Correlation of Catecholamine Levels in the Bed Nucleus of the Stria Terminalis and Reduced Sexual Behavior in Middle-Aged Male Rats

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The correlation between dopamine (DA) and norepinephrine (NE) levels in the bed nucleus of the stria terminalis (BNST) and male sexual behavior was examined in middle-aged rats. Male rats (18–19 months) were divided into: (a) Group MIE, consisting of rats showing mounts, intromissions, and ejaculations; (b) Group MI, composed of rats showing mounts and intromissions, but no ejaculation; and (c) Group NC, consisting of noncopulators. Young adult rats (4–5 months) displaying complete copulatory behavior were used as the control. Tissue levels of DA, NE, and DA metabolites in the BNST were measured by high-pressure liquid chromatography. DA, but not NE, levels in MIE rats were significantly lower than those in young controls. DA and NE levels in MIE rats were significantly higher than those in NC rats. These results suggest that DA and NE in the BNST might play an important role in the control of male sexual behavior in middle-aged rats.

Key Words: Aging—Bed nucleus of the stria terminalis—Dopamine—Norepinephrine—Male sexual behavior—Rat.

Male sexual behavior declines with advancing age (1–5). Our previous study has further characterized qualitative variations in the sexual behavior of male rats aged 18–19 months; some rats already fail to exhibit any sexual performance at this age, other animals show only mounts and intromissions, but no ejaculation, and, interestingly, the others still display the same complete copulatory pattern seen in young male rats (6). Even with the ability of showing the complete copulatory pattern in some middle-aged male rats, there are significant differences between these animals and young males in various behavioral parameters, such as frequencies and latencies for mounting, intromission, and ejaculation. This indicates that, during aging, the differential decline in male sexual behavior occurs in subpopulations of male rats, which provides us an ideal model to study the mechanisms whereby the aging process involves the control of male sexual behavior.

Sexual behavior is mainly controlled by a well-organized neural circuit that connects a variety of brain regions, but the activation of this neural circuit is tightly regulated by hormonal milieu (7). Lack of sex steroids after castration (8) or genetic knockout of steroid receptors (9) impairs male sexual behaviors in rodents. However, the primary cause of the age-related deficits in mating behavior is believed to be deterioration of the central nervous system, rather than alterations in peripheral sex steroid hormones (3,4). In agreement with this viewpoint, we have previously found that, in middle-aged rats, loss of male sexual performance is associated with a decrease in the number of gonadotrophin-releasing hormone (GnRH) neurons in the forebrain (10) and a reduction in monoamine levels in the nucleus accumbens (NAcc) (11). Thus, our observations support that decline of male sexual performance at middle age might be due to alterations in the neural systems controlling sexual behavior.

The bed nucleus of the stria terminas (BNST) belongs to the limbic system and has reciprocal connections with the medial preoptic area (MPOA) and medial amygdala (MA) (12). These three brain regions all play important, but distinct roles in the regulation of male sexual behavior (13,14). In rodents, the MPOA residing in the hypothalamus controls the copulatory behavior, and the MA receiving the projections from both the main and accessory olfactory bulbs mediates the female odor-elicited chemoinvestigative behavior and noncontact erection (15). In male rats, lesion of the BNST causes moderate defects in copulation with reduced numbers of intromission and ejaculation as well as increased interintromission intervals and postejaculatory refractory periods, but severely impaired noncontact erection (13). This observation suggests that the BNST may regulate male sexual behavior through its connections with the MA and MPOA. In addition, the BNST contains abundant androgen receptors (16), and implanting testosterone in the BNST restores mating behaviors in castrated males (17). It further indicates that the BNST is essential for integrating both the chemosensory and hormonal signals to activate copulatory behavior in males (18,19).

While dopamine (DA) is believed to facilitate male sexual behavior in terms of motivation, copulation, and the genital reflex (8,20,21), norepinephrine (NE) appears to stimulate male sexual behavior, because blocking the a2 adrenergic
autoreceptor by yohimbine restores mating behaviors in sexually satiated male rats (8,22). The BNST receives DA innervation from the ventral tegmentum (23,24) and contains dense NE terminals projected from the medullary A1, A2 neurons, and the locus ceruleus (A6), suggesting that both DA and NE neurotransmission might influence the BNST function (25–27). Recently, male sexual performance has been reported to be related to tissue levels of DA, but not of NE, in the MPOA and/or arcuate nucleus, and ejaculatory behavior might be associated with critical DA tissue levels in the MPOA and/or arcuate nucleus in middle-aged rats (28). However, little is known about roles of DA or NE in the BNST in the control of male sexual behavior and whether their levels in the BNST change during aging. The aim of the present study was therefore to test the hypothesis that the different types of male sexual performance observed in middle-aged rats are correlated with different levels of DA and NE in the BNST.

