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Research Report

The expression of brain sexual dimorphism in artificial selection of rat strains

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Abstract

Central nervous system sex differences have two morphological patterns. In one pattern, males show larger measurements (volume, number of neurons) than females (male > female; m > f) and, in the other, the opposite is true (female > male; f > m). The bed nucleus of the stria terminalis (BST) is a unique model for the study of sex differences because it has dimorphic and isomorphic subdivisions, with the former showing the two sexually differentiated morphological patterns. Meanwhile, other CNS structures, like the locus coeruleus (LC), present the f > m pattern. The philogenetic maintenance of the two patterns of sexual differentiation can help to disentangle the functional meaning of sex differences. Laboratory rat strains, whether albino or pigmented, descend from the Wistar strain through artificial selection. The present work compares the BST and LC of Wistar and Long–Evans rats. The medial posterior subdivision of the BST (BSTMP) is sexually dimorphic (m > f pattern) in the original (Wistar) and derived (Long–Evans) strains, while the lateral anterior and medial anterior subdivisions of the BST and the LC only present sex differences (f > m pattern) in the ancestor Wistar strain. Isomorphic BST regions are the same in both strains. The fact that the BSTMP, which is implicated in male copulatory behavior, is sexually dimorphic in both strains, as well as in other species, including humans, indicates the relevance of this structure in male sexual behavior in mammals.

Theme: Neural basis of behavior
Topic: Hormonal control of reproductive behavior

Keywords: Brain sex difference; Bed nucleus of the stria terminalis; Locus coeruleus; Strain difference; Wistar rat; Long Evans rat; Sex difference; Artificial selection; Vomeronasal system

1. Introduction

A relevant aspect of any theory on the physiological and behavioral functions of brain sexual dimorphism has to do with its continuance in strains and species. Sex differences in rats have been described with respect to different morphological parameters such as volume, number of cells, shape, and anatomical asymmetry [18–20,24,25,28,29] whether the same (or the homologous) brain nucleus or structure presents sexual dimorphism in several strains or along the phylogenetic line is very important to help disentangle the functional meaning of sex differences. In this sense, there are brain nuclei, like the sexually dimorphic nucleus of the preoptic area (SDN-POA), that present sexual dimorphism in several species like quails [5,78] and doves [71], or sheep [62], or hamsters [26], and polygamous montane voles [68] and rats [24,25,40], or rhesus monkeys [8], and human beings [38,73].

Comparing brain sexual dimorphism in different strains and species is also important for testing general hypotheses related to sexual dimorphism. For instance, it has been proposed that the Accessory Olfactory (or Vomeronasal) System (VNS), which is related to reproductive behavior

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[33,34], is sexually dimorphic in mammals [27,65–67]. The SDN-POA is contained in the medial preoptic area (MPA), which in turn is a tertiary projection of the vomeronasal organ. To a much lesser extent, sex differences have been demonstrated with respect to the medial posterior division of the bed nucleus of the stria terminalis (BSTMP), a region that has been related to male rat copulatory behavior [9,22]. The BST, a secondary projection structure of the VNS [69], can be a unique model for the study of sex differences and their evolution because it has dimorphic and isomorphic subdivisions in rats and the former present both different morphological patterns, m > f and f > m. In the medial posterior subdivision (BSTMP), male Wistar rats show a larger subdivision volume and a higher number of neurons than do females [16,29]. These results have also been reported in the Sprague–Dawley strain [10]. On the contrary, in the lateral and medial anterior subdivisions (BSTLA and BSTMA), females show a larger subdivision volume and/or higher number of neurons than do males [16,29]. The BST also presents several isomorphic regions in the lateral subdivision: the BST lateral posterior region (BSTLP) and the BST lateral juxtacapsular region (BSTLjx). With regards to the BST, and as far as we know, sex differences have been shown in the Wistar [16,29,60] and Sprague–Dawley [10] strains, in guinea pigs [37], in chickens [44], and in human beings [3,46,83].

