Neuroscience Letters 487 (2011) 177–181

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Measurements of neuron soma size and density in rat dorsal striatum, nucleus accumbens core and nucleus accumbens shell: Differences between striatal region and brain hemisphere, but not sex

John Meitzen*, Kelsey R. Pflepsen, Christopher M. Stern, Robert L. Meisel, Paul G. Mermelstein

Department of Neuroscience, University of Minnesota, Minneapolis, MN, United States

ARTICLE INFO

Article history:
Received 2 August 2010
Received in revised form
27 September 2010
Accepted 7 October 2010

Keywords:
Striatum
Sexual dimorphism
Rat
Nucleus accumbens
Lateralization
Morphometry

ABSTRACT

Both hemispheric bias and sex differences exist in striatal-mediated behaviors and pathologies. The extent to which these dimorphisms can be attributed to an underlying neuroanatomical difference is unclear. We therefore quantified neuron soma size and density in the dorsal striatum (CPu) as well as the core (AcbC) and shell (AcbS) subregions of the nucleus accumbens to determine whether these anatomical measurements differ by region, hemisphere, or sex in adult Sprague–Dawley rats. Neuron soma size was larger in the CPu than the AcbC or AcbS. Neuron density was greatest in the AcbS, intermediate in the AcbC, and least dense in the CPu. CPu neuron density was greater in the left in comparison to the right hemisphere. No attribute was sexually dimorphic. These results provide the first evidence that hemispheric bias in the striatum and striatal-mediated behaviors can be attributed to a lateralization in neuronal density within the CPu. In contrast, sexual dimorphisms appear mediated by factors other than gross anatomical differences.

Lateralizations and sex differences in neural circuitry and behavior occur across animal taxa and brain region. These brain dimorphisms can take many forms, including but not limited to differences in neuroanatomy, neurochemistry, and electrophysiology [37,3]. In some model systems, these differences are dramatic and have a fairly straightforward connection to behavior [39,34]. In other areas, these differences may be more subtle, although no less important. One of these regions is the mammalian striatum, including the dorsal striatum (CPu) and the core and shell subregions of the nucleus accumbens (AcbC and AcbS, respectively). Though clear lateralizations [40,12,7,6,32,4,5,19] and sex differences [24,2,11] exist in the striatum and striatal-related behaviors and pathologies, whether this is reflected in striatal neuron morphology remains largely unknown. Given that differences in neuronal soma size and density have previously been found between hemispheres and sexes in other brain regions, we decided to quantify these neuronal attributes in the CPu, AcbC and AcbS of adult gonadectomized male and female rats. Gonadectomized rats were used to avoid any potential confounds induced by steroid sex hormones, which exert activational effects on striatal neurons [2,23,14] beyond baseline sex differences [2,11]. We found that neuron soma size was larger in the CPu than the AcbC or AcbS. Neuron density was greatest in the AcbS, intermediate in the AcbC, and least dense in the CPu. While no attribute was sexually dimorphic, one lateralization was detected: neuron density in the CPu was greater in the left versus right hemisphere. These results indicate that the different striatal regions can be distinguished based upon neuron soma size and density, provide an anatomic basis for previous findings regarding lateralization in the striatum and striatal-mediated behavior, and that sex differences in striatal-mediated behaviors are driven by factors other than neuron soma size and density.

1. Animals

The Institutional Animal Care and Use Committee at the University of Minnesota approved all procedures used in this study. Four adult male and four adult female Sprague–Dawley rats were purchased from Harlan Laboratories. Rats were gonadectomized on day 60 of life at Harlan Laboratories. The absence of gonads was verified post-mortem. Food and water were available *ad libitum*, and animals were maintained on a 14-h light, 10-h dark cycle in a climate controlled colony.

2. Brain histology

On day 75 of life, animals were deeply anesthetized using pentobarbital (200 mg/kg, *i.p.*) and perfused transcardially with saline

* Corresponding author at: Dept. of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church St SE, Minneapolis, MN, 55455, United States.
Tel.: +1 612 625 0784; fax: +1 612 626 5009.
E-mail address: meitz010@umn.edu (J. Meitzen).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.10.017

until liver clearance, and then with 300 ml of 4% paraformaldehyde over 12 min. The anticoagulant heparin (0.5 ml of 1000 UPS units/ml) was injected into the left ventricle prior to perfusion. Brains were post-fixed overnight in 4% paraformaldehyde at 4 °C, cryoprotected in 30% sucrose solution in 0.1 M phosphate-buffered saline (PBS), and then sectioned coronally (50 μm) on a freezing microtome. The right hemisphere of each brain was nicked to distinguish hemispheres. Every other section was mounted onto slides, stained with cresyl violet, and imaged using a Leica DM4000B light microscope coupled to a Leica DFC500 digital camera. The cresyl violet stain was used to provide continuity with previous studies, because the stain effectively distinguishes between neurons and glia (discussed further below), and because other laboratories have found no difference in the morphology of D1 and D2 expressing medium spiny neurons [33]. Given the distribution of soma sizes measured by this study and the preponderance of medium spiny neurons in the striatum [35,10], the vast majority of the neurons measured in this study were medium spiny neurons.