METHODS

Animals and Behavioral Screening

Twenty-five middle-aged (18–19 months) and seven sexually experienced young adult (4–5 months) male Long-Evans rats were used. The animals were housed in a temperature-controlled room (22 ± 1°C) with a reversed 14:10 hour light/dark cycle (lights on at 10:00 pm) with food and water provided ad libitum. All middle-aged rats had, on various occasions, served as breeders or as sexual partners in other experiments. Before initiation of the present study, all the middle-aged rats had been rested sexually for approximately 6 months, and the young rats had been used only once as sexual partners at the age of 3 months and had been rested for approximately 1 month. All procedures were in accordance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Before beginning the experiment, the male sexual behavior of all rats was observed as previously described (6). The tests for each male rat began at 1 pm, and the observation period lasted 30 minutes. During the test, the room was dimly illuminated by a red lamp (10 watts). Each subject was first placed in a 45 × 35 × 35 cm Plexiglas test chamber for 5 minutes to habituate, and then two sexually receptive young female rats (3–5 months) were introduced. These females had been ovariectomized and received (subcutaneously) a 5 mm Silastic capsule filled with estradiol benzoate (Sigma, St. Louis, MO). Both females were replaced by others every 15 minutes to prevent the male from becoming satiated to the same female partners during the test. A total of eight female Long-Evans rats were used as sexual partners for the behavioral test. Because full male sexual behavior capacity is not always manifested in a single test, particularly in older animals, three copulatory tests were performed at intervals of 5 days. For a single copulatory test, the mount latency and intromission latency were computed as the time from the beginning of the test until the first response, and the ejaculation latency was calculated as the time from the initial intromission to ejaculation. The numbers of mounts, intromissions, and ejaculations in a 30-minute observation period were used to calculate the mount frequency, intromission frequency, and ejaculation frequency, respectively. Based on sexual performance, the middle-aged animals were assigned to one of three groups. The MIE group (n = 8) consisted of rats that showed a complete copulatory pattern, that is, mounts, intromissions, and ejaculations; the MI group (n = 8) consisted of rats that showed mounts and intromissions, but no ejaculation; and the NC group (n = 9) showed no copulatory behavior. Young adult rats (n = 7) displaying complete copulatory behavior were used as controls.

Measurement of Monoamines

All animals were killed by decapitation 1 week after the last sexual behavior test, and their brains were rapidly removed, immediately frozen in −20°C isopentane, and stored at −80°C. Brains were cut into serial sections of 200 μm on the frontal plane using a cryostat. The BNST was micropunched according to Palkovits’ method (29), and its location, schematically represented on the coronal diagrams (30), is shown in Figure 1. The micropunched tissue was homogenized in 0.1 N perchloric acid at room temperature and centrifuged at 3000 × g for 15 minutes. The supernatant was separated for 3 minutes. The supernatant was separated for 3 minutes.

Figure 1. Location of the micropunched sites in the bed nucleus of the stria terminalis schematically represented as circular areas on coronal diagrams of rat brain (modified from reference 30).
External standards of DA, DOPAC, HVA, and NE (Sigma) were dissolved in 0.1 N perchloric acid and run simultaneously with each experiment. The detection limit of our HPLC system for monoamines, defined as a peak height/noise ratio $>2$, was 40 pg.

**Data Analysis**

For individual animals, BNST levels of DA, DOPAC, HVA, and NE were normalized with levels of total protein and expressed as nanograms per milligram of protein. Statistical analyses of differences for the levels of DA, DOPAC, HVA, and NE as well as the ratios of DOPAC/DA, HVA/DA, and [DOPAC + HVA]/DA were performed by one-way analysis of variance (ANOVA) followed by a Scheffé test for post hoc comparison. A confidence level of $p < .05$ was considered statistically significant.

**RESULTS**

**Male Sexual Behavior**

These behavioral data were reproduced from our previously published article with permission (28). As shown in Figure 2, three qualitatively different types of male sexual performance were observed among middle-aged rats based on the presence of specific components of copulatory behavior during an observation period of 30 minutes. The MIE group showed a complete copulatory pattern, that is, mounts, intromissions, and ejaculations; the MI group displayed mounts and intromissions, but no ejaculation; and the NC group showed no copulatory behavior. In addition, the MIE group displayed higher mount and intromission frequencies, but shorter mount and intromission latencies than the MI group did (Figure 2). Although the MIE rats were able to display complete sexual behavior, their mount frequency, intromission frequency, ejaculation frequency, and ejaculation latency were significantly different from those of young controls.

**Levels of DA and its Metabolites in the BNST**

Among the three groups of middle-aged rats, DA levels in the BNST in MIE rats were markedly higher than those in NC rats [$F(1,16) = 10.879, p < .05$] (Figure 3A). Although DA levels in MI rats were lower than those in MIE rats, the difference was not statistically significant. There were no
significant differences between the three groups of middle-aged animals in levels of the main DA metabolites, DOPAC (Figure 3B), or HVA (Figure 3C). A statistically significant difference was seen in DA and HVA levels \( F(1,14) = 23.915 \) and \( F(1,11) = 19.425 \), respectively, \( p < .01 \) in the BNST between MIE and young rats (Figure 3, A and C).

In addition, as shown in Table 1, the DOPAC/DA ratio in the BNST was higher in NC rats than in MIE rats \( F(1,16) = 17.671, p < .01 \) or MI rats \( F(1,16) = 8.908, p < .05 \). In contrast, the HVA/DA and [DOPAC + HVA]/DA ratios were not significantly different between the three groups of middle-aged animals. There was no significant difference in these ratios between MIE rats and young rats (Table 1).