Another structure, the locus coeruleus (LC), sends rich noradrenergic projections to the VNS [12,76], and it, also, has been demonstrated to be sexually dimorphic in the Wistar strain [28] but not in Sprague–Dawley rats [4]. Moreover, sex differences in the LC have also been reported in humans [7]. In the LC, which is implicated in a variety of physiological and behavioral functions, including reproduction [2,52], Nissl [28,58] and dopamine-β-hydroxylase [49] studies have shown that females have a larger volume and a higher number of neurons than do males. The origin and maintenance of these differences seem to depend on the action of estradiol [14,28].

Laboratory rat strains, whether albino or pigmented, descend from the Wistar strain as a consequence of artificial selection [56,84], a selection process that helped Darwin to understand and then define sexual selection [13]. In the present study, in order to test the consistency of sex differences for structures related to sexual behavior and reproductive physiology, we compare the BST and the LC between Wistar rats and their descendants, the pigmented Long–Evans rat strain.

2. Materials and methods

2.1. Subjects

Adult male and female Wistar rats (Charles River, Criffa; Saint-Aubin-Les-Elbeuf, France) and adult male and female Long–Evans rats (Janvier, Madrid, Spain), weighing 250–300 g, were divided into 4 groups (7 rats each) according to strain (Wistar and Long–Evans) and sex (male and female). They were housed in standard cages in same-sex and strain groups and maintained in 12:12 light:dark cycle (lights off at 8:00 PM). Food and water were provided ad libitum. The stock room was kept at 22 ± 2 °C. Animal care and handling throughout the experimental procedures were in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

2.2. Histology

At the age of 90 days, the animals were deeply anesthetized with an intraperitoneal injection (250 mg/kg) of tribromoethanol and perfused intracardially with saline (0.9%) followed by 4% paraformaldehyde. The brains were removed and stored in paraformaldehyde for 2 days followed by 3–5 days in 30% sucrose at 4 °C. The brains were frozen and coronally sectioned at a thickness of 50 μm (fore and midbrain) or 40 μm (pons and cerebellum). All sections were stained with a 0.1% solution of cresyl violet (Merck) brought to pH 4 with glacial acetic acid.

2.3. Stereology

Stereological methods were used to determine the volume and neuron number of the BST subdivisions and the LC. The Cavalieri principle was used to estimate volume [51] and the total number of neurons was estimated using the disector and fractionator techniques [30,31,72]. To measure these morphological parameters, we used a Diaplan Leitz microscope with a computer-controlled stage (MultiControl 2000; Mörzhäuser Wetzlar, Germany) allowing randomly chosen steps to be generated on the x and y axes. This specially fitted rotating stage allows the slices to be shifted by 360°, independently of the x–y movements. The stereological software package (GRID; Interactivision, Denmark) makes it possible to superimpose the required grid patterns over the microscope image. Finally, an electronic microcator (Heidenhain, Germany) with a resolution of 0.5 μm is attached to the microscope so the z axis measurements of the stage can be taken.

The BSTMA, BSTLA, BSTMP, BSTLp, BSTLjx, and LC volumes were estimated using the classic Cavalieri principle [51]. On each coronal section (only every other section, with first one being randomly selected), a set of points (generated by the GRID system) was systematically placed and the points that coincided within the area studied were counted. The total volume was obtained by multiplying the number of points by the area associated with each point and by the distance between two sections counted. This distance was obtained from the product of the cut thickness (50 μm BST, 40 μm LC) multiplied by the sampling interval.

The number of neurons in the BSTMA, BSTLA, BSTMP, BSTLp, BSTLjx, and LC was estimated using the optical fractionator, which combines the optical disector and fractionator techniques [30,31,72]. The sections were cut with a cryostat to a thickness of 50 μm (BST) and 40 μm (LC), but their actual width after using the microcator was

approximately 20–25 μm. The optical disector was used as follows: at ×186 magnification, frames (disectors) were generated over the area with a horizontal step of 90 μm and a vertical step of 90 μm for the BST and a horizontal step of 90 μm and a vertical step of 50 μm for the LC. All of the frames that included nuclei surface were considered. The profiles (cell nuclei) that were completely enclosed in the test frame ($A[f]$ = 842 μm^2 for all subdivisions of the BST and for the LC) and those intersected by the inclusion edges at a ×4323 magnification were counted. The height of the disector was 12 μm. Finally, the total number of neurons in both structures was obtained applying the fractionator formula:

$$\sum Q = 1/\text{ssf} \times 1/\text{asf} \times 1/\text{hsf}$$

In this formula, $\sum Q$ is the total number cell nuclei counted; ssf is the section sampling fraction; asf is the area sampling fraction; and hsf is the height sampling fraction. The counting unit was the nucleolus. An observer, unaware of the group membership of each specimen, determined the volume and the number of neurons in each slide.

