Neonatal castration of male rats affects the dendritic response to differential environments in granule neurons of the hippocampal dentate gyrus

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An earlier report from our laboratory demonstrated that there were sex differences in the dendritic tree of granule neurons from the rat dentate gyrus (Juraska, Fitch, Henderson, & Rivers, 1985). To investigate the hormonal basis for these sex differences, we castrated or sham-operated male rats within hours of birth. At weaning, rats from both hormonal groups were assigned to either a complex or an isolated environment. After 1 month in the differential environments, the rats' brains were stained with Golgi-Cox, and the dendritic fields of granule neurons were quantified from 28 animals. Castrated males showed a response to the environments (complex > isolated) that was similar to that of normal females from previous work. This plasticity did not occur in the sham-operated males. However, the castrated males had fewer dendrites than did sham-operated males overall, so that there were no differences between the sham-operated and castrated males in the complex environment, where sex differences (female > male) had been previously found. Therefore, males that were neonatally castrated partially mirrored females in their pattern of dendritic sex differences in dentate granule neurons.

Sex differences in brain structure are well documented in areas of the brain associated with reproduction in several species and are also found in other brain areas, such as the cortex and hippocampus, that are less clearly linked to reproductive functions (see review in De Vries, De Bruin, Uylings, & Corner, 1984). In our laboratory, we have found sex differences in neuronal structure in several brain regions. These include the visual cortex, in which there are differences in dendritic branching (Juraska, 1984) and in the axons of the corpus callosum (Juraska & Kopcik, 1988), and the hippocampus, in which there are dendritic differences in the dentate granule neurons (Juraska, Fitch, Henderson, & Rivers, 1985) and CA3 pyramidal neurons (Juraska, Fitch, & Washburne, in press). The possible hormonal basis for these differences has not been established.

In the neural areas studied thus far in mammals, androgens appear to be the primary determinants of sex differences, either directly or indirectly through aromatization to estrogen (Arnold & Gorski, 1984). Perinatal androgen levels appear to be the single most important hormonal influence on sex differences in the neural areas that have been studied, although there is growing recognition that pubertal hormones also have effects (Arnold & Breedlove, 1985; Bloch & Gorski, 1987). It would seem that

manipulating androgens would be a logical starting point for investigating the hormonal basis of the sex differences we have previously described in the hippocampus.

The dendritic sex differences in the dentate granule neurons (Juraska et al., 1985) were investigated in the present study. The sex differences in these neurons can be viewed from two perspectives. First, a difference exists between the sexes in their reactions to a postweaning environment that is relatively complex as opposed to an isolated one. We describe this as a difference in plasticity. Female rats had more dendritic material in their dentate granule neurons when raised in a complex environment than when raised in isolation; neurons from male rats did not display these differences. Second, sex differences occurred within each environment. Males had slightly more dendritic material than females when both were from the isolated environment. On the other hand, females had more dendritic material than males when both were raised in the complex environment. The present study was a start at understanding the possible hormonal basis of these two patterns of sex differences. We castrated males at birth and examined their granule neurons after rearing them in the two environments.

There are two ways that neonatal castration could influence the dendritic field of the granule neurons. First, the growth of dendrites could be altered, presumably because of the lack of testosterone during development. The second possible consequence of castration would involve the interactions with or response to the differential environments. Puberty occurs during the differential rearing period, so the lack of postpubertal testosterone could influence the neural response to these environments. Less

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direct effects on the response to the environment could also occur due to the absence of developmental testosterone. An effect of castration on the response to the environment appears likely, since the rearing environment was a critical variable in the sex differences that we previously reported in the dentate granule neurons. In this study, we found that both dendritic growth and plasticity were influenced by neonatal castration.

METHOD

Long-Evans hooded rats (descended from Simonsen Laboratories, Gilroy, CA, stock) were bred in the laboratory. The testes were removed under cryoanesthesia from 24 male rats from 12 litters within 6 h of birth. Twenty-four male littermates of these castrated males were sham-operated such that an incision was made. All rats were warmed to 37°C and returned to their mothers until weaning age (23–25 days).

At weaning, 12 littermate sets, consisting of 2 sham-operated and 2 castrated males, were assigned to the environments. From each litter, one rat from each hormonal condition was assigned to the complex environment and one to the isolated condition where they remained for 1 month.