**Levels of NE in the BNST**

NE levels in the BNST in NC rats were significantly lower than those in MIE rats \( F(1,16) = 10.886, p < .05 \), but not those in MI rats (Figure 3D). However, BNST NE levels in MIE rats were not significantly different from those in young rats or MI rats.

**DISCUSSION**

One of the main findings reported here is that tissue levels of both DA and NE in the BNST decrease in association with impairment of sexual performance in middle-aged male rats. In the present study, NC rats showing no copulatory behavior had significantly lower levels of both DA and NE in the BNST than the MIE group, but MI rats lacking ejaculation showed no difference in DA or NE levels in the BNST. Our data suggest that DA and NE levels in the BNST might not be responsible for loss of the ejaculatory reflex, but may be prerequisite to the performance of male sexual behavior in rats.

Reduction of neurotransmitter content can be due to either enhanced release or decreased synthesis. The lower DA contents in the middle-aged groups are possibly due to decreased synthesis by aging. Increased brain levels of DOPAC are commonly taken to indicate enhanced dopaminergic activity (32). In the present study, no difference in DOPAC levels was seen among all four groups, suggesting no difference in the activity of DA neurons in the BNST in these animals. Although we did not observe any change in levels of DOPAC or HVA among these three groups of middle-aged rats, after normalizing to DA levels, a higher ratio of DOPAC/DA in the BNST was noticed in the NC group, suggesting that DA synthesis and/or metabolism in the BNST might also be critical for the regulation of DA neurotransmission in mating behaviors.

Regardless of age, young and MIE rats both showed a complete male sexual behavior pattern, but MIE rats displayed lower frequencies and longer latencies of mounting, intromission, and ejaculation (6). As compared to young rats, significantly lower levels of DA and HVA, but not DOPAC or NE, in the BNST were seen in the MIE group, and such a decrease in DA and HVA might be the cause for the deficits in copulatory proficiency seen in middle-aged rats. This result also suggests that, during aging, DA synthesis and metabolism in the BNST might become decreased and increased, respectively. Furthermore, in the BNST, the DA system seems to be much more vulnerable than the NE system during the aging process.

The lower BNST DA levels found in the middle-aged rats may be associated with the age-related decline in male

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**Table 1. DOPAC/DA, HVA/DA, and [DOPAC + HVA]/DA Ratios in the Bed Nucleus of the Stria Terminals of Young and Middle-Aged Male Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>DOPAC/DA Ratio</th>
<th>HVA/DA Ratio</th>
<th>[DOPAC + HVA]/DA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>0.68 ± 0.05</td>
<td>0.45 ± 0.07</td>
<td>1.13 ± 0.11</td>
</tr>
<tr>
<td>MIE</td>
<td>0.97 ± 0.10**</td>
<td>0.31 ± 0.04</td>
<td>1.16 ± 0.11</td>
</tr>
<tr>
<td>MI</td>
<td>1.43 ± 0.16*</td>
<td>0.44 ± 0.04</td>
<td>1.81 ± 0.17</td>
</tr>
<tr>
<td>NC</td>
<td>2.56 ± 0.42</td>
<td>0.56 ± 0.08</td>
<td>1.62 ± 0.12</td>
</tr>
</tbody>
</table>

*Notes: All values are expressed as the mean ± standard error of the mean.*

*p < .05 and **p < .01, significantly different from the NC group.

DOPAC = 3,4-dihydroxyphenylacetic acid; DA = dopamine; HVA = homovanillic acid; MIE = mounts, intromissions, and ejaculations; MI = mounts and intromissions, but no ejaculation; NC = noncopulatory.
sexual behavior seen in these animals. The significant difference in sexual performance between the MIE and NC groups is certainly not due entirely to the difference in catecholamine levels in the BNST observed in this study. Catecholamine contents in other brain areas related to sexual behavior, such as the MPOA, might also influence copulatory performance (8).

Lesions of the BNST in male rats cause moderate defects in copulatory behavior, with decreased numbers of intromission and ejaculation, but prolonged intromissory intervals and postejaculatory refractory periods (13). Unlike the males with BNST lesions, NC rats cannot display any copulatory behavior. Similarly, these middle-aged male rats show little sniffing and/or pursuit of female rats. The difference in behavioral deficits might be due to extensive deteriorations that occur in the BNST and other brain regions, such as NAcc, MPOA, and MA, of NC rats. In addition, deterioration in other neurotransmitter and neuropeptide systems might also contribute to the aging process that causes complete loss of copulatory behavior in male rats. In support of this opinion our previous study has demonstrated that low DA levels are also found in the NAcc of NC rats (11).

Our results strongly suggest that the different patterns of male sexual performance observed in the middle-aged rats may be related to the decline in catecholamine levels in the BNST. Furthermore, middle-aged male rats showing spontaneous declines in their ability to perform various sexual behaviors also provide a useful model for us to study and understand the mechanisms for the neural control of male sexual behavior.

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