2.4. Statistical analysis

Volume and number of neurons within each region were analyzed using non-parametric analyses (Kruskal–Wallis one-way analysis of variance) because the data did not show homogeneity of variance; this was followed by the Mann–Whitney U test to compare group medians.

3. Results

3.1. Bed nucleus of the stria terminalis

3.1.1. Sexually dimorphic subdivisions of the BST

3.1.1.1. Lateral anterior subdivision of the BST (BSTLA). The Kruskal–Wallis analyses detected a statistically significant group effect with respect to the number of neurons [$H(3)$ = 15.57; $P < 0.001$]. As can be seen in Fig. 1A, there are sex differences in the Wistar strain, since females have a higher

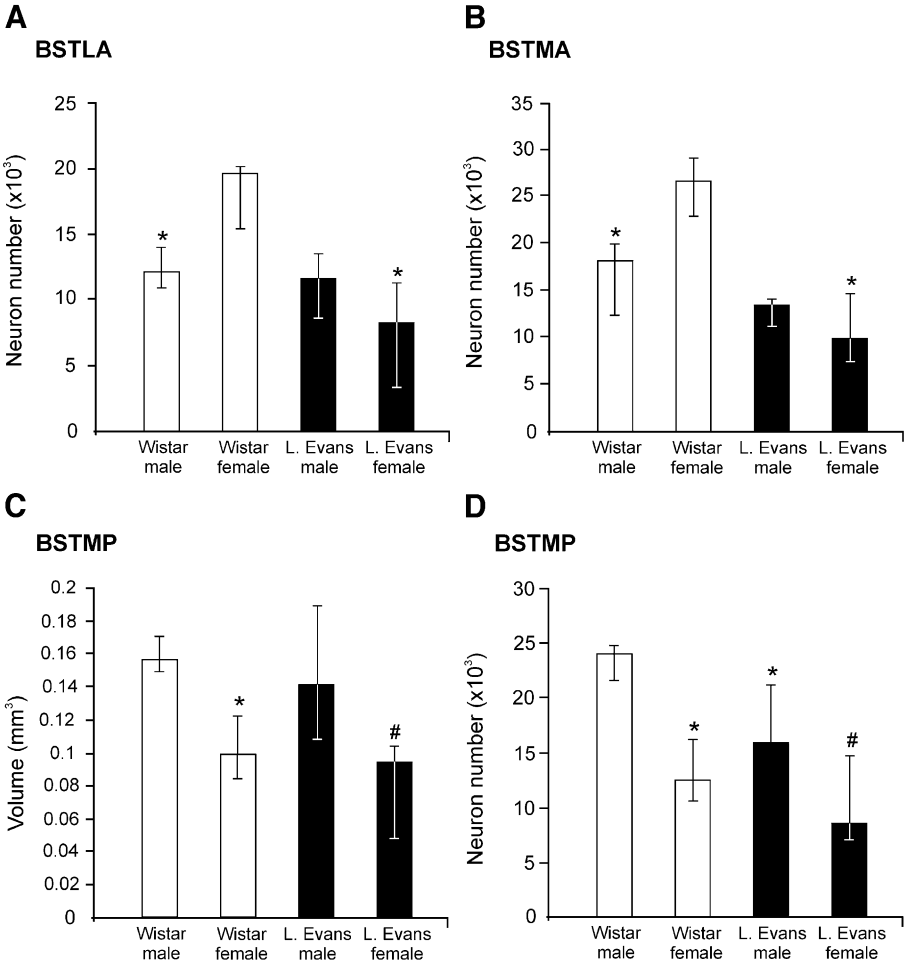

Fig. 1. Graphs show the neuron number in (A) BSTLA, (B) BSTMA, and (D) BSTMP and the volume of the BSTMP (C). WM: Wistar males; WF: Wistar females; LEM: Long–Evans males; LEF: Long–Evans females. *At least $P < 0.05$ with respect to WF (graphs A and B) and with respect to WM (graphs C and D). #At least $P < 0.05$ with respect to LEM (graphs C and D). Results are expressed as median and 25th and 75th percentiles.

