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BRAIN RESEARCH

Short Communication

Sex differences in neuronal morphology in the killifish hypothalamus

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ABSTRACT

This study examined the neuroarchitecture of the male and female killifish (*Fundulus heteroclitus*) hypothalamus to evaluate whether sexual dimorphism of this brain region exists in fishes as it does in mammals and other vertebrates. The rostral medulla, a brain region distinct from the hypothalamic–pituitary–gonadal axis, was also examined to determine if any observed differences were region-specific. With the use of Golgi–Cox impregnation, five dendritic characteristics were measured from neurons of both the hypothalamus and medulla including: spine density, number of branch points, dendrite length, surface area and volume. Dendritic spines are associated with excitatory synapses, and changes in density are associated with a variety of normal and pathological changes. Consistent with mammalian studies, we found that adult female killifish have 25% greater dendritic spine densities in the hypothalamus than male killifish (densities of 0.34 ± 0.06 μm^{-1} and 0.25 ± 0.08 μm^{-1}, respectively). By contrast, no statistically significant difference between males and females was detected in spine densities in the rostral medulla. This finding supports the conclusion that hypothalamic sexual dimorphism is conserved in killifish.

Sexual dimorphism of the CNS has been described in all vertebrate classes, with the mammalian brain the most extensively studied. During development, steroid hormones from the differentiated gonads organize the brain into a masculine or feminine form. This process of aromatase-induced conversion of testosterone to estradiol is well-documented in mammals, as are the resulting morphological sex differences. Sexual differentiation is manifested partly through morphological sex differences in the ultrastructure of organelles, dendritic organization and the volume of distinct cell groups within the brain (Arnold and Gorski, 1984; De Vries, 2004; MacLusky and Naftolin, 1981; Simerly, 2002). A variety of neuroanatomical, morphometric sex differences have been described and include differences in the size of entire brain regions, volume of distinct sub-nuclei or projections, sex differences in soma size, dendritic length, branch number, spine synapses and total dendrite surface area. Morphometric sex differences of the largest magnitude generally occur in the hypothalamic nuclei of mammals (Amateau and McCarthy, 2004; Ayoub et al., 1983; Cherry et al., 1992; Commins and Yahr, 1984; Dohler et al., 1982; Gorski et al., 1980; Handa et al., 1985; Hines et al., 1985; Matsumoto

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Fig. 1 – Sexual dimorphic characteristics of the adult male (A) and female (B) *F. heteroclitus*. Male killifish are typically olive green and pale yellow in color with vertical silver bars and iridescent spots scattered along the body and fins. In spawning condition, the yellow coloring on the ventral half of the male changes from a pale to a brilliant yellow. Female killifish are typically brownish green in color, and their dorsal region is darker than their ventral region. The female exhibits black vertical bars, and no markings are found on the fins. The black bars fade when the female is in spawning condition.

and Arai, 1980, 1981, 1983, 1986; Mong et al., 1999; Tobet et al., 1986) and birds (Balthazart and Adkins–Regan, 2002) or in the song control nuclei of birds (Schlinger and Brenowitz, 2002; Wade and Arnold, 2004). Dendritic spines are the primary site of excitatory synaptic input and are a readily quantifiable marker of synapses. The density and/or number of spines on dendrites of hypothalamic neurons are markedly dimorphic, being higher in the preoptic area and ventromedial nucleus of males compared to females (Amateau and McCarthy, 2004; Pozzo-Miller and Aoki, 1991) but lower in the arcuate nucleus of males compared to females (Matsumoto and Arai, 1981, 1986; Mong et al., 1999). Thus, there is considerable regional heterogeneity in the directionality of dendritic spine density sex differences within the mammalian hypothalamus, and, in each instance, the role of estradiol is critical. CNS sexual dimorphisms of avian species are found in portions of the forebrain, collectively known as the vocal control region, and involve those nuclei related to song control. These nuclei are significantly larger in the male than in the female, with an increase in number and size of neurons (Arnold and Gorski, 1984). Differences between the two genders in the volume of preoptic nuclei within the CNS of amphibians and reptiles have also been reported. For example, in toads (*Bufo japonicus*), the regions of the brain involved in mate calling, the anterior portion of the preoptic nucleus and the amygdala pars medialis, are significantly larger in males than in females (Takami and Urano, 1984). Relatively little is known about the process of gender variation in the brains of fishes or the resulting morphological differences. To date, Bass (1992, 1996) provides the only evidence of gender difference in fish neuronal architecture. Similar to findings in birds and amphibians, Bass observed that the neuronal structures involved with reproductive vocalizations differ between male and female plainfin midshipman (*Porichthys notatus*). This species has two male morphs with divergent vocalizing patterns, which also differ in their neuronal architecture. The long hums of the Type I morph are notably distinguishable from the short grunts of both the Type II morph and the female. The dendrites and soma of the sonic motoneurons that innervate the sonic muscles are one to three times larger in Type I males than they are in both Type II males and females (Bass, 1992, 1996).

As part of the hypothalamic–gonadal–pituitary axis, the hypothalamus contributes greatly to reproductive function and behavior in vertebrates. The hypothalamus has been found to be the most sexually dimorphic region of the brain in higher vertebrate classes, however, no publications to date have focused on gender differences in hypothalamic neuronal structure in fishes. Thus, the objective of this study was to

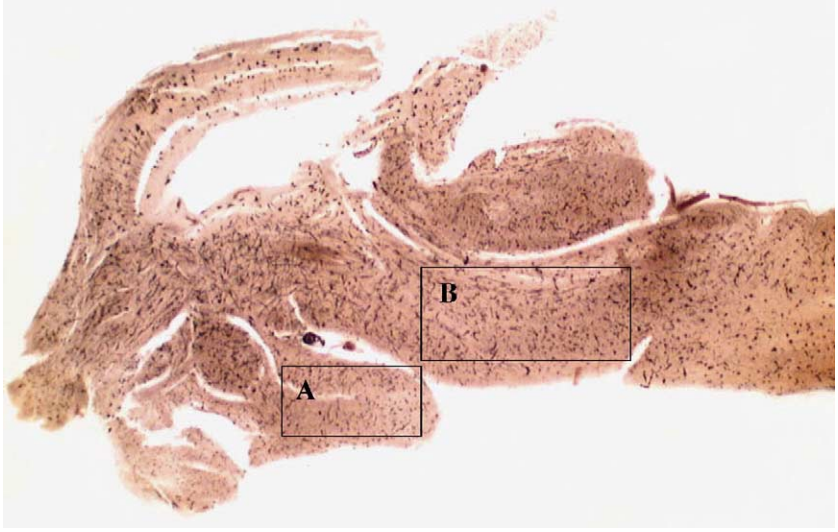

Fig. 2 – Sagittal section of a Golgi–Cox impregnated killifish brain. Anatomical regions analyzed for neuronal sexual dimorphisms included the hypothalamus (region labeled A) and the rostral medulla (region labeled B). Scale bar = 1 mm.