3. Morphometry

Morphometric methods were adapted from previous studies [21,27,36,38], and followed the principles of unbiased stereology [30]. Neuronal density and soma size were measured in both hemispheres and along the rostral-caudal axis of the CPu, AcbC and AcbS. Neuron density was measured using protocols adapted from previous studies [38], including those that analyzed striatal tissue [27,36]. Briefly, following previous analyses [27], a large counting box (199 μm × 149 μm) was placed within the CPu, AcbC or AcbS in a systemic and random manner. A large box was used to minimize sampling variance by ensuring equal sampling of both patch and matrix. Patch and matrix medium spiny neurons have similar morphology and intrinsic membrane properties [17,18]. At least two of these large fields were counted in each hemisphere, and at least 200 neurons were counted per hemisphere per region. Neuronal nucleoli were used as the unit of count to obtain estimates of neuron density that were unbiased by soma size or shape. Nucleoli that were bisected by either the left boundary or the upper boundary of the counting frame were counted, whereas those that were bisected by the right boundary or the lower boundary were not counted. Along the *z*-axis, nucleoi were counted if they were bisected by the upper boundary but not the lower. To account for sectioning artifacts at the tissue edge (e.g., lost nucleolus caps), nucleoli in the top and bottom 3 μm of the section were not included in any count. The size of neuronal nucleoli did not differ between groups, so it is unlikely that splitting errors introduced asymmetric bias to our data [29,15]. Density was calculated by dividing neuron count by the volume of the tissue sampled and averaging across sampling boxes. The reliability of our neuron density measurements was assessed by having an experimenter blindly re-measure neuron density in multiple fields across subjects. Neurons were distinguished from glia by the clear presence of a nucleolus, a well-defined nuclear envelope, nongranular cytoplasm, and/or an obvious axon hillock, as in previous studies [21,38,20,28]. Measurements were made blind to treatment group.

Methods for measuring neuron size were adapted from previous studies [21,36,38]. Neuron size was measured using the cross-sectional area of the soma. A minimum of 100 neurons per region were measured, and this sample size was sufficient to encompass the entire range of variability in striatum soma area. Neurons were distinguished from glia as described above, and all measurements were made blind to treatment group.

4. Statistics

We used a one-way (two-tailed) ANOVA and Tukey's post hoc tests to make comparisons between brain regions, and two-way repeated measures ANOVA and Tukey's post hoc tests to make comparisons between sex and hemisphere within a brain region. Software used was Prism 4.03 (Graphpad, La Jolla, CA) and SigmaStat 3.00 (SPSS, Chicago, IL). Probability values less than 0.05 were considered *a priori* as significant. Data are presented as mean ± SEM.

5. Results

We first compared neuron soma size and density across striatal regions independently of sex or hemisphere using a one-way ANOVA with Tukey's post hoc test. We found that neuron soma size was larger in the CPu compared to the AcbC or AcbS (Fig. 1A; $p < 0.0001$ for both; $F_{2,21} = 36.70$), and did not differ between the AcbC or AcbS ($p > 0.05$). Neuron density likewise varied across striatal regions. Neurons were denser in the AcbS compared to both the CPu and the AcbC (Fig. 1B; $p < 0.001$ for both; $F_{2,21} = 59.84$), and in the AcbC compared to the CPu ($p < 0.001$). These data indicate that different striatal regions can be distinguished based upon neuron size and density.

After determining that neuron soma size and density differed between the CPu, AcbC and AcbS, we compared these measures between hemisphere and sex in each striatal region using two-way repeated measures ANOVAs (Table 1). We found no differences in neuron soma size between sex, hemisphere, or the interaction between sex and hemisphere in the CPu (Fig. 2A), AcbC (Fig. 2B) and AcbS (Fig. 2C).

A difference was detected, however, in neuron density. Neuron density was lateralized in the CPu, with neurons being denser in the left hemisphere than the right (Fig. 3A, $p < 0.05$; $F = 7.152$). No differences in neuron density were found between hemispheres in the AcbC (Fig. 3B) or the AcbS (Fig. 3C). We also found no differences in neuron density between sex or the interaction between

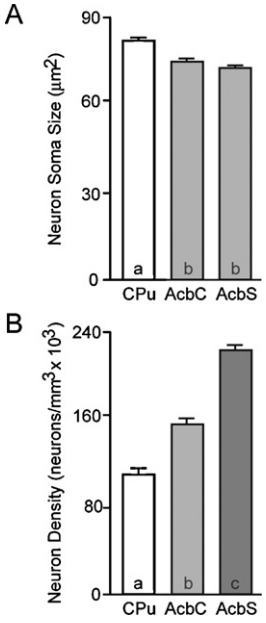

Fig. 1. Neuron soma size and density differed between the CPu, AcbC and AcbS. (A) Neuron soma size was significantly increased in the CPu compared to the AcbC and AcbS ($p < 0.0001$ for both). (B) Neurons were denser in the AcbS compared to both the CPu and the AcbC ($p < 0.001$ for both), and in the AcbC compared to the CPu ($p < 0.001$).