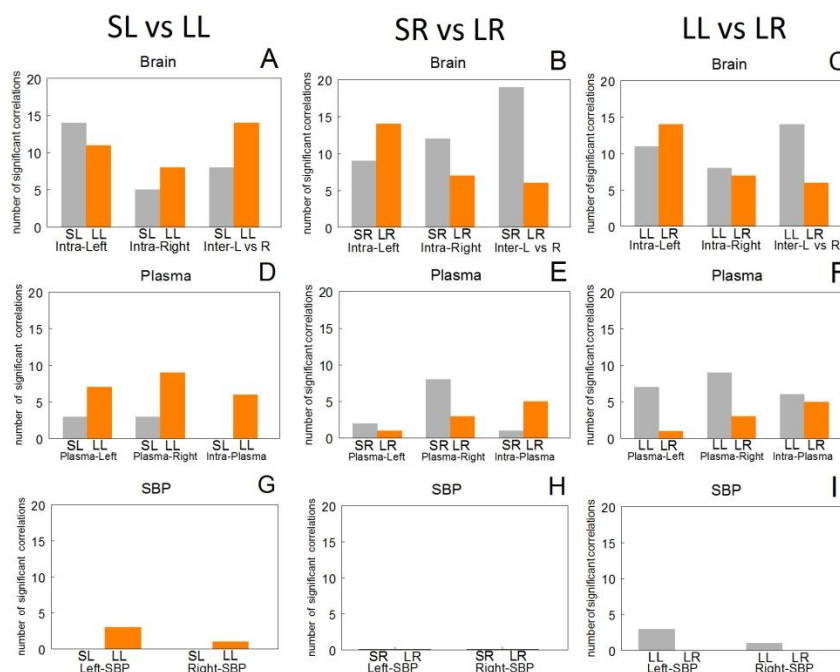


Figure 4. Total number of intra- (Intra-Left or Intra-Right) and inter-hemispheric (Inter-Left vs. Right) significant correlations between brain aminopeptidase activities (Brain) (A–C); between plasma aminopeptidase activities versus left (Plasma-Left) or right (Plasma-Right) brain aminopeptidase activities and Intra-Plasma aminopeptidase activities between themselves (Plasma) (D–F); between left (Left-SBP) or right (Right-SBP) aminopeptidase activities versus systolic blood pressure (SBP) in Sham Left (SL), Sham Right (SR), Lesioned Left (LL) or Lesioned Right (LR) spontaneously hypertensive rats (G–I).

Except for the intra-location correlations (intra-P, intra-H, intra-A or intra-PI), Figure 5 represents all the significant correlations that were obtained in all the groups analyzed corresponding to Tables 1 to 4. The pattern of correlations differs between all the groups studied. In the sham left group, we can highlight the positive correlations between left mPFC and left hippocampus (A) and negative correlations between left hippocampus and right amygdala (B). In the sham right group, we can highlight the global predominance of positive correlations, particularly between the left amygdala and left hippocampus and between right mPFC and right amygdala (A), but especially between the left and right amygdala and between left hippocampus and right amygdala (B). In animals with a left lesion, we highlight the negative correlations between the left amygdala and left hippocampus, the positive correlations between plasma and left amygdala or mPFC, and positive correlations between plasma and right mPFC (A). In this group, we also highlight the positive correlation between the left amygdala and right hippocampus, both positive and negative between the left amygdala and right amygdala and negative between left mPFC and right amygdala (B). However, especially noteworthy is that in hypertensive animals, this is the only group that correlates the brain with SBP, specifically the negative correlation with the left amygdala and positive with the right (B). In the group of animals with a right lesion, we can highlight the positive correlations between the left amygdala and left hippocampus, positive between left mPFC and plasma, negative between right hippocampus and plasma (A), and negative between left hippocampus and right amygdala (B).