In the complex environment condition (EC), 12 rats were group housed in a large wire-mesh cage (110×81.5×71 cm) with various objects that were changed daily. In addition, each EC group was allowed 30 min per day in an open field (1.2×1.2 m) with a new arrangement of objects. Rats from each hormonal condition were housed separately. (There were two complex environments.) This was done to replicate previous research in which male and female rats were housed separately (Juraska et al., 1985), and because the differences in play fighting between the hormonal groups (Meaney & Stewart, 1981b) could be more stressful for the castrated males. In the isolated condition (IC), rats were housed individually in standard stainless-steel laboratory cages (25×22×30 cm). These cages were located in the same room as the EC cages, and IC rats could see into the room. Food and water were constantly available, and the light:dark cycle was 12:12 h. All handling of the rats occurred during the light cycle.

The rats were sacrificed at approximately 55 days of age. This age is past puberty for male rats, as evidenced both by a rise in serum testosterone at 35 days and by behavioral indices (Meaney & Stewart, 1981a; Sachs & Meisel, 1979). Fifty-five days is also the age of the rats for which we previously described sex differences in dentate granule cells (Juraska et al., 1985). All rats were anesthetized with sodium pentobarbital and bled through the jugular vein. The brains were stained with Golgi-Cox according to Glaser and Van der Loos (1981). After staining was judged complete, based on test sections (cut by hand with a razor blade), coronal slabs containing the hippocampus were embedded in celloidin. To maintain a constant orientation across animals, coronal sections were cut at 150 µm at an angle that maximized the extent of the apical dendrites of overlying cortical pyramidal neurons within the section.

Seven littermate sets (28 animals) were used, and 20 neurons were sampled per animal. Neurons were traced with the aid of a camera lucida at 500× on tissue that was coded so that the person drawing did not know an animal's group. Neurons were chosen such that their cell bodies were in the middle of the section to minimize branches cut off at the plane of section. To ensure reliability among the different individuals drawing neurons, a separate experimenter made spot checks of neuron tracings.

Granule cells were sampled such that half (10) of the cells from each animal had their cell bodies in the upper one-third of the granule cell layer and the other half in the lower two-thirds of the cell layer. This sampling method takes into account the variability inherent in this cell population (Green & Juraska, 1985). The sampling area

was restricted. Cells were sampled from the dorsal blade only in a restricted anterior–posterior zone starting 150 µm from the most anterior level of the posterior commissure and extending 900 µm in a posterior direction. Only cells dorsal to the midpoint of the medial geniculate were used.

Dendritic branching was analyzed in two ways. First, the intersections between dendrites and a series of concentric rings centered on the cell body, calibrated at 20-µm intervals, were counted as in previous work (Juraska et al., 1985). Second, each branch segment was counted and its length measured. Counts were made at each order from the cell body, such that branches originating at the cell body were first order (1°); after one bifurcation, second order (2°); and so forth. For dendritic length measurements, each branch was classified according to whether the segment bifurcated, terminated within the section without bifurcating, or was cut off or obscured. No correction was made for the compression of a three-dimensional branch length into a two-dimensional tracing. Although branch length is distorted in absolute terms, the relative differences found are valid since the compression works against finding differences by increasing the variability of measured length.

Analyses of variance (ANOVAs) were performed on each concentric ring and branching measure using hormonal condition, environment, hemisphere, litter, and somal position as factors, with neurons nested within each animal. All analyses used animal, rather than neuron, as the unit of analysis. To investigate significant interactions, we performed post hoc tests by examining each group individually in an ANOVA. The SAS statistical programs were used.

RESULTS

Concentric Ring Analysis

The concentric ring analysis determines where dendrites are relative to the cell body. This analysis revealed that castration affected both dendritic plasticity and extent. Figure 1 illustrates the results of the concentric ring analysis. There was an environmental effect in Rings 4, 5, and 6 (EC > IC, $p < .03$). Rings 10 through 14 showed an effect of the hormonal condition (sham-operated > castrated, $p < .04$). The hormonal condition and environment factors interacted significantly ($p < .05$) in Rings 5, 6, 8, 9, and 10. Post hoc tests revealed that there was an environmental difference in the castrated rats (EC > IC, $p < .03$) in Rings 5, 6, 8, and 9. Also, there was a hormonal group difference in the isolated rats (sham-operated > castrated, $p < .05$) in Rings 6, 9, and 10. Thus, there were two major consequences of castration: (1) Castrated males displayed plasticity to the differential environments in the middle of the dendritic tree, whereas little plasticity was found in the sham-operated males (Rings 4, 5, and 6 would not have had a significant environmental effect if only the sham-operated males had been examined). (2) Castration decreased the extent of the middle and outer dendritic tree. The overall pattern of results is illustrated by the total number of ring intersections in Figure 2, even though the interaction shown in this figure fell just short of statistical significance ($p < .06$).

