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Testosterone regulates the density of dendritic spines in the male preoptic area

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ABSTRACT

Male-typical behavior is dependent on testosterone. Castrated males gradually stop mating and engaging in sexual behaviors. Castrates treated with testosterone regain motivation and sex behaviors over time. Although this effect is well characterized, the specific mechanisms by which testosterone treatment recovers sexual behaviors remain unknown. The medial preoptic area (MPOA) is a likely site for testosterone's action on copulation. The integrity of the area is essential for the expression of male sex behavior; and the MPOA is densely populated with receptors for gonadal steroids. Moreover testosterone appears to regulate synaptic efficacy in the MPOA. Exposure to sexually relevant stimuli stimulates the MPOA but only in the presence of circulating testosterone. Sites afferent to the area respond to similar exposure independent of the hormonal milieu suggesting that testosterone mediates communication between the MPOA and its afferents. The protracted time course suggests that the effects of steroidal manipulation are mediated by structural changes. The present experiment evaluated this hypothesis by comparing dendritic spine density among Syrian hamsters that were castrated, castrated and treated with testosterone, or were left gonadally intact. Brains were sectioned and stained using the rapid Golgi stain protocol (FD Neurotechnologies, Baltimore), and the spine density, dendrite length, and the number of branches were compared among groups. Intact and testosterone replaced animals had more spines and greater spine density but did not differ in dendrite length and branching from castrated animals. These results suggest that existing dendrites increase the number of spines available for synapse formation but do not extend their dendrites in response to testosterone treatment.

Introduction

Testosterone plays an essential role in the development and maintenance of male sexual behaviors. Without testosterone, a male fails to mature sexually or express male-typical behaviors as an adult. In rodents, chemosensory signals originating from a female are also critical for the execution of male sex behaviors (Meredith, 1986; Murphy and Schneider, 1970). The chemosensory pathway that mediates this response is well documented and highly conserved.

The pathway originates in the main and accessory olfactory bulbs (Meredith, 1986), and proceeds through the medial (Me) and cortical (ACo) amygdala and the bed nucleus of the stria terminalis (BST) (Gomez and Newman, 1992) to converge on the medial preoptic area (MPOA) (Been and Petrulis, 2011). The MPOA plays a critical role in the regulation of mating behavior. Bilateral destruction of the MPOA impairs copulation in male rats, as well as most all mammalian species, and all species of birds, reptiles, amphibians, and fish that have been investigated (Dominguez and Hull, 2005; Paredes and Baum, 1995). The MPOA is a unique area whose nuclear groups are species specific. In the hamster the critical site for the regulation of male sexual behavior in the MPOA is a small, steroid receptor-dense, sub-region in its lateral aspect, the magnocellular division of the medial preoptic nucleus (the MPN mag) (Maragos et al., 1989); see Fig. 1. The MPN mag receives projections from the BST, Me and ACo and is densely populated with receptors for both androgens and estrogens (Wood and Newman, 1995). Destruction of the MPN mag disrupts male mating behavior, but leaves investigatory behaviors intact (Powers et al., 1987). Data suggest that testosterone regulates synaptic transmission between the extended amygdala and the MPOA that mediates responses to female pheromones. For example, exposure to pheromones stimulates the MPN mag in males with circulating testosterone but fails to do so in castrated males (Fiber and Swann, 1996). The amygdala and BST are stimulated in both castrates and intacts suggesting that the loss of testosterone leads to a loss of neural transmission between MPOA and the MeA/BST.

Structural synaptic plasticity is the fundamental trait of an adapting network (Stahnisch and Nitsch, 2002). Changes in synaptic efficacy underlie a wide variety of behavioral changes from cognition to hormone release and may play a role in the hormonal regulation of mating. Morphological changes associated with changes in the hormonal environment have been demonstrated in many regions of the brain (Parducz et al., 2006), Dendritic spines—small protrusions on dendrites—are at the heart of current studies of morphological plasticity. These structures play a critical role in synaptic transmission

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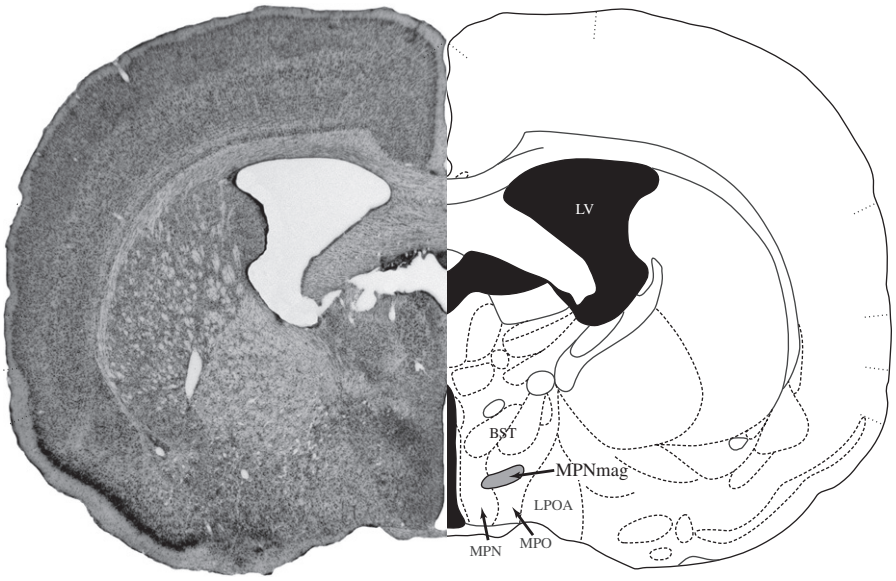