number of neurons in the BSTLA than male rats ($P < 0.002$), but not in the Long–Evans strain, in which male and female present a similar number of neurons (see also Fig. 2A). Males of both strains have a similar number of neurons (Fig. 1A) while the Wistar females have more neurons than the Long–Evans females ($P < 0.004$) or Long–Evans males ($P < 0.004$). When strains were compared, a statistically significant group effect was also detected in the number of neurons [$H(1) = 9.47$; $P < 0.002$], since Wistar rats had more neurons than Long–Evans rats. However, since the males of both strains present a similar number of neurons, the strain differences can be explained by the Wistar female rats having more neurons than the Long–Evans female rats.

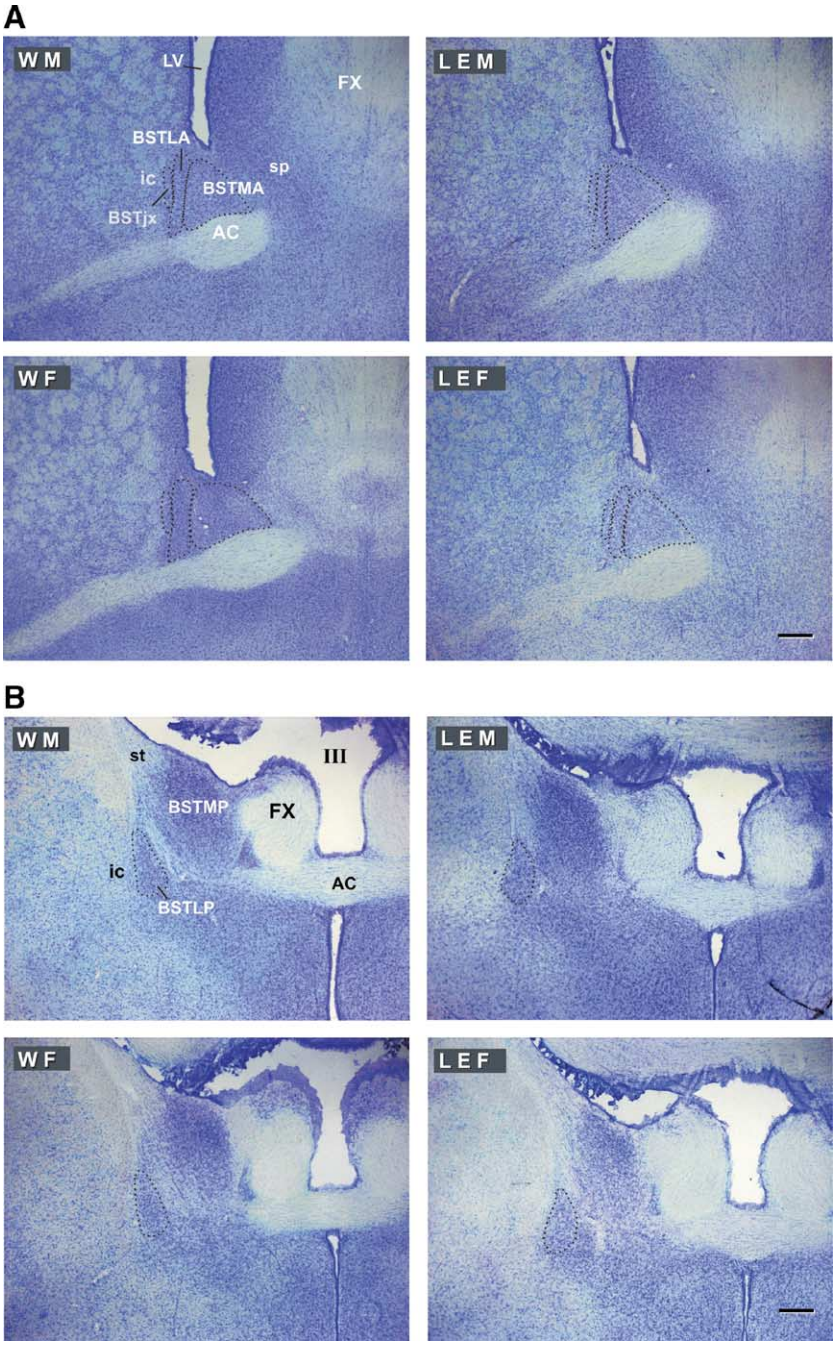

Fig. 2. Coronal sections illustrating (A) BSTLA, BSTMA, and BSTjx subdivisions (interaural 9.20 mm; Bregma 0.20 mm) [57] and (B) BSTMP and BSTLP subdivisions (interaural 8.20 mm; Bregma 0.20 mm) [57] in the experimental groups. WM: Wistar males; WF: Wistar females; LEM: Long–Evans males; LEF: Long–Evans females; AC: anterior commissure; BSTjx: juxtacapsular subdivision of the BST; BSTLA: lateral anterior subdivision of the bed nucleus of the stria terminalis (BST); BSTLP: lateral posterior subdivision of the BST; BSTMA: medial anterior subdivision of the BST; BSTMP: medial posterior subdivision of the BST; FX: fornix; ic: internal capsule; LV: lateral ventricle; III: third ventricle; sp: septum. Scale bar: 300 μm.

3.1.1.2. Medial anterior subdivision of the BST. Kruskal–Wallis analyses showed a statistically significant group effect with respect to the number of neurons [$H(3) = 16.27$; $P < 0.001$]. There are sex differences in the Wistar strain BSTMA, since the females have more neurons than do the males ($P < 0.006$), but no sex differences occur in the Long–Evans rats, since the male and female rats of that strain have a similar number of neurons. Males of both strains have a similar number of neurons (Fig. 1B) but the female Wistar rats have more neurons than do female Long–Evans ($P < 0.004$; see also Fig. 2A). A statistically significant group effect was also observed in the comparison of neuron number between the strains [$H(1) = 11.87$; $P < 0.001$], since Wistar had more neurons than Long–Evans rats. However, since the males of both strains had a similar number of neurons, the strain differences can also be explained by female Wistar rats having more neurons in the BSTMA than the female Long–Evans rats, as also occurred in the BSTLA.