The position of the cell body within the granule cell layer significantly affected the distribution of the dendrites within the tree, as found in previous work (Green & Juraska, 1985; Juraska et al., 1985). In Rings 1 through 5, neurons whose cell bodies were within the upper one

third of the granule cell layer had more intersections ($p < .004$), whereas in Rings 9 through 15, the neurons in the lower two-thirds of the layer had more dendrites that intersected the concentric rings ($p < .02$). This pattern was similar to that reported in previous work with male and female rats (Juraska et al., 1985).

Examination of the significant interactions ($p < .05$) between hormonal conditions and layer position revealed that the sham-operated males had replicated previous work more closely than had the castrated males, who appeared to have a somewhat altered distribution of dendritic material between neurons in the upper one-third and the lower two-thirds of the granule cell layer. In Rings 3 through 6, neurons from castrated males did not show the significant differences in intersections due to layer position that were found in sham-operated males. In Rings 8, 10, and 11, castrated males had fewer dendritic intersections from neurons in the upper one-third of the layer than in the lower layer and than in the sham-operated rats. There were no differences in these rings due to layer position in the sham-operated males. Thus, the decreased concentration of dendrites in the outer dendritic tree of the castrated males illustrated in Figure 1 is disproportionate

Figure 1. The average number of intersections per neuron between the dendritic field and each ring in a series of concentric rings. Statistical significance for each ring is notated with an E for an effect of the environment, an H for an effect of hormonal condition, and an I for an interaction between the environment and hormonal condition.

Figure 2. The sum, averaged per neuron, of the intersections between a series of concentric rings and the dendritic field.

tionately due to a decrease in the dendrites of neurons high in the cell body layer.

The hemisphere factor was significant only once: there was an interaction between hemisphere and environment in Ring 12 ($p < .03$). A difference in only one ring can probably be attributed to chance. This is further confirmation of our previous observation (Juraska et al., 1985) that there are no systematic differences in granule cell dendrites due to their location in the right or left hemisphere.

Branch Analysis

Dentate granule cells do not branch with the regularity of cortical neurons; therefore, the branch order analysis is not as powerful as it is for the cortical neurons for which it was developed. Consequently, the differences between castrated and sham-operated males are not as evident in the branch analysis as in the concentric ring analysis. There was an environmental effect ($EC > IC$) in the number of fourth-order branches ($p < .03$), seventh-order branches ($p < .02$), and total number of branches ($p < .04$). Castrated males had more first-order branches ($p < .02$). The previously reported effects of layer position were replicated here: in branches from orders 1 through 3, there were more dendritic branches in neurons from the upper one-third than from the lower two-thirds ($p < .0001$), and in branch orders 4 through 7, there were more dendritic branches in neurons from the lower two-thirds than from the upper one-third ($p < .03$).

In the analysis of length, sham-operated males had longer whole dendrites (bifurcating plus terminal branches) ($p < .03$). The length of cut/obscured branches showed a hormonal condition $\times$ environment interaction

($p < .03$): castrated IC males had shorter branches than sham-operated IC males, but no differences were found between sham-operated and castrated EC males.

DISCUSSION

Neonatal castration of the male rat alters both the form and plasticity of the dentate granule cell dendrites. Castrated males mimic to some degree the plasticity in response to the differential environments that is seen in normal female rats, in contrast to the lack of plasticity seen in intact or sham-operated male rats. Thus, in terms of dendritic plasticity, castrated males appear to be feminized in this brain area. In addition, there is another effect: castration results in a smaller dendritic tree. This effect is especially obvious in the attenuated dendritic tree of castrated males from the isolated environment. Castration also affects the rats from the complex environment. Castrated rats do not significantly exceed sham-operated males at any level of the dendritic tree (although the trend is in that direction). Yet, in previous work females had more dendritic material than normal males in the complex environment. Thus, both EC and IC castrated males, while feminized in terms of plasticity, do not have as extensive a dendritic tree as do comparably housed females.