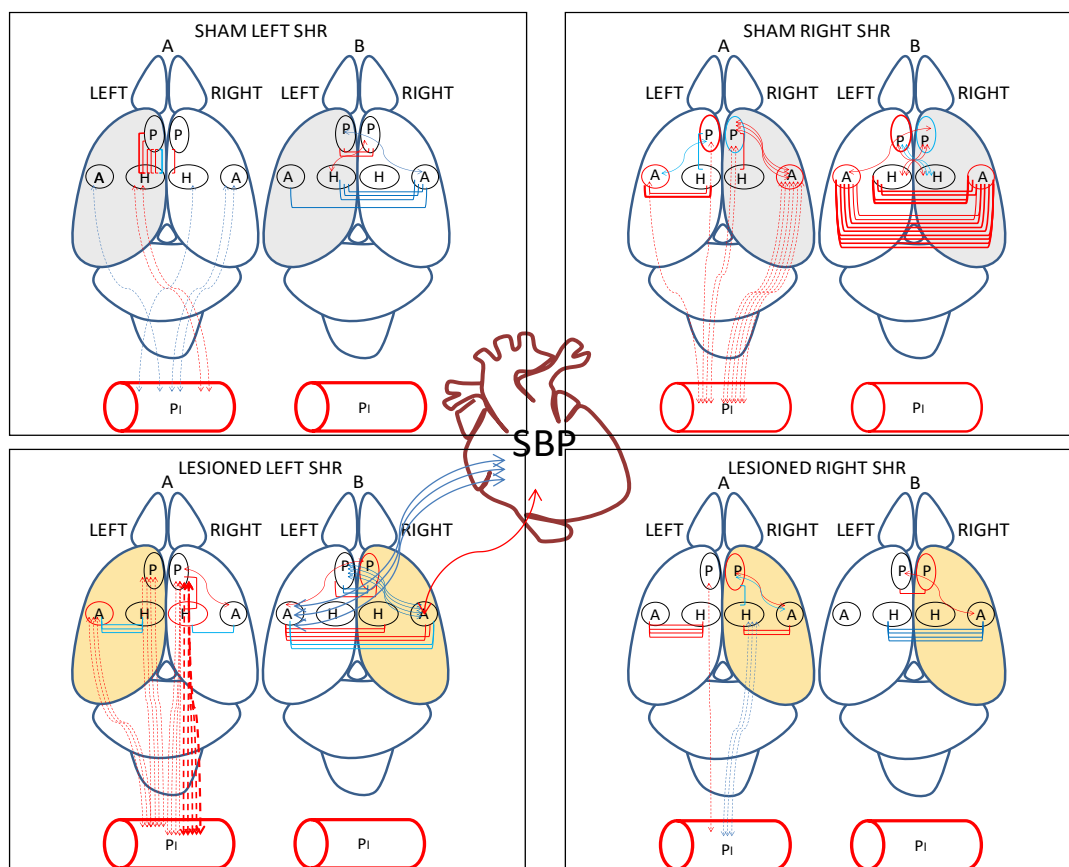


Figure 5. Schematic representation of the observed significant correlations: between brain aminopeptidase activities; between plasma aminopeptidase activities versus brain aminopeptidase activities; between brain aminopeptidase activities versus systolic blood pressure (SBP) in the four groups studied of spontaneously hypertensive rats. Positive correlations in red and negative correlations in blue. Continue lines: significant correlations between brain locations and between brain versus SBP. Dotted lines: significant correlations between brain versus plasma. The thickness of lines is proportional to the degree of significance. Above rat brain: A, intra-hemispheric and brain vs. plasma correlations, B, inter-hemispheric correlations. The hemisphere was injected with saline for sham animals in grey. The hemisphere was injected with 6-OHDA for lesioned animals in light orange. P, medial prefrontal cortex; A, amygdala; H, hippocampus; PI, plasma.

4. Discussion

Regarding the role in the emotional processing of brain asymmetry and neurovisceral asymmetry, considering the role of the interaction of DA with peptidergic or not peptidergic neurotransmitters, and with respect to the role of blood pressure levels, we can highlight some previous contributions: It has been described that there is an increase in anxiety and depression in hypertensive humans and SHR [40,41]. Emotional processing differs between normotensive and hypertensive patients [10]. The mPFC is important in such emotional processing [51]. Cognitive alterations have been described in hypertensive conditions in both animals and humans [52,53]. It is also necessary to take into account the existence of different functional consequences after left or right hemispheric damage, such as lower sensorimotor function after left damage and better cognitive function after left than after right damage [54]. On the other hand, cognitive alterations have been described in Parkinson's disease that involves the hippocampus [55] and it has also been described that the amygdala participates in cardiovascular regulation [56]. Emotional alterations have also been described in hypertensive patients, alterations in which disruptions in neuro-visceral communication may be involved [42]. The consequences of the coexistence of Parkinson's and hypertension have also been described, raising the possibility of the possible benefit of antihypertensive treatments in Parkin-

son's patients [43]. Following 6-OHDA administration, corticotropin-releasing hormone and neurotensin mRNA expression is decreased in the amygdala and the bed nucleus of stria terminalis [57]. Dopamine depletion causes asymmetric responses depending on gender and type of behavior [58]. Postmortem human brains demonstrate an asymmetry in opiate expression in the anterior cingulate cortex, an area involved in emotional and pain processing [59]. After DA depletion with 6-OHDA, CCK levels decrease more in SHR than in WKY [60]. A lower amount of DA in Am, Hc and mPFC of SHR than WKY has also been described [61]. As previously indicated, DA coexists with CCK and NT [37]. In addition, more DA receptors have also been described in SHR than in WKY [62] and brain angiotensin II, by means of its AT1 receptors, regulates dopaminergic neurotransmission [63], clearly involving the RAAS in neurodegenerative diseases [64].

After analyzing the present results, it is clear that the behavior of the SBP clearly differs between normotensive WKY [39] and SHR animals. While in WKY, the SBP does not vary after the left or right lesion, in the SHR the pressure does not vary after the left lesion but increases after the right one. In addition, the SR and LR groups have lower SBP than the SL and LL groups (Figure 2).

Regarding the role of angiotensin peptides: Ang II is the main hypertensive peptide; Ang III has similar or less hypertensive properties than Ang II and is the main brain Ang peptide that stimulates the secretion of vasopressin; Ang IV, without hypertensive functions, is involved in the regulation of brain local blood flows and has an anxiolytic effect acting on the amygdala; Ang 2–10 may counterbalance the hypertensive effect of Ang II and Ang III in the brain [35]. According to the results obtained in plasma (Figure 3) and the role of aminopeptidases in RAAS (Figure 1), in LL there is a reduction of GluAP and AlaAP which could suggest a lower metabolism of Ang II and Ang III increasing consequently their hypertensive action. In LL there is a reduction of CysAP, increasing, therefore, the hypertensive action of vasopressin and also diminishing AspAP activity which could reduce the formation of Ang 2–10 moderating its hypertensive counterbalancing. In contrast, in LR there is a marked increase in GluAP and AlaAP which suggests the higher formation of Ang III and Ang IV with which we can think of a lower hypertensive effect than in the LL group. In LR there is no change in vasopressinase (CysAP) activity and an increase in AspAP raising the formation of Ang 2–10 with a possible counterbalance of Ang II and Ang III. Taken together, our results in plasma would be compatible with higher SBP in LL and lower SBP in LR.