3.1.1.3. Medial posterior subdivision of the BST. There was a statistically significant group effect with respect to the volume [$H(3) = 17.38$; $P < 0.001$] and number of neurons [$H(3) = 18.60$; $P < 0.0001$]. Wistar and Long–Evans rats show sexual dimorphism, with males of both strains having a larger volume (Figs. 1C and 2B; Wistar, male vs. female; $P < 0.002$; Long–Evans, male vs. female, $P < 0.006$) and greater number of neurons (Fig. 1D; Wistar, male vs. female; $P < 0.002$; Long–Evans, male vs. female, $P < 0.009$) than females. Comparing males between strains, one can see that the Wistar males have more neurons than the Long–Evans males ($P < 0.006$), although the BSTMP volume is similar in both strains. This difference in neuron number explains the strain differences found in the analysis [$H(1) = 5.07$; $P < 0.02$].

3.1.2. Isomorphic subdivisions of the BST

The volume and number of neurons in the Lateral juxtacapsular (BSTLjx) and the lateral posterior (BSTLP) subdivisions of the BST are similar in both rat sexes and strains (Figs. 2A and B).

3.2. Locus coeruleus

There was a statistically significant group effect for both volume [$H(3) = 7.57$; $P < 0.05$] and number of neurons in the LC [$H(3) = 10.98$; $P < 0.01$]. The Wistar rat LC is sexually dimorphic since the females show a larger LC volume (Figs. 3A and 4; $P < 0.009$) and greater number of neurons than do males (Fig. 3B; Wistar: $P < 0.01$); the Long–Evans strain does not show these sex differences (Fig. 4). Males of both strains present a similar LC volume and number of neurons (Figs. 3A and B). However, the female Wistar rats have a larger volume and number of neurons than do female Long–Evans rats (volume: $P < 0.01$; number of neurons: $P < 0.01$). When the strains are compared, there is a statistically significant group effect for the number of neurons [$H(1) = 7$; $P < 0.008$], and Wistar rats have generally more LC neurons than do Long–Evans rats. Although males of both strains have a similar number of neurons, the strain differences in neuron number are explained by the fact that Wistar females have more neurons than Long–Evans females.