Fig. 1. The location of the MPN mag in a coronal section through the MPOA of a Syrian hamster (adapted from Morin and Wood 2001).

as more than 90% of excitatory synapses terminate on spines and appear to be a very important component of synaptic plasticity in the brain (Nimchinsky et al., 2002). These changes are very dynamic. Changes in spine density have been observed to occur over days, during the course of the rat estrus cycle (Woolley and McEwen, 1992). Recent advances have even allowed observation of spine formation and loss over the course of hours using two-photon microscopy on mice cortical cells in vivo (Yang et al., 2011).

Sex steroids play powerful roles in the regulation of spine plasticity. Estradiol mediates the fluctuation of hippocampal cell spine density during the estrous cycle of adult female rats (Woolley and McEwen, 1992), probably by acting on inhibitory interneurons that express estrogen receptors in the CA1 region (Rudick and Woolley, 2001). Data collected by Gould et al. (1990) demonstrate that female sex hormones modulate the number and shape of dendritic spines on cells of the rat hippocampus. Gonadectomized females exhibited decreased spine density in CA1 pyramidal cells that was prevented with estradiol treatment. The presence of estrogen receptors in the CA1 region suggests that the steroid plays a direct role in these changes. This hypothesis is further supported by the lack of changes in spine density in the granule cells of the rat hippocampus that lack steroid receptors (Gould et al., 1990). Changes in spine density can be attributed to an increase in the number of spines in the CA1 region per se, because no similar increase in branching or dendrite length has been found (Woolley and McEwen, 1994). These effects are not limited to the hippocampus. Similar results have also been reported in steroid rich regions of the limbic system and hypothalamus (Cooke and Woolley, 2005). Testosterone has also been shown to increase the spine density of neurons in the medial amygdala and dorsal hippocampus of rats (Cunningham et al., 2007; Li et al., 2012). To date the role of testosterone in the regulation of spine density in the preoptic area has not been examined.

The goal of this experiment was to determine the effect of testosterone on synaptic density of the MPN mag in males, by comparing the density of spines in intact males to those in castrates and castrates treated with testosterone. The study examines the hypothesis that testosterone regulates connectivity in the MPN mag by influencing the density of dendritic spines in that region.

Methods

All procedures were approved by the Lehigh's Institutional Animal Care and Use committee.

Animals

Adult male Syrian hamsters 60–75 days of age were obtained from stock bred in our animal facility and maintained on a 14:10 light/dark cycle with food and water freely available at all times. Each hamster was randomly placed in one of 3 groups: intact ($n = 7$), castrated + vehicle (referred to as "castrates") ($n = 7$), or castrated + T ($n = 7$). Animals in the castrated and castrated + T groups were castrated as described below at 60–70 days of age. Nine weeks post castration each of the males in the castrated + T, and castrated + vehicle groups was injected subcutaneously with 500 μg testosterone in 0.1 ml mineral oil (+T), or 0.1 ml mineral oil (+ vehicle) every other day for the next 3 weeks. This time course was chosen based on previous work which found that, in hamsters, castration will cause the loss of mating behaviors over the course of as much as 9 weeks (Whalen and Luttge, 1971), and the subsequent replacement of testosterone by injection or implant will recover these behaviors over the course of about 3 weeks (Debold and Clemens, 1978).

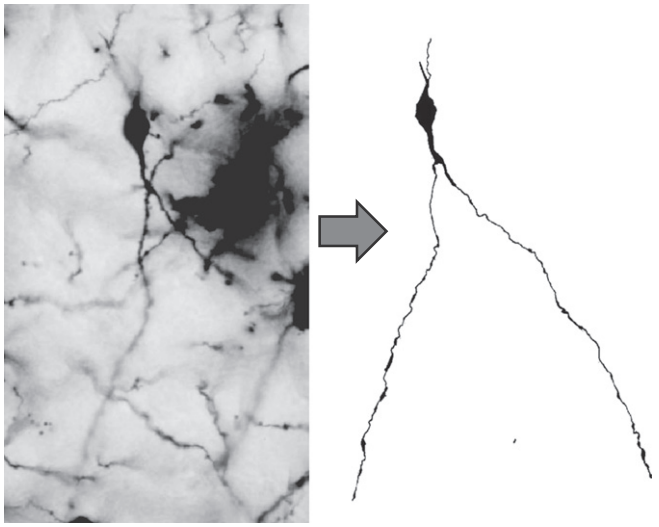

Fig. 2. An example of a neuron and its tracing for analysis. Neurons were traced using a scope with a tracing tube as section thickness and lack of clarity precluded automated means of analysis.