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Monoamine levels in the nucleus accumbens correlate with male sexual behavior in middle-aged rats

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Abstract

The correlation between monoamine levels in the nucleus accumbens (NAcc) and male sexual behavior was studied in middle-aged rats. Male rats (18–19 months) were assigned to three groups: (1) Group MIE consisted of rats showing mounts, intromissions, and ejaculations; (2) Group MI was composed of rats showing mounts and intromissions, but no ejaculation; and (3) Group NC were non-copulators showing no sexual behavior. Young adult rats (4–5 months), displaying complete copulatory behavior, were used as the control group. Levels of dopamine (DA), serotonin, and norepinephrine and their metabolites in the NAcc were measured by high-pressure liquid chromatography with electrochemical detection. No difference was seen in DA levels between MIE rats and young controls, whereas DA levels in NC rats were significantly lower than those in both MIE and MI rats. Serotonin levels in NC rats were significantly higher than those in MIE and MI rats. Conversely, norepinephrine levels in NC rats were lower than those in MIE rats. These results suggest that monoamine levels in the NAcc correlate with sexual performance in male rats and that changes in NAcc monoamine levels might affect male sexual behavior in middle-aged rats.

Keywords: Aging; Nucleus accumbens; Dopamine; Serotonin; Norepinephrine; Male sexual behavior; Rat

1. Introduction

A gradual decline in male sexual behavior with age and age-related deficits of sexual behavior in male rats have been extensively documented (Chambers and Phoenix, 1984; Gray et al., 1981; Hsu et al., 1986; Larsson, 1958; Smith et al., 1992). A deterioration of the central nervous system rather than alterations in peripheral sex hormones is generally accepted as the main reason for these changes (Meisel and Sachs, 1994). Our previous study (Tsai et al., 1994a) showed that qualitatively different types of sexual behavior can be seen in male rats aged 18–19 months; some of them already fail to exhibit any sexual performance at this age, other animals show only mounts and intromissions, but no ejaculation, and interestingly, others are still able to display the same complete copulatory pattern seen in young males. There were also significant differences among these groups in parameters of male sexual performance, such as mount frequency, intromission frequency, mount latency, and intromission latency. Furthermore, male sexual performance is associated with changes in the number of gonadotropin-releasing hormone neurons in the forebrain in middle-aged rats (Tsai et al., 1997). Thus, these distinctly different types of male sexual performance might be due to changes in the neural systems controlling sexual behavior.

The nucleus accumbens (NAcc), which is principally innervated by dopaminergic neurons of the ventral tegmental area, is suggested to play a key role in sexual arousal and copulatory activity. Numerous studies have indicated that sexual motivation and copulation in male rats are associated with dopamine (DA) release in the NAcc during sexual behavior (Damsma et al., 1992; Fiorino et al., 1997; Fiorino and Phillips, 1999; Fumero et al., 1994; Mas et al., 1990; Pfaus et al., 1990; Pfaus and Phillips, 1991; Pleim et al., 1990; Robinson et al., 2001; Wang et al., 1995). Thus, we

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hypothesized that changes in the tissue levels of DA in the NAcc could correlate with the different types of male sexual performance seen in middle-aged rats.

DA is widely believed to facilitate male sexual behavior (Hull et al., 2002, 2004). However, little is known about whether the DA content of the NAcc affects male sexual behavior and, although stimulation of NAcc DA receptors stimulates sexual motivation and onset of copulation (Everitt, 1990; Moses et al., 1995), there is no direct evidence that changes in NAcc DA levels influence male copulatory behavior. In addition, since both serotonin (5-HT) and norepinephrine (NE) are reported to be involved in the regulation of male sexual behavior (Hull et al., 2002), it was also of interest to examine whether changes in tissue levels of both 5-HT and NE in the NAcc influenced sexual performance in male rats. Thus, the aim of the present study was to investigate whether the different types of male sexual performance observed in middle-aged rats correlated with differences in tissue levels of various monoamines in the NAcc.

2. Methods

2.1. Animals and behavioral screening

Twenty-five middle-aged (18–19 months) and seven young adult (4–5 months) sexually experienced male Long-Evans rats were used. The animals were housed in a temperature-controlled room (22±1 °C) with a reversed 14 h:10 h light/dark cycle (lights on at 2000 h) with free access to food and water. 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 rested sexually for approximately 6 months, while young rats were used only once as sexual partners at the age of 3 months and had rested for approximately 1 month.