4. Discussion

The results of the present study with respect to the LC in Wistar rats confirm our previous reports on this strain; females have a larger LC volume and more neurons than males. However, these results were not replicated in the Long–Evans strain. Babstock et al. [4] were also unable to replicate sex differences in the overall LC volume in the Sprague–Dawley strain. Thus, in rats, the expression of sex differences in the LC seems to be present in the ancestor strain but not in Long–Evans or Sprague–Dawley strains, which are both derived from the Wistar strain.

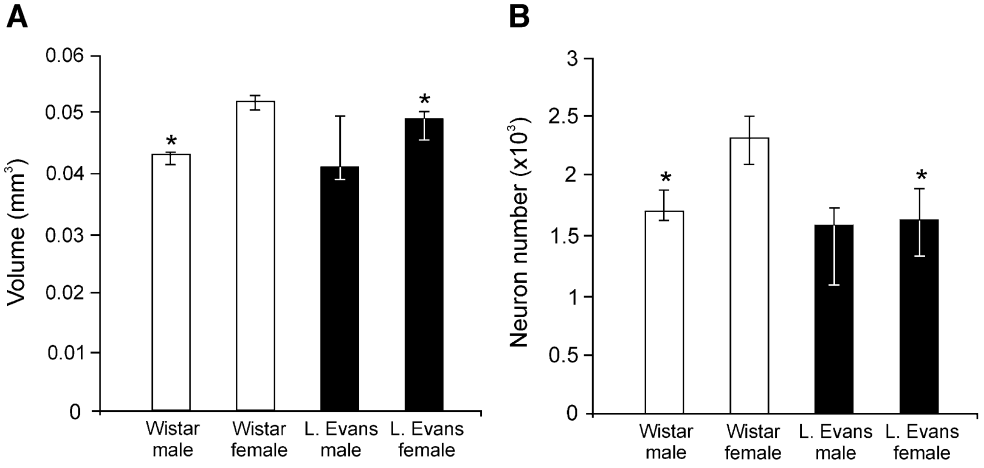

Fig. 3. Graphs show (A) the volume and (B) the number of neurons in the locus coeruleus. WM: Wistar males; WF: Wistar females; LEM: Long–Evans males; LEF: Long–Evans females. *At least $P < 0.05$ with respect to WF (graphs A and B). Results are expressed as median and 25th and 75th percentiles.

