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Research Report

Neuronal composition of the magnocellular division of the medial preoptic nucleus (MPN mag) is sex specific in the Syrian Hamster (*Mesocricetus auratus*)

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ABSTRACT

The magnocellular division of the medial Preoptic nucleus (MPN mag) plays a critical role in the regulation of male sexual behavior in the hamster. Results from previous studies indicated that the number of neurons in the MPN mag is greater in males than females but failed to find significant differences in the volume of the nucleus suggesting that other elements in the nucleus may be greater in the female. The results of the present study, using NeuN to identify neurons, are in line with this hypothesis. The data show that (1) neurons in the MPN mag display two distinct phenotypes, those with a single nucleolus and those with multiple nucleoli; (2) the percentage of each phenotype is sex specific, differing over the course of development and (3) there is no sex difference in the number of glial cells at any age. Sex differences in the numbers of each type are correlated with developmental milestones and suggest that morphological changes are influenced by changes in circulating gonadal steroids during development.

1. Introduction

Sex differences in behavior have been described in a variety of species but the mechanisms within the brain that underlie these differences are not completely understood. Males and females of most species display sex specific behaviors such as parenting, aggression and sexual behavior (Cooke et al., 1998) suggesting that the networks that subserve these behaviors are sexually dimorphic. A number of morphological parameters have been correlated with sex differences in behaviors in both bird and rodent brains. Nuclei in the network that mediates song production are larger in males than in females in the canary and zebra finch (Nottebohm and Arnold, 1976). Differences within the song nuclei are correlated with the male's need to sing as a courtship ritual; females do not sing. Correlation of sexual dimorphisms with behavior finds less support in the rodent hypothalamus. There are dramatic sex differences within subdivisions of the medial preoptic area (MPOA), a large region implicated in the regulation of male sex behavior (Cooke et al., 1998). For instance, the sexually dimorphic nucleus (SDN) is 5× larger in volume in male rats than their female counter parts (Gorski et al., 1980) and the pars compacta (SDApc) of the

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Abbreviations: MPN mag, Magnocellular division of the medial preoptic nucleus; MPOA, Medial preoptic area; FHVS, Female hamster vaginal secretions; P, postnatal day; PBS, phosphate buffered saline; SDA, Sexually dimorphic area; BST, Bed nucleus of the stria terminalis; SDN, Sexually dimorphic nucleus; LMAN, Lateral magnocellular nucleus of the anterior neostriatum

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sexually dimorphic area is robust in males and small to nonexistent in female gerbils (Commins and Yahr, 1984). But the role of these areas in behavior is not clear. Lesions of the SDN do not affect copulation in sexually experienced rats, (Arendash and Gorski, 1983), and lesions of the SDApc have no effect on copulation in gerbils (Yahr and Gregory, 1993). Moreover, areas that do play a role in copulatory behavior, such as the dorsal MPOA (dMPOA) of the rat and the medial and lateral aspect of the sexually dimorphic area (SDA) in gerbils, are not sexually dimorphic in volume (Arendash and Gorski, 1983; Commins and Yahr, 1984; Yahr and Gregory, 1993).