Before beginning the experiment, the male sexual behavior of all the rats was observed using a previously described method (Tsai et al., 1994a). During the sexual behavior test, the animal room was dimly illuminated by a red lamp (10 W). Each subject was placed in a $45\times35\times35\,cm^3$ Plexiglas test chamber and allowed 5 min to habituate, then two sexually receptive young female rats (3–5 months) that had been ovariectomized and implanted subcutaneously with a 5 mm estradiol benzoate-filled Silastic capsule were placed in the chamber. Both females were replaced by others every 15 min to prevent the male from becoming bored by exposure to the same female partners. The tests were begun at 1300 h and the observation period lasted 30 min for each male rat. Since 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. After behavioral testing, the middle-aged animals were assigned to one of three groups based on sexual performance. The MIE group consisted of rats that showed a complete copulatory pattern, i.e. mounts, intromissions, and ejaculations, the MI group was composed of rats that showed mounts and intromissions, but no ejaculation, and the NC group showed no copulatory behavior. Young adult rats, displaying complete copulatory behavior, were used as controls.

2.2. Measurement of monoamines

Animals were sacrificed by decapitation one week after the last sexual behavior test and the brain rapidly removed and immediately frozen in −20 °C isopentane. Serial 200-μm-thick cryostat sections were cut on the frontal plane and the NAcc was micropunched according to Palkovits' (1973) method. The micropunched tissue was homogenized at room temperature in 0.1 N perchloric acid and centrifuged at 3000×*g* for 3 min, then the supernatant was assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and NE by high pressure liquid chromatography (HPLC) with electrochemical detection (Tsai et al., 1994b). A 12.5-cm-long, 5-μm C-18 reverse phase column (Waters Chromatography Division, USA) was connected to a carbon electrode set at a potential of +0.75 V relative to the Ag/AgCl reference electrode, in turn connected to an LC-4 amperometric detector (Bioanalytic System Inc., USA) in the HPLC system. The detection limit for monoamines, defined as a ratio of peak height to noise greater than 2, was 40 pg.

The mobile phase consisted of 1.75 g of heptanesulfonic acid, 0.1 g of disodium EDTA, 3.5 ml of triethylamine, 4 ml of phosphoric acid, and 40 ml of acetonitrile made up to 1 l in

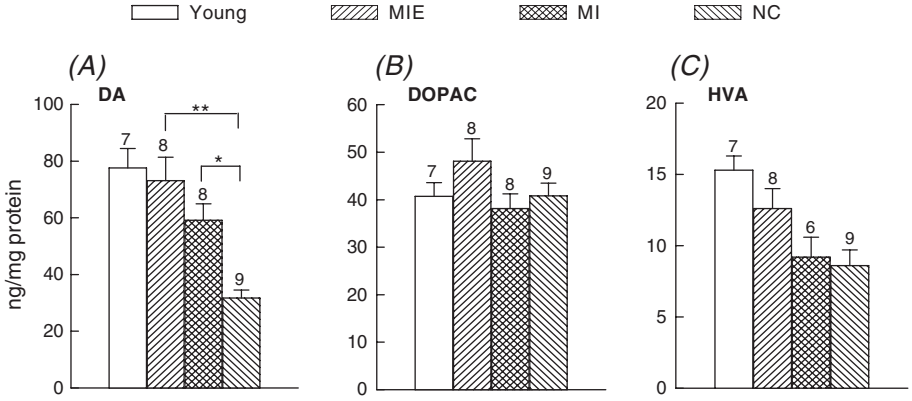

Fig. 1. Concentrations of DA (A), DOPAC (B), and HVA (C) in the NAcc of young and middle-aged male rats. Each bar represents the means±S.E.M. The number at the top of each bar indicates the number of rats used in the group. *$P<0.05$ and **$P<0.01$, significantly different from the NC group.

Table 1
DOPAC/DA, HVA/DA and DOPAC plus HVA/DA ratios in the nucleus accumbens of young and middle-aged male rats

Group	DOPAC/DA ratio	HVA/DA ratio	[DOPAC+HVA]/DA ratio
Young	0.56±0.07	0.21±0.02	0.77±0.09
MIE	0.73±0.11**	0.19±0.03	0.92±0.13**
MI	0.70±0.09**	0.18±0.02	0.82±0.11**
NC	1.36±0.12	0.27±0.01	1.62±0.12

All values are expressed as means±S.E.M.
** Significantly different from the NC rats ($P<0.01$).

Table 2
5-HIAA/5-HT ratio in the nucleus accumbens of young and middle-aged male rats

Group	5-HIAA/5-HT ratio
Young	2.18±0.18
MIE	1.48±0.16
MI	1.42±0.19
NC	1.44±0.22

All values are expressed as means±S.E.M.

distilled water and was filtered and degassed just prior to use. External standards of DA, DOPAC, HVA, 5-HT, 5-HIAA, and NE were dissolved in 0.1 N perchloric acid and run simultaneously with each experiment. The protein content of the tissue pellets solubilized in 0.5 N NaOH was assayed by the method of Lowry et al. (1951).