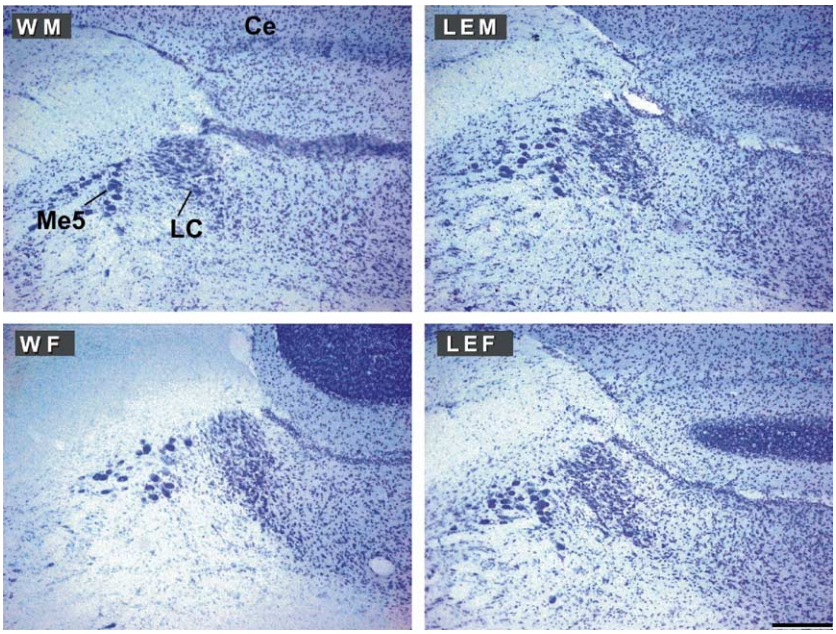

Fig. 4. Coronal sections illustrating locus coeruleus nucleus (interaural −1.04 mm; Bregma −10.04 mm) [57] in the experimental groups. WM: Wistar males; WF: Wistar females; LEM: Long–Evans males; LEF: Long–Evans females Ce: cerebellum; LC: locus coeruleus; Me5: mesencephalic trigeminal nucleus. Scale bar: 200 μm.

With respect to the BST, the present study investigated three types of subdivisions. First, those subdivisions in which Wistar females have larger morphological measurements than males (BSTMA and BSTLA) [16,29]; second, a subdivision in which Wistar males have larger morphological parameters than the females (BSTMP) [29]; and, last, two BST subdivisions, BSTLP and BSTLjx, in which no sex difference have been reported in Wistar rats [16,29].

The Wistar BST data replicate almost all the results previously reported in the literature. We have found that Wistar females present larger morphological measurements (subdivision volume and neuron number) than do males in the BSTLA [16]. The only previous report in the BSTMA also showed sex differences but was focused only on volume [16]. The present study in Wistar rats finds no sex differences in this parameter but does find differences in the number of neurons, since females have more neurons than males. Earlier findings with respect to the Wistar BSTMP were also replicated and males show larger morphological parameters than do females [16]. In addition, the same results were obtained in the isomorphic regions of the BST. As previously reported [16,29], no sex differences were found in the BSTLP or the BSTLjx.

Sex differences were seen in the BSTMP but not in the BSTLA, BSTMA, or LC in Long–Evans rats. Like Wistar rats, the Long–Evans males have a larger volume and greater number of neurons in the BSTMP than do females. However, contrary to the results observed in the Wistar rats, no sex differences were seen in the BSTLA or the BSTMA. The BSTLP and the BSTLjx are isomorphic in both strains. Thus, in the BST, strain differences with respect to sex differences are circumscribed to the BSTMA and the BSTLA, the two BST subdivisions in which the female has greater morphological parameters than do male Wistar rats. The same occurred in the Long–Evans LC, a structure that in Wistar females also shows larger morphological values than do males.

Wistar and Long–Evans strains have a similar number of neurons in their isomorphic structures, but the strain differences emerge in the sexually dimorphic structures. In the female > male morphological patterns (BSTMA, BSTLA, LC), strain differences are produced because the Wistar female has more neurons than the Long–Evans female. In the male > female pattern (BSTMP), strain differences are seen because the Wistar males have more neurons than the Long–Evans males. Thus, strain differences in the brain morphological parameters seem to be the result of sexual dimorphism.

Our findings in this study, and other reports in the literature, indicate the following general tendency: First, in the BSTMP, which presents a male > female morphological pattern, sex differences are seen in different strains: Wistar rats ([16,29] and the present experiment), Sprague–Dawley rats [10], Long–Evans rats (present experiment), and even guinea pigs [37]. This pattern of sex difference (male > female) was confirmed by other authors or extended to other measurements such as vasopressine-ir and/or galanine-ir cells in Wistar [59,60] and Sprague–Dawley rats [35,77], AR-ir cells in Wistar rats [36] and Syrian hamsters [80], and testosterone target neurons [48] in Sprague–Dawley rats. Second, the female > male morphological pattern reported in the LC, BSTMA, and the BSTLA [14,16,28,29,49] and confirmed in this study in Wistar rats is not seen in Long–Evans (this study) or Sprague–Dawley rats [4]. Third, the

isomorphic BST divisions in the Wistar rats are also isomorphic in the Long–Evans strain.