In agreement with Zhai and Feng [60], the present results show that just the surgical injury produced by the introduction of the cannula and the subsequent injection of saline differs depending on whether we carried out the surgery in the left or right hemisphere. In this sense, in the present study with SHR, it is remarkable the large number of inter-hemispheric correlations involving the amygdala in the sham right animals compared to the sham left (Figure 5). This pattern clearly differs when we selectively deplete DA in the left or right hemisphere, showing that this new factor modifies the entire left or right pattern of correlations that we observed in the left or right sham animals.

Especially interesting is the behavior of the correlations between left or right hemispheric locations, SBP and plasma (Figures 6 and 7), compared with the results previously obtained with normotensive WKY animals [39]. The SBP mainly correlates with the LL group in WKY and exclusively with the LL group in SHR. In WKY LL, the right amygdala correlates positively with SBP but the left hippocampus correlates negatively. In SHR LL, the pattern is similar: the right amygdala correlates positively with SBP but the left amygdala correlates negatively. These results highlight the importance of left DA depletion as well as the relevant role of the amygdala in cardiovascular regulation [56] in which there could be a balance or imbalance between the regulatory role of the left and right amygdala in the control of blood pressure. This suggestion could be supported if we analyze individually the consequences of each significant correlation involving the amygdala in LL SHR (Figure 6). In these LL animals, there is a positive correlation between GluAP of the right amygdala and the levels of SBP: the higher GluAP, the higher

SBP. GluAP metabolizes Ang II to Ang III (Figure 1) which may imply an increase in SBP. There is a negative correlation between AspAP of the left amygdala and SBP, the higher AspAP, the lower SBP. AspAP metabolizes Ang I to Ang 2–10 which may counterbalance the effects of Ang II and Ang III contributing to a reduction of SBP. There is a negative correlation between CysAP of the left amygdala and SBP, the higher CysAP, the lower SBP, this is in agreement with a reduction of vasopressin due to the high levels of CysAP also contributing to lower SBP. There is also a slight significant correlation between GluAP of the left amygdala and SBP, the higher GluAP, the lower SBP. In this case, the increase in Ang III in the left amygdala, in contraposition with the positive correlation of GluAP of the right amygdala with SBP, would imply lower SBP. Taking together, in LL SHR, the right amygdala would be related to an increase in SBP and the left would be a decrease.

In WKY [39], the amygdala also has a relevant role correlating significantly with SBP. In SL, CysAP of the left amygdala presents a negative correlation with SBP, the higher CysAP, the lower SBP. In SR, GluAP of the right amygdala correlates positively with SBP, the higher GluAP, the higher SBP. In LL, GluAP of the right amygdala correlates negatively with SBP, the higher GluAP, the lower SBP and also in this group, CysAP of the right amygdala has a highly significant positive correlation with SBP, the higher CysAP, the higher SBP. In conclusion, these results support the role of the amygdala in cardiovascular control and extend it to the concept of bilateral asymmetrical control.

LESIONED LEFT SHR CORRELATIONS

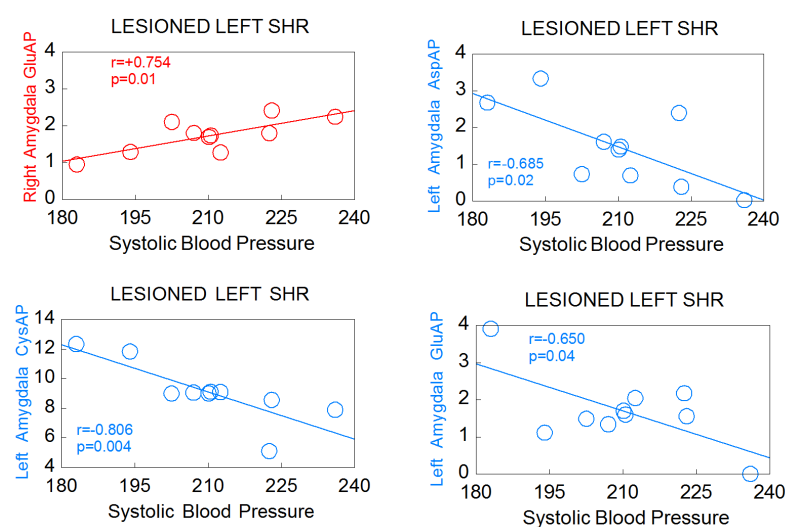


Figure 6. Significant correlations between aminopeptidase activities expressed as nmol of the corresponding L-amino acid- β -naphthylamide, hydrolyzed per min per mg of proteins in the left (blue) or right (red) amygdala and systolic blood pressure in lesioned left spontaneously hypertensive rats (SHR) (see Table 3). Negative correlations are in blue. Positive correlations are in red. Pearson's correlation coefficients (r) and p values are indicated in each figure.

With plasma, the pattern is reversed in LR SHR animals (Figure 7): While the left mPFC correlates positively with plasma GluAP, the right hippocampus correlates negatively with plasma aminopeptidases. In WKY, the negative correlation between the right hemisphere and plasma in the LR is maintained, but there is no correlation with the left hemisphere in this group. In the WKY LL, there is no specific pattern. We can therefore affirm that the response to left or right DA depletion is different in normotensive WKY rats than in SHR (Figure 8).

In general terms, there is a greater number of correlations in SHR than in WKY. In comparison with WKY, in SL SHR the left intra-hemispheric correlations increase, and

the inter-hemispheric ones are inverted to negative, however, in SR SHR, inter-hemispheric and right intra-hemispheric increase. As occurred in the SL, in the SHR LL, compared to the WKY LL, the left intra-hemispheric correlations increase and the inter-hemispheric ones are inverted, but with greater intensity. In the LR, the left intra-hemispheric correlations increase in SHR, and the inter-hemispheric correlations are inverted with respect to the WKY. If we look at Figure 8, it seems clear that left DA depletion (LL) is the one that induces a greater number of correlations, particularly in SHR. It is also necessary to highlight that in WKY, all groups correlate between brain and SBP, especially after left DA depletion. In SHR, only after left DA depletion do brain-SBP correlations appear.