In hamsters, the magnocellular subdivision of the medial preoptic area (MPN mag) plays a critical role in male typical sexual behavior. Electrolytic lesions that include the MPN mag eliminate copulation in hamsters (Powers et al., 1987). Several lines of evidence suggest that the MPN mag serves to integrate internal hormonal information, indicating reproductive state, with external cues from reproductive partners. Exposure to pheromonal cues stimulates cells in the MPN mag (Fiber and Swann, 1996; Wood and Newman, 1995b). The MPN mag contains receptors for both estrogen and androgens (Wood and Newman, 1995a) and gonadal steroids restore mating behavior in castrated males when they are implanted near the MPN mag (Wood and Williams, 2001) if the nucleus also receives pheromonal input (Wood and Newman, 1995b). The response to pheromones is sex specific. Only the MPN mag of males shows c-Fos protein in response to exposure by female hamster vaginal secretions (FHVS) (Fiber and Swann, 1996) suggesting that the MPN mag is sexually differentiated. Consistent with this hypothesis, the results of a rigorous stereological examination of cresyl violet stained tissue indicated that the MPN mag of adult male hamsters have significantly more neurons than those of females (Govek et al., 2003). Surprisingly, the volume of the MPN mag did not differ between the sexes suggesting additional elements (glial cells and/or neuropil) were greater in the female. The goal of the present experiment was to identify that element using the neuronal marker NeuN in conjunction with cresyl violet counterstain. Previous studies, designed to identify the time course of sex differences, reported the development of sex differences in cell number but failed to determine if the cells were neurons or glia (Govek and Swann, 2007). The current study extends these findings by examining the development of sex differences in neuronal types in the MPN mag.

2. Results

At ages p5/p10 main effects of age, sex, and cell type are all significant (F (1, 24)=26, $p<0.001$), (F (1, 24)=4.6, $p=0.042$), (F (1, 24)=752.1, $p<0.001$). The only significant interaction in the younger ages is between age and cell type (F (1, 24)=144.45, $p<0.001$), where multiple nucleolus neurons are greater in number than glial cells at both ages but the difference is larger for the p5 than for the p10 hamsters. At ages p30/p45/p60 there were significant interactions between cell type and sex (F (1.73, 74.46)=35.21, $p<0.001$) and between cell type and age (F (3.463, 74.46)=14.61, $p<0.001$); the different sexes were sexually dimorphic in neuronal type after p30 and numbers of cell type changed over time (Fig. 3). Interactions were determined using the Greenhouse–Geisser method.

2.1. *Single nucleolus*

As shown in Fig. 3, there were no neurons containing a single nucleolus at postnatal day 5; this neuronal type emerged by postnatal day 10. The number of single nucleolus neurons was not sexually dimorphic at p10 ($p=0.724$). By postnatal day 30 there was a significant sex difference in the number of neurons with a single nucleolus; males had more than females ($p=0.011$). Postnatal day 45 and p60 showed similar results ($p<0.001$ for both ages).

2.2. *Multiple nucleoli*

Neurons with multiple nucleoli were present at all ages and decreased over time (the number decreased more for males over time). At postnatal days 5, 30, 45, and 60 females had more multiple nucleolus neurons than males ($p=0.005$, $p=0.003$, p=0.030, $p=0.002$). At postnatal day 10 there was no sex difference in the number of neurons with multiple nucleoli.

2.3. *Total neuron number*

The number of both neuronal types was added to determine total neuron number. No sex differences were found in the total number of neurons except at p5 in which females had more than males ($p<0.006$ as shown in Fig. 4).

2.4. *Total cell number*

At postnatal day 5 females had a significantly greater total number of cells than males ($p=0.016$). The total number of cells decreased from postnatal day 5 to postnatal day 10 in females and males ($p<0.001$ and $p=0.017$ respectively). By p10 there were no differences in the number of cells ($p=0.756$); females lost more total cells than males by p10. There were also no significant sex differences in total cell number at ages p30, p45, or p60 between males and females ($p=0.119$, $p=0.798$, $p=0.942$).

2.5. *Glia*

There were no sex differences in the number of glial cells at any age ($p=0.531$). Number of glial cells decreased over time and slowly steadied by p30.

2.6. *Age*

The number of neurons with multiple nucleoli significantly decreased in both male and female hamsters during development. From p5 to p10 there was a significant decrease ($p<0.001$ for both), again from p10 to p30 ($p<0.001$ for males and $p=0.042$ for females), and from p30 to p45 ($p=0.016$ for males and $p=0.002$ for females). There was no significant decrease in neurons with multiple nucleoli from p45 to p60 for either male or female hamsters ($p=0.874$ and $p=0.517$).