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Research Report

Sexual dimorphism in hybrids rats

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ABSTRACT

Laboratory rat strains descend from Wistar rats as a consequence of artificial selection. Previously we reported that the medial posterior division of the bed nucleus of the stria terminalis (BSTMP) was sexually dimorphic in Wistar and Long–Evans strains while the medial anterior division (BSTMA) and the locus coeruleus (LC) only showed sex differences in the ancestor Wistar strain. The lateral posterior division (BSTLP) was isomorphic in both strains. The present work studies the number of neurons in the BSTMP, BSTMA, BSTLP and LC of male and female Wistar and Long–Evans rats (F_0) and their hybrid F_1 and F_2 generations. The BSTMP is sexually dimorphic in the F_0, F_1 and F_2 generations while sex differences in the LC are only seen in F_0 Wistar rats but not in the F_0 Long–Evans or the F_1 and F_2 hybrid generations. Sex differences in the BSTMA are seen in F_0 Wistar but not in F_0 Long–Evans rats and completely disappear in the F_2 generations. The number of neurons in the LC of both males and females decreased in heterozygotic individuals (F_1) but increased in homozygotic (F_2). However, the number of neurons in the BSTMP changes significantly over the generations, although the ratio of neurons (female/male) is stable and unaffected in homo- or heterozygosis. Thus, the mechanism that regulates the neuronal female/male ratio would be different from the one that controls the number of neurons. The facts that sex differences in the BSTMP are not affected by homo- or heterozygosis and that they are seen in several mammalian orders suggest the existence of a "fixed" type of brain sex differences in the Mammalia Class.

1. Introduction

Sex differences in the mammalian brain have two main characteristics: First, they appear in neural networks, such as the vomeronasal pathway, which controls physiological and behavioral aspects of reproduction; and second, they take one of two opposite morphological patterns (Guillamon and Segovia, 1996; Segovia and Guillamon, 1993; Segovia et al., 1999). In some CNS structures males have larger morphological measurements (number of neurons, volume, etc.) than females, while in others the opposite is true. In the rat, the male>female pattern is characteristic of rat brain structures that receive vomeronasal input such as the accessory olfactory bulb, bed nucleus of the accessory olfactory tract, medial amygdala, posteromedial cortical amygdaloid nucleus, medial posterior division of the bed nucleus of the stria terminalis and the medial preoptic area (for a review, see Arnold and Gorski, 1984; Guillamon and Segovia, 1996; Segovia and Guillamon, 1993; Simerly, 2002), while the female>male pattern is seen in the anteroventral periventricular nucleus

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(Orikasa and Sakuma, 2003; Simerly et al., 1998), parastrial nucleus (Del Abril et al., 1990) and in the brain stem (i.e., locus coeruleus [LC]; Guillamon et al., 1988b).

Brain sex differences have also been reported in mice. The male>female pattern has been described in a cell group medial to the medial extension of the bed nucleus of the stria terminalis in two mice strains (Brown et al., 1999) and in the principal nucleus of the bed nucleus of the stria terminalis (Forger et al., 2004). The female>male pattern was found in cells located beneath the anterior commissure (Brown et al., 1999) and in the anteroventral periventricular nucleus (Forger et al., 2004; Simerly et al., 1998). Furthermore, these two patterns of sexual dimorphism have also been seen in rabbits (Bisenius et al., 2006; Segovia et al., 2006).

The bed nucleus of the stria terminalis (BST) is a useful model for the study of brain sex differences because it has dimorphic and isomorphic subdivisions. For instance, in Wistar rats the medial posterior division (BSTMP) presents the male>female pattern (Del Abril et al., 1987; Garcia-Falgueras et al., 2005; Guillamon et al., 1988a), while the medial anterior (BSTMA) and the lateral anterior (BSTLA) show a female>male pattern (Del Abril et al., 1987; Garcia-Falgueras et al., 2005; Guillamon et al., 1988a). The lateral posterior region (BSTLP) is isomorphic (Del Abril et al., 1987; Garcia-Falgueras et al., 2005). Moreover, BSTMP is one of the structures that receive vomeronasal input (Shipley et al., 2004) and, in the rat, has been related to the control of male copulatory behavior (Claro et al., 1995; Emery and Sachs, 1976). In Wistar rats, the LC, a structure that sends rich noradrenergic projections to olfactory structures (Shipley et al., 1985), is sexually dimorphic (Garcia-Falgueras et al., 2005; Guillamon et al., 1988b; Luque et al., 1992; Pinos et al., 2001), but not in the Sprague–Dawley (Babstock et al., 1997) and the Long–Evans (Garcia-Falgueras et al., 2005) strains.