Sprague–Dawley and Long–Evans strains descend from Wistar rats [56,84]. Thus, it seems that the male > female morphological pattern, expressed in the BSTMP, is maintained in the descendent Long–Evans strain, but the female > male morphological pattern (BSTMA, BSTLA and LC) seen in Wistar rats is lost in the derived strains: the Long–Evans and Sprague–Dawley (at least for the LC in this strain) rats [4]. The isomorphic regions of the BST are similar in Wistar and Long–Evans rats. It should be remembered that, of all these BST subdivisions, only the BSTMP belongs to the VNS [15] and projects to the medial preoptic area [15,70]. Thus, only the VNS structure is sexually dimorphic in the two strains studied here. It should be stressed that the VNS is implicated in the control of physiological and behavioral aspects of reproduction [6,9,17,22,23,33,34,39,41,45,47,50,53,61,63,64,82] and our observations in this study also support the hypothesis that the VNS is sexually dimorphic [27,65–67].

The male > female sexual morphological pattern seen in the BSTMP in the Wistar and Long–Evans strains is seen in other species of mammals like guinea pigs [37] and humans [3,11,46,83] as well as in some bird species [1,43,54,55,79]. In rats, lesions of the whole BST in Long–Evans rats [22] or a lesion circumscribed to the BSTMP in Wistar rats [9] disrupt copulatory behavior in males. Thus, it seems that a BSTMP lesion is sufficient to affect copulatory behavior.

In humans, the BST is sexually dimorphic with larger morphological measurements in men than women in the central subdivision (BSTc) ([46,83]; for review [75]) and in the darkly stained posteromedial component of the BST (BSTdsmp) [3]. The BSTc has been related to gender identity since male-to-female transsexuals have a feminized BSTc that is smaller than in heterosexual men. The rat oval nucleus would be homologous to the human BSTc because of its anatomical location and similar peptide expression [21,32,42,81]. Correspondingly, and due to its anatomical location and staining affinity, the rat BSTMP could be considered homologous to the human BSTdsmp.

It seems that the inbred artificial selection, which was an inspiring model for Darwinian sexual selection, of the Long–Evans strain has preserved sex differences in a structure related to male copulation (BSTMP) while the female > male sexually dimorphic pattern (LC, BSTMA, BSTLA) is lost in the Long–Evans and another derived strain, the Sprague–Dawley rats (at least the LC in the later strain). Can any functional hypothesis be advanced from this observation? Reports in the literature show that the BSTc correlates with sexual identity in humans since it is smaller in women and male-to-female transsexuals [46,74,83], and the BSTMP seems to be involved with male copulatory behavior [9,22]. Several years ago, we advanced the hypothesis that the functional meaning of the male > female morphological pattern is to facilitate the expression of male sexual behavior while inhibiting the expression of female behavior [65]. Although much more work is needed, it seems that this pattern is so firmly established in the BST that it is preserved through strains and species.

Is it possible to suggest a tentative hypothesis to explain strain differences in brain sexual dimorphism? Selection has induced brain sexual dimorphism in such a way that some brain nuclei present a constant pattern of sex differences independently of strain and species, while other nuclei show less stability and their sex differences can disappear not only between species but between strains as our results suggest.

Moreover, it might be the case that evolutionary mechanisms have led to the most appropriate level of sexual dimorphism for species survival, probably acting on brain behavioral and physiological mechanisms that control reproduction. Thus, when brain sexual dimorphisms reach a homogeneous biological base (high degree of homozygosis), the sexually dimorphic pattern of a cerebral nucleus (i.e., m > f or f > m) would be maintained constant through strains and species because it would be the most appropriate pattern for reproductive purposes. It would be a fixed pattern of high stability. That could be the case for the m > f morphological pattern in the BSTMP, which is the same in Wistar, Long–Evans, and Sprague–Dawley rats, guinea pigs and is also seen in birds, lagomorphs [in preparation], and human beings.

The mechanisms of artificial selection used for the selection of rat strains are based on crossings with wild males and on specific morphological, physiological, and behavioral characteristics that obviously carry over other characteristics that are indirectly selected. If a determined brain nucleus has a heterogeneous biological base (high degree of heterozygosis), during artificial selection, it would be possible to obtain strains that differ with respect to sexual dimorphism. That could be the case for the LC, BSTMA, and BSTLA, in which sexual dimorphism appears in Wistar rats but not in Long–Evans and Sprague–Dawley rats.

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