LESIONED RIGHT SHR CORRELATIONS

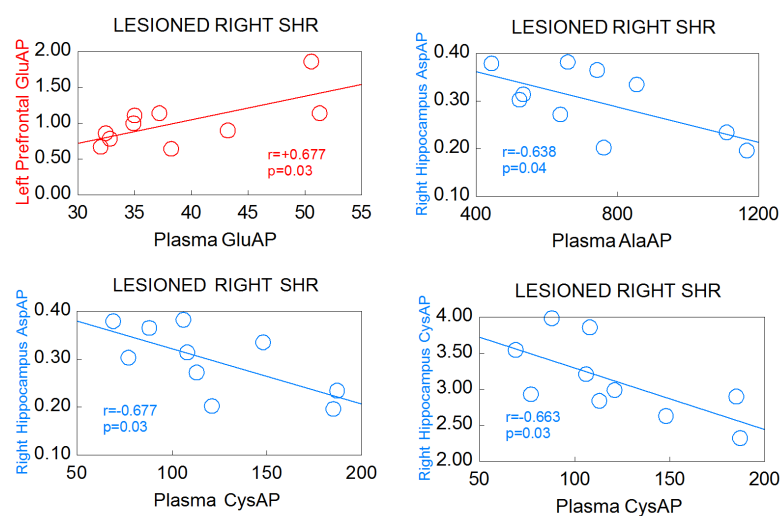


Figure 7. Significant correlations between aminopeptidase activities expressed as nmol of the corresponding L-amino acid- β -naphthylamide, hydrolyzed per min per mg of proteins in the left prefrontal cortex (red) or right hippocampus (blue), and plasma aminopeptidase activities, expressed as pmol of the corresponding L-amino acid- β -naphthylamide, hydrolyzed per min per mg of proteins, in lesioned right spontaneously hypertensive rats (SHR) (see Table 4). Negative correlations are in blue. Positive correlations are in red. Pearson's correlation coefficients (r) and p values are indicated in each figure.

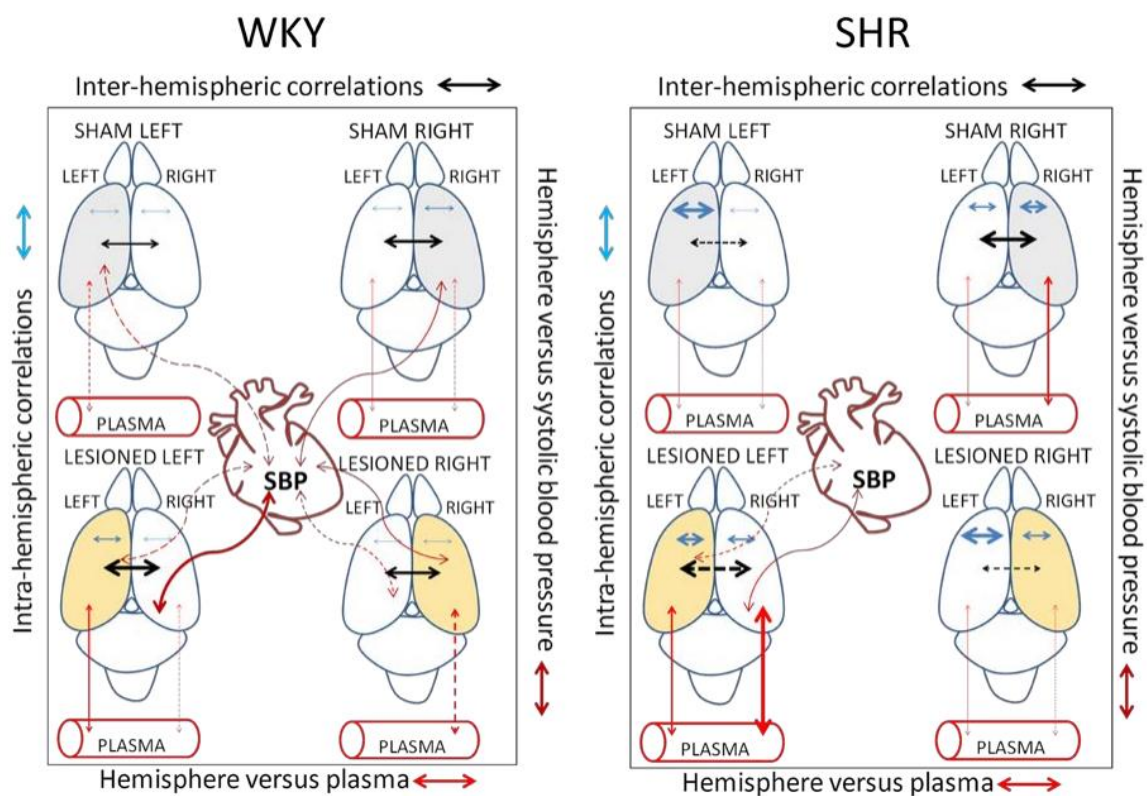


Figure 8. Schematic comparison of the main correlations in WKY and SHR. Intra-hemispheric correlations: blue arrows. Inter-hemispheric correlations: black arrows. Left or right hemisphere versus systolic blood pressure correlations: dark orange. Left or right hemisphere versus plasma correlations: red arrows. Continuous lines: positive correlations. Dotted lines: negative correlations. The thickness of lines is proportional to the number of correlations and the degree of significance.