In a recent paper we compared sex differences in the number of neurons in the BST and LC in Wistar and Long–Evans rats (Garcia-Falgueras et al., 2005). In that work we found that the BSTMP is sexually dimorphic (male>female) in both strains while the BSTMA, BSTLA and LC only show differences (female>male, in all cases) in the Wistar strain. The lateral juxtacapsular (BSTLjx) and the lateral posterior (BSTLP) BST subdivisions are isomorphic in the Wistar and the Long–Evans strains (Garcia-Falgueras et al., 2005).

It seems that selection has induced brain sexual dimorphism in some brain nuclei, like the BSTMP, that present a constant pattern of sexual dimorphism independently of strain and species (Allen and Gorski, 1990; Chung et al., 2000; Del Abril et al., 1987; Forger et al., 2004; Garcia-Falgueras et al., 2005; Guillamon et al., 1988a; Hines et al., 1985; Kruijver et al., 2000; Segovia et al., 2006; Stefanova and Ovtscharoff, 2000), while other nuclei, like the LC, show less stability and the sex differences may disappear not only in different species but also between strains of the same species (Garcia-Falgueras et al., 2005; Segovia et al., 2006).

Some studies have related sexual brain differences with genes. Forger et al. (2004) have related sex differences in the BSTMP with the *bax* gene and Brown et al. (1999) also related sex differences in the BST of SF-1 gene-disrupted mice. These works suggest a role of genes in sexual differentiation of the brain.

The production of hybrids, and the consequent increment of genetic variability, is another strategy to study the effect of genetic background on brain sex differences. We have seen a stable male>female pattern in the number of neurons in the BSTMP that appears in several species and strains. However, the female>male pattern for the number of neurons in the LC and the BSTMA seems to be unstable because it is seen in Wistar but not in Long–Evans rats. In the present work, we study the effect of heterozygosis of the two sexually dimorphic patterns over two successive generations. This was achieved by counting the number of neurons in the BSTMP, BSTMA, BSTLP and LC of male and female hybrids obtained by crossing individuals of the Wistar and the Long–Evans strains. The individuals are classified according to two criteria: matrilineal ascendance and molecular characteristics identified by the study of specific highly polymorphic DNA regions known as simple sequence length polymorphisms (SSLPs) or microsatellite DNA. These 1–6 simple nucleotide repeat sequences are often used as genetic markers for genotyping different strains.

2. Results

2.1. *The characterization of Long–Evans and Wistar strains*

With respect to the four molecular markers that characterized the Wistar and the Long–Evans strains, the transitions from F_0 to F_1 and from F_1 to F_2 had values of 100% homozygosis (F_0), 100% heterozygosis (F_1) and a partial recuperation of homozygosis (F_2) for the cited markers (Table 1).

The use of molecular markers restricts the number of subjects per group because only the homozygotic individuals are selected for analysis in F_2. Thus, it was not possible to apply both criteria (matrilineal and molecular) in all the experimental groups, but in those in which it was possible, the results obtained reinforce each other. In particular, we were able to apply both criteria to subjects whose brain structures did not show sex differences in F_2. In LC, BSTMA and BSTLP, there were no statistically significant differences in the number of neurons between the subjects grouped by the matrilineal criterion and those grouped by the molecular marker criterion: LC (WWLE, $t(19)=1.14$, n.s.; LELEW; $t(21)=0.04$, n.s.); BSTMA (WWLE: $t(14)=0.97$, n.s.; LELEW: $t(21)=0.80$, n.s.); BSTLP (WWLE: $t(12)=1.44$, n.s.; LELEW: $t(21)=0.98$, n.s.).

2.2. *Sex differences and comparison of the number of neurons between Wistar and Long–Evans strains*

2.2.1. *Bed nucleus of the stria terminalis*

There was a statistically significant main effect of sex ($F_{1,18}=24.02$; $p<0.0001$) in the BSTMP showing that males have more neurons than females in this structure in both Wistar and Long–Evans strains (Tables 2 and 3). There were statistically significant main effects of strain in the BSTMA ($F_{1,18}=6.77$; $p<0.02$) and the BSTLP ($F_{1,18}=11.01$; $p<0.005$) showing that Wistar rats have more neurons than Long–Evans rats in the BSTMA, while inspection of the means showed that female Wistar rats have more neurons in the BSTMA than the other groups (at least $p<0.05$) (Table 2). Thus, the Wistar strain showed sex differences (female>male) in the BSTMA (Table 2). With respect to the BSTLP, the Long–Evans females have more neurons than male and female Wistar rats ($p<0.05$ in both