These results imply that the granule cell dendritic tree in normal female rats is influenced not only by the lack of testosterone during development, but also by either the developmental or postpubertal ovarian steroids. Evidence has been accumulating for years that the ovarian hormones play a role in development (Dohler et al., 1984; Gerall, Dunlap, & Hendricks, 1973; Stewart & Cygan, 1980; Toran-Allerand, 1986). There is also recent evidence that adult estrogen and progesterone can alter the sexually dimorphic nucleus in a female direction in adult, gonadectomized males (Bloch & Gorski, 1987). Thus the structure of the female rat brain may not be simply a result of the absence of steroids.

The timing of the testosterone effects cannot be elucidated from this study since the castrated males lacked testosterone during both postnatal development and postpuberty. It is not clear from the existing literature at which age testosterone would be more likely to have its effects. Greater female plasticity in the dentate gyrus has been described by Loy and Milner (1980) when they found that fimbrial/fornix transection resulted in more axon sprouting in females than in males. They later demonstrated that neonatal castration, but not adult castration, mimicked this effect in males (Milner & Loy, 1982). Also, Meyer, Ferres-Torres, and Mas (1978) found that prepubertal castration did not alter spine development in the dentate gyrus as it did in CA1. However, in the present study, plasticity was exhibited in response to environments that were instituted at weaning and maintained past the time of puberty. It is easier to understand how pubertal testosterone might play a direct role than to understand the indirect role that would be required for developmental testosterone to be responsible for the interactions with the

environment. It is plausible that developmental testosterone could more readily influence the overall growth of the dendritic tree. Still, the timing of testosterone effects on the dendritic tree awaits further research.

Regardless of the age at which it is effective, it is not known how testosterone interacts with dentate granule cells. Low levels of dihydrotestosterone bind in the adult hippocampus, but the cellular location has not been delineated (Stumpf & Sar, 1978). Around the time of puberty, there are no estrogen receptors in the dentate granule cells (Loy, Gerlach, & McEwen, 1988). However, many of the neurons afferent to the granule cells bind estradiol, including the interneurons of the infragranular hilar area and layers 2 and 3 of the entorhinal cortex, the neurons of origin of the perforant pathway (Loy et al., 1988). The cells of the medial septal area/diagonal band also bind estradiol (Pfaff & Keiner, 1973). It appears likely that the gonadal steroids influence the granule cells transsynaptically.

The effects of castration may be even more indirect than through afferents to the dentate gyrus. The lack of testosterone could affect other brain areas that alter the behavior of the animal and this, in turn, could alter the type of information reaching the hippocampus. In general, female rats tend to be more active than male rats. Neonatal castration increases the activity of male rats to near the level of females; this does not occur with later castration (Bengelloun, Nelson, Zent, & Beatty, 1976). It should be noted that, in spite of the greater activity of female rats, we have observed only minor differences in the interactions of intact male and female rats with the complex environment (Juraska & Meyer, 1986). Thus, the possibility that the effects of testosterone on the dentate granule cells are mediated via behavior remains open.

We do not know what the effects of the dendritic changes observed here would be on a behavior such as maze performance that requires an intact hippocampus (e.g., Olton & Werz, 1978). One problem is that the effects of castration are not restricted to the dentate granule cells. Just as sex differences can be found at some level in every part of the brain that has been examined, testosterone effects are undoubtedly widespread. This makes examination of the one-to-one relationship between a neural change and any complex behavior very difficult.

It should be noted that some of testosterone's effects on the dentate gyrus could occur through changes in cell number. Perinatal testosterone has been implicated in sexual dimorphisms in the SDN-mPOA and the SNB of the rat (reviewed by Arnold & Gorski, 1984). Cell division in the rat dentate gyrus continues during the postnatal period (Schlessinger, Cowan, & Gottlieb, 1975) when testosterone levels are high. Wimer and Wimer (1985) found sex differences in granule cell number in three out of six mouse strains. However, Yanai (1979) failed to find sex differences in granule cell number in the two strains of rats that he examined. This makes it less likely that there are cell number differences between the castrated and sham-operated rats.

The present study demonstrated that neonatal castration affects the growth and plasticity of the dendritic tree of dentate granule cells in the rat. There are still many unanswered questions on the role of gonadal steroids in this area. What other hormonal condition must occur to totally feminize the granule cell: developmental or postpuberal estrogen? progesterone? Does testosterone act directly on the cell or through other brain areas? Clearly, postnatal testosterone levels alone are not sufficient to account for the entire pattern of sex differences.

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