In conclusion, the present results with SHR and the previous ones with WKY demonstrate that selective unilateral depletion of a specific neurotransmitter changes radically the bilateral pattern of response, in these cases involving proteinase activities, suggesting functional changes depending on the bilateral consequences. These results reveal a high complexity in the bilateral response to physiological and pathological changes in the organism, without being able to establish yet a specific pattern of response. Perhaps, the most interesting results suggest an important role for the asymmetrical functioning of the amygdala in cardiovascular control, particularly when the left hemisphere was depleted of dopamine and an asymmetrical behavior in the interaction between the medial prefrontal cortex, hippocampus and amygdala with plasma, depending on the left or right depletion of dopamine which supports an asymmetric neurovisceral integration of the organism. In general terms, our results lead us to speculate on the concept of cerebral asymmetry, or rather neuro-visceral asymmetry, as an intrinsic property of the organism whose intensity or modification of the predominant side depends on multiple environmental, physiological and/or pathological factors [26,27]. This gives rise to a variable bilateral dynamic process in which many questions remain to be answered, such as what is the functional advantage of asymmetry over symmetry?; what emotional consequences derive from the bilateral cerebral and neuro-visceral biochemical modifications observed?; or how to connect the consequences of these modifications with the blood pressure control? However, it is necessary to indicate that the present study lacks parallel investigations of emotional behavior that could be interesting to understand the functional significance of brain asymmetry involving aminopeptidases. In addition, unilateral lesions in the analyzed areas could provide interesting suggestions about the role of these areas and aminopeptidases in blood pressure control. It would

also be interesting to look into the analysis of the bilateral behavior of interactions between the limbic zones after treatments with RAAS inhibitors.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of interest: The authors declare no conflict of interest.

References

1. Bennett, M.R. The prefrontal–limbic network in depression: Modulation by hypothalamus, basal ganglia and midbrain. *Prog. Neurobiol.* **2011**, *93*, 468–487. <https://doi.org/10.1016/j.pneurobio.2011.01.006>.
2. Cools, R.; Arnsten, A.F.T. Neuromodulation of prefrontal cortex cognitive function in primates: The powerful roles of monoamines and acetylcholine. *Neuropsychopharmacology* **2022**, *47*, 309–328. <https://doi.org/10.1038/s41386-021-01100-8>.
3. Šimić, G.; Tkalčić, M.; Vukić, V.; Mulc, D.; Španić, E.; Šagud, M.; Olucha-Bordonau, F.E.; Vukšić, M.R.; Hof, P. Understanding Emotions: Origins and Roles of the Amygdala. *Biomolecules* **2021**, *11*, 823. <https://doi.org/10.3390/biom11060823>.
4. Anderson, M.C.; Floresco, S.B. Prefrontal-hippocampal interactions supporting the extinction of emotional memories: The retrieval stopping model. *Neuropsychopharmacology* **2022**, *47*, 180–195. <https://doi.org/10.1038/s41386-021-01131-1>.
5. Battaglia, S.; Harrison, B.J.; Fullana, M.A. Does the human ventromedial prefrontal cortex support fear learning, fear extinction or both? A commentary on subregional contributions. *Mol. Psychiatry* **2022**, *27*, 784–786. <https://doi.org/10.1038/s41380-021-01326-4>.
6. Güntürkün, O.; Ströckens, F.; Ocklenburg, S. Brain Lateralization: A Comparative Perspective. *Physiol. Rev.* **2020**, *100*, 1019–1063. <https://doi.org/10.1152/physrev.00006.2019>.
7. Lubben, N.; Ensink, E.; Coetzee, G.A.; Labrie, V. The enigma and implications of brain hemispheric asymmetry in neurodegenerative diseases. *Brain Commun.* **2021**, *3*, fcab211. <https://doi.org/10.1093/braincomms/fcab211>.
8. Yuvaraj, R.; Murugappan, M. Hemispheric asymmetry non-linear analysis of EEG during emotional responses from idiopathic Parkinson’s disease patients. *Cogn. Neurodynamics* **2016**, *10*, 225–234. <https://doi.org/10.1007/s11571-016-9375-3>.
9. Aberg, K.C.; Doell, K.C.; Schwartz, S. Hemispheric Asymmetries in Striatal Reward Responses Relate to Approach-Avoidance Learning and Encoding of Positive-Negative Prediction Errors in Dopaminergic Midbrain Regions. *J. Neurosci.* **2015**, *35*, 14491–14500. <https://doi.org/10.1523/jneurosci.1859-15.2015>.
10. Consoli, S.M.; Lemogne, C.; Roch, B.; Laurent, S.; Plouin, P.-F.; Lane, R.D. Differences in Emotion Processing in Patients with Essential and Secondary Hypertension. *Am. J. Hypertens.* **2010**, *23*, 515–521. <https://doi.org/10.1038/ajh.2010.9>.
11. Park, G.; Thayer, J.F. From the heart to the mind: Cardiac vagal tone modulates top-down and bottom-up visual perception and attention to emotional stimuli. *Front. Psychol.* **2014**, *5*, 278. <https://doi.org/10.3389/fpsyg.2014.00278>.
12. Thayer, J.F.; Lane, R.D. A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* **2000**, *61*, 201–216. [https://doi.org/10.1016/s0165-0327\(00\)00338-4](https://doi.org/10.1016/s0165-0327(00)00338-4).
13. Thayer, J.F.; Mather, M.; Koenig, J. Stress and aging: A neurovisceral integration perspective. *Psychophysiology* **2021**, *58*, e13804. <https://doi.org/10.1111/psyp.13804>.
14. O’Connor, A.T.; Haspula, D.; Alanazi, A.Z.; Clark, M.A. Roles of Angiotensin III in the brain and periphery. *Peptides* **2022**, *153*, 170802. <https://doi.org/10.1016/j.peptides.2022.170802>.
15. Ferrario, C.M.; Groban, L.; Wang, H.; Sun, X.; VonCannon, J.L.; Wright, K.N.; Ahmad, S. The renin–angiotensin system bio-molecular cascade: A 2022 update of newer insights and concepts. *Kidney Int. Suppl.* **2022**, *12*, 36–47. <https://doi.org/10.1016/j.kisu.2021.11.002>.

16. Gouveia, F.; Camins, A.; Ettcheto, M.; Bicker, J.; Falcão, A.; Cruz, M.T.; Fortuna, A. Targeting brain Renin-Angiotensin System for the prevention and treatment of Alzheimer's disease: Past, present and future. *Ageing Res. Rev.* **2022**, *77*, 101612.
17. Wright, J.W.; Harding, J.W. Contributions by the Brain Renin-Angiotensin System to Memory, Cognition, and Alzheimer's Disease. *J. Alzheimer's Dis.* **2019**, *67*, 469–480. <https://doi.org/10.3233/JAD-181035>.
18. Jo, Y.; Kim, S.; Ye, B.S.; Lee, E.; Yu, Y.M. Protective Effect of Renin-Angiotensin System Inhibitors on Parkinson's Disease: A Nationwide Cohort Study. *Front. Pharmacol.* **2022**, *13*, 837890. <https://doi.org/10.3389/fphar.2022.837890>.
19. Urmila, A.; Rashmi, P.; Nilam, G.; Subhash, B. Recent Advances in the Endogenous Brain Renin-Angiotensin System and Drugs Acting on It. *J. Renin-Angiotensin-Aldosterone Syst.* **2021**, *2021*, 9293553. <https://doi.org/10.1155/2021/9293553>.
20. Kantak, K.M. Rodent models of attention-deficit hyperactivity disorder: An updated framework for model validation and therapeutic drug discovery. *Pharmacol. Biochem. Behav.* **2022**, *216*, 173378. <https://doi.org/10.1016/j.pbb.2022.173378>.
21. Correia, B.S.; Nani, J.V.; Waladares Ricardo, R.W.; Stanisic, D.; Costa, T.B.; Hayashi, M.A.; Tasic, L. Effects of Psychostimulants and Antipsychotics on Serum Lipids in an Animal Model for Schizophrenia. *Biomedicines* **2021**, *9*, 235. <https://doi.org/10.3390/biomedicines9030235>.
22. Tayebati, S.K.; Tomassoni, D.; Amenta, F. Spontaneously hypertensive rat as a model of vascular brain disorder: Microanatomy, neurochemistry and behavior. *J. Neurol. Sci.* **2012**, *322*, 241–249. <https://doi.org/10.1016/j.jns.2012.05.047>.
23. Schirru, M.; Véronneau-Veilleux, F.; Nekka, F.; Ursino, M. Phasic Dopamine Changes and Hebbian Mechanisms during Probabilistic Reversal Learning in Striatal Circuits: A Computational Study. *Int. J. Mol. Sci.* **2022**, *23*, 3452. <https://doi.org/10.3390/ijms23073452>.
24. Tanaka, M.; Szabó, .; Spekker, E.; Polyák, H.; Tóth, F.; Vécsei, L. Mitochondrial Impairment: A Common Motif in Neuropsychiatric Presentation? The Link to the Tryptophan–Kynurenine Metabolic System. *Cells* **2022**, *11*, 2607. <https://doi.org/10.3390/cells11162607>.
25. Panov, G. Dissociative Model in Patients with Resistant Schizophrenia. *Front. Psychiatry* **2022**, *13*, 845493. <https://doi.org/10.3389/fpsy.2022.845493>.
26. Ramírez-Sánchez, M.; Prieto, I.; Segarra, A.B.; Banegas, I.; Martínez-Cañamero, M.; Domínguez-Vías, G.; de Gasparo, M. Brain Asymmetry: Towards an Asymmetrical Neurovisceral Integration. *Symmetry* **2021**, *13*, 2409. <https://doi.org/10.3390/sym13122409>.
27. Wu, X.; Kong, X.; Vatansver, D.; Liu, Z.; Zhang, K.; Sahakian, B.J.; Robbins, T.W.; Feng, J.; Thompson, P.; Zhang, J. Dynamic changes in brain lateralization correlate with human cognitive performance. *PLoS Biol.* **2022**, *20*, e3001560. <https://doi.org/10.1371/journal.pbio.3001560>.
28. McManus, C. Half a century of handedness research: Myths, truths; fictions, facts; backwards, but mostly forwards. *Brain Neurosci. Adv.* **2019**, *3*, 2398212818820513. <https://doi.org/10.1177/2398212818820513>.
29. Esteves, M.; Lopes, S.S.; Almeida, A.; Sousa, N.; Leite-Almeida, H. Unmasking the relevance of hemispheric asymmetries—Break on through (to the other side). *Prog. Neurobiol.* **2020**, *192*, 101823. <https://doi.org/10.1016/j.pneurobio.2020.101823>.
30. Shi, G.; Li, X.; Zhu, Y.; Shang, R.; Sun, Y.; Guo, H.; Sui, J. The divided brain: Functional brain asymmetry underlying self-construal. *NeuroImage* **2021**, *240*, 118382. <https://doi.org/10.1016/j.neuroimage.2021.118382>.
31. Alba, F.; Iribar, C.; Ramírez, M.; Arenas, C. Un método fluorimétrico para la determinación de aminopeptidasas cerebrales. A fluorimetric method for the determination of brain aminopeptidasas. *Arch. Neurobiol.* **1989**, *52*, 169–173.
32. Hui, K.-S.; Lajtha, A. *Handbook of Neurochemistry*, 2nd ed.; Lajtha, A., Ed.; Plenum: New York, NY, USA, 1983; Volume 4, pp. 1–19.
33. Alba, F.; Ramírez, M.; Iribar, C.; Cantalejo, E.S.; Osório, C. Asymmetrical distribution of aminopeptidase activity in the cortex of rat brain. *Brain Res.* **1986**, *368*, 158–160. [https://doi.org/10.1016/0006-8993\(86\)91054-1](https://doi.org/10.1016/0006-8993(86)91054-1).
34. Alba, F.; Ramirez, M.; Cantalejo, E.S.; Iribar, C. Aminopeptidase activity is asymmetrically distributed in selected zones of rat brain. *Life Sci.* **1988**, *43*, 935–939. [https://doi.org/10.1016/0024-3205\(88\)90270-6](https://doi.org/10.1016/0024-3205(88)90270-6).
35. Patel, S.; Rauf, A.; Khan, H.; Abu-Izneid, T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. *Biomed. Pharmacother.* **2017**, *94*, 317–325. <https://doi.org/10.1016/j.biopha.2017.07.091>.
36. Salio, C.; Lossi, L.; Ferrini, F.; Merighi, A. Neuropeptides as synaptic transmitters. *Cell Tissue Res.* **2006**, *326*, 583–598. <https://doi.org/10.1007/s00441-006-0268-3>.
37. Seroogy, K.; Ceccatelli, S.; Schalling, M.; Hökfelt, T.; Frey, P.; Walsh, J.; Dockray, G.; Brown, J.; Buchan, A.; Goldstein, M. A subpopulation of dopaminergic neurons in rat ventral mesencephalon contains both neurotensin and cholecystokinin. *Brain Res.* **1988**, *455*, 88–98. [https://doi.org/10.1016/0006-8993\(88\)90117-5](https://doi.org/10.1016/0006-8993(88)90117-5).
38. Repova, K.; Aziriova, S.; Krajcirovicova, K.; Simko, F. Cardiovascular therapeutics: A new potential for anxiety treatment? *Med. Res. Rev.* **2022**, *42*, 1202–1245. <https://doi.org/10.1002/med.21875>.
39. Banegas, I.; Prieto, I.; Segarra, A.B.; Vives, F.; MartínezCañamero, M.; Durán, R.; Luna, J.d.D.; de Gasparo, M.; Domínguez Vías, G.; Ramírez-Sánchez, M. Asymmetric Interaction of Neuropeptidase Activities between Cortico-Limbic Structures, Plasma and Cardiovascular Function after Unilateral Dopamine Depletions of the Nigrostriatal System. *Biomedicines* **2022**, *10*, 326. <https://doi.org/10.3390/biomedicines10020326>.
40. Player, M.S.; Peterson, L.E. Anxiety Disorders, Hypertension, and Cardiovascular Risk: A Review. *Int. J. Psychiatry Med.* **2011**, *41*, 365–377. <https://doi.org/10.2190/pm.41.4.f>.

41. Hinojosa, F.R.; Spricigo, L., Jr.; Izídio, G.S.; Brüske, G.R.; Lopes, D.M.; Ramos, A. Evaluation of two genetic animal models in behavioral tests of anxiety and depression. *Behav. Brain Res.* **2006**, *168*, 127–136. <https://doi.org/10.1016/j.bbr.2005.10.019>.
42. Yoris, A.; Legaz, A.; Abrevaya, S.; Alarco, S.; López Peláez, J.; Sánchez, R.; García, A.M.; Ibáñez, A.; Sedeño, L. Multicentric evidence of emotional impairments in hypertensive heart disease. *Sci. Rep.* **2020**, *10*, 14131. <https://doi.org/10.1038/s41598-020-70451-x>.
43. Tulbă, D.; Avasilichioaiei, M.; Dima, N.; Crăciun, L.; Bălănescu, P.; Buzea, A.; Băicuș, C.; Popescu, B.O. Shared Molecular Targets in Parkinson's Disease and Arterial Hypertension: A Systematic Review. *Biomedicines* **2022**, *10*, 653. <https://doi.org/10.3390/biomedicines10030653>.
44. Turgut, M.; Jellestad, F.K.; Mathisen, J.R.; Thorsen, T.; Wester, K.; Aakvag, A. Asymmetry of sympathetic activity in a rat model of Parkinson's disease induced by 6-hydroxydopamine: Haemodynamic, electrocardiographic and biochemical changes. *Res. Exp. Med.* **1997**, *197*, 281–292. Correction in: *Res. Exp. Med.* **2000**, *200*, 67. <https://doi.org/10.1007/s004330050077>.
45. Banegas, I.; Segarra, A.B.; Prieto, I.; Vives, F.; de Gasparo, M.; Duran, R.; De Dios Luna, J.; Ramírez-Sánchez, M. Asymmetrical response of aminopeptidase A in the medial prefrontal cortex and striatum of 6-OHDA-unilaterally-lesioned Wistar Kyoto and spontaneously hypertensive rats. *Pharmacol. Biochem. Behav.* **2019**, *182*, 12–21. <https://doi.org/10.1016/j.pbb.2019.05.007>.
46. Banegas, I.; Prieto, I.; Vives, F.; Alba, F.; de Gasparo, M.; Duran, R.; de Dios Luna, J.; Segarra, A.B.; Hermoso, F.; Ramírez, M. Asymmetrical response of aminopeptidase A and nitric oxide in plasma of normotensive and hypertensive rats with experimental hemiparkinsonism. *Neuropharmacology* **2009**, *56*, 573–579. <https://doi.org/10.1016/j.neuropharm.2008.10.011>.
47. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*, 4th ed.; Elsevier Academic Press: Amsterdam, The Netherlands; London, UK, 1998.
48. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. <https://doi.org/10.1006/abio.1976.9999>.
49. Banegas, I.; Prieto, I.; Segarra, A.B.; Vives, F.; de Gasparo, M.; Duran, R.; Luna, J.D.D.; Ramírez-Sánchez, M. Bilateral distribution of enkephalinase activity in the medial prefrontal cortex differs between WKY and SHR rats unilaterally lesioned with 6-hydroxydopamine. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2017**, *75*, 213–218. <https://doi.org/10.1016/j.pnpbp.2017.02.015>.
50. Banegas, I.; Prieto, I.; Vives, F.; Alba, F.; de Gasparo, M.; Duran, R.; Segarra, A.B.; Ramírez, M. Lateralized response of oxytocinase activity in the medial prefrontal cortex of a unilateral rat model of Parkinson's disease. *Behav. Brain Res.* **2010**, *213*, 328–331. <https://doi.org/10.1016/j.bbr.2010.05.030>.
51. Sullivan, R.M. Hemispheric Asymmetry in Stress Processing in Rat Prefrontal Cortex and the Role of Mesocortical Dopamine. *Stress* **2004**, *7*, 131–143. <https://doi.org/10.1080/102538900410001679310>.
52. Gironacci, M.M.; Vicario, A.; Cerezo, G.; Silva, M.G. The depressor axis of the renin–angiotensin system and brain disorders: A translational approach. *Clin. Sci.* **2018**, *132*, 1021–1038. <https://doi.org/10.1042/cs20180189>.
53. Jennings, J.R.; Heim, A.F. From Brain to Behavior: Hypertension's Modulation of Cognition and Affect. *Int. J. Hypertens.* **2012**, *2012*, 701385. <https://doi.org/10.1155/2012/701385>.
54. Zhai, Z.; Feng, J. Left-right asymmetry influenced the infarct volume and neurological dysfunction following focal middle cerebral artery occlusion in rats. *Brain Behav.* **2018**, *8*, e01166. <https://doi.org/10.1002/brb3.1166>.
55. Wang, J.-W.; Liu, J.; Wang, Z.-L.; Gao, F.; Yang, J.; Wang, X.-C.; Guo, Y.; Wang, Y.; Ma, B.-R.; Wang, H.-S.; et al. Activation and blockade of 5-HT4 receptors in the dorsal hippocampus enhance working and hippocampus-dependent memories in the unilateral 6-hydroxydopamine lesioned rats. *Behav. Brain Res.* **2022**, *431*, 113952. <https://doi.org/10.1016/j.bbr.2022.113952>.
56. Yamanaka, K.; Waki, H. Conditional Regulation of Blood Pressure in Response to Emotional Stimuli by the Central Nucleus of the Amygdala in Rats. *Front. Physiol.* **2022**, *13*, 820112. <https://doi.org/10.3389/fphys.2022.820112>.
57. Day, H.E.; Vittoz, N.M.; Oates, M.M.; Badiani, A.; Watson, S.J., Jr.; Robinson, T.E.; Akil, H. A 6-Hydroxydopamine lesion of the mesostriatal dopamine system decreases the expression of corticotropin releasing hormone and neurotensin mRNAs in the amygdala and bed nucleus of the stria terminalis. *Brain Res.* **2002**, *945*, 151–159. [https://doi.org/10.1016/s0006-8993\(02\)02747-6](https://doi.org/10.1016/s0006-8993(02)02747-6).
58. Sullivan, R.M.; Dufresne, M.M.; Siontas, D.; Chehab, S.; Townsend, J.; Laplante, F. Mesocortical dopamine depletion and anxiety-related behavior in the rat: Sex and hemisphere differences. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2014**, *54*, 59–66. <https://doi.org/10.1016/j.pnpbp.2014.05.002>.
59. Watanabe, H.; Fitting, S.; Hussain, M.Z.; Kononenko, O.; Iatsyshyna, A.; Yoshitake, T.; Kehr, J.; Alkass, K.; Druid, H.; Wadensten, H.; et al. Asymmetry of the Endogenous Opioid System in the Human Anterior Cingulate: A Putative Molecular Basis for Lateralization of Emotions and Pain. *Cereb. Cortex* **2015**, *25*, 97–108. <https://doi.org/10.1093/cercor/bht204>.
60. Kimura, Y.; Miyake, K.; Kitaura, T.; Kihira, K.; Fukuchi, H. Changes of Cholecystokinin Octapeptide Tissue Levels in Rat Brain Following Dopamine Neuron Lesions Induced by 6-Hydroxydopamine. *Biol. Pharm. Bull.* **1994**, *17*, 1210–1214. <https://doi.org/10.1248/bpb.17.1210>.
61. Nakamura, K.; Shirane, M.; Koshikawa, N. Site-specific activation of dopamine and serotonin transmission by aniracetam in the mesocorticolimbic pathway of rats. *Brain Res.* **2001**, *897*, 82–92. [https://doi.org/10.1016/s0006-8993\(01\)02096-0](https://doi.org/10.1016/s0006-8993(01)02096-0).
62. Kirouac, G.J.; Ganguly, P.K. Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: An autoradiographic analysis. *Neuroscience* **1993**, *52*, 135–141. [https://doi.org/10.1016/0306-4522\(93\)90188-1](https://doi.org/10.1016/0306-4522(93)90188-1).

-
63. Basmadjian, O.M.; Occhieppo, V.B.; Marchese, N.A.; Baiardi, G.; Bregonzio, C. Brain Angiotensin II Involvement in Chronic Mental Disorders. *Protein Pept. Lett.* **2017**, *24*, 817–826. <https://doi.org/10.2174/0929866524666170728144801>.
 64. Almeida-Santos, A.F.; Kangussu, L.M.; Campagnole-Santos, M.J. The Renin-Angiotensin System and the Neurodegenerative Diseases: A Brief Review. *Protein Pept. Lett.* **2017**, *24*, 841–853. <https://doi.org/10.2174/0929866524666170822120258>.