2.3. Data analysis

Statistical analysis of differences in the levels of DA, DOPAC, HVA, 5-HT, 5-HIAA, and NE and of the DOPAC/DA, HVA/DA, [DOPAC+HVA]/DA, and 5-HIAA/5-HT ratios was performed by one-way analysis of variance (ANOVA) followed by a Scheffè's test for post-hoc comparison. P values less than 0.05 were taken as the level of significant difference.

3. Results

3.1. Levels of DA and DA metabolites in the NAcc

DA levels in the NAcc in MIE and MI rats were markedly higher than those in NC rats ($F(1,16)=21.458$, $P<0.01$ and $F(1,16)=9.387$, $P<0.05$, respectively) (Fig. 1A). 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 (Fig. 1B) and HVA (Fig. 1C). When monoamine levels in the NAcc in young rats were measured to detect any age-related effects, no statistically significant difference was seen in the levels of DA or its metabolites in the NAcc between young and MIE rats (Fig. 1).

In addition, as shown in Table 1, the DOPAC/DA and [DOPAC+HVA]/DA ratios, but not the HVA/DA ratio, in the NAcc were higher in NC rats than in MIE ($F(1,16)=16.998$, $P<0.01$ and $F(1,16)=16.552$, $P<0.01$, respectively) and MI rats ($F(1,16)=18.824$, $P<0.01$ and $F(1,16)=20.790$, $P<0.01$, respectively) and there was no significant difference in these ratios between MIE rats and young rats (Table 1).

3.2. Levels of 5-HT and its metabolite in the NAcc

5-HT levels were significantly lower in MIE and MI rats than those in NC rats ($F(1,16)=13.555$, $P<0.05$ and $F(1,16)=10.004$, $P<0.05$, respectively), but there was no significant difference between MIE and MI rats (Fig. 2A). There were no statistical differences in NAcc levels of 5-HIAA, the main 5-HT metabolite (Fig. 2B), or in the 5-HIAA/5-HT ratio between the three middle-aged groups (Table 2).

No significant differences in NAcc levels of 5-HT (Fig. 2A), 5-HIAA (Fig. 2B) or in the 5-HIAA/5-HT ratio (Table 2) were seen between MIE rats and young rats.

3.3. Levels of NE in the NAcc

NE levels in the NAcc in NC rats were significantly lower than those in MIE rats ($F(1,16)=11.068$, $P<0.05$), but not those in MI rats (Fig. 2C). However, NAcc NE levels in MIE rats were not significantly different from those in young rats or MI rats.

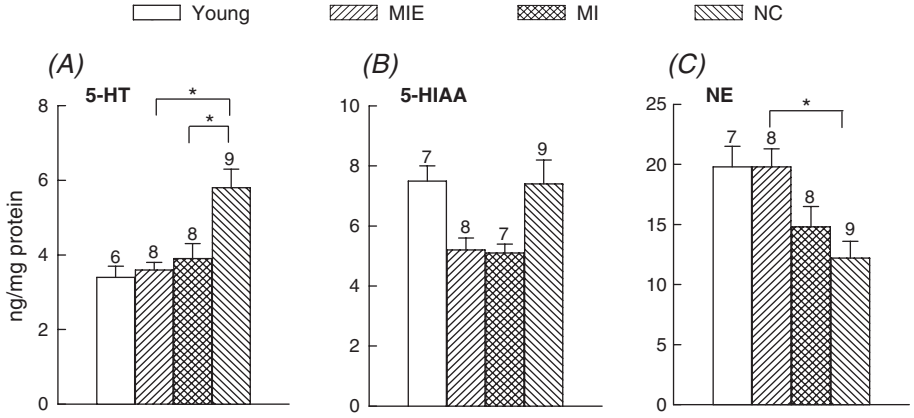

Fig. 2. Concentrations of 5-HT (A), 5-HIAA (B), and NE (C) in the NAcc of young and middle-aged male rats. Each bar represents the means±S.E.M. The number at the top of each bar is the number of rats used in the group. *$P<0.05$, significantly different from the NC group.

4. Discussion

The main findings reported here are that male sexual behavioral performance in rats is correlated with changes in tissue levels of monoamines (DA, 5-HT, and NE) in the NAcc, instead of age per se. Despite an age difference of 14 months between the young and MIE rats, both groups showed a complete male sexual behavior pattern and similar NAcc monoamine levels. In contrast, both NAcc monoamine levels and sexual performance in MIE rats were significantly different from those in the NC group, suggesting that the different components comprising male sexual behavior are affected differentially by senility and might be determined by tissue levels of NAcc monoamines.

In our previous study, although both young and MIE rats showed a complete sexual behavior pattern, young rats displayed markedly stronger sexual performance than MIE rats, revealed by the higher number and shorter latency of mounting, intromission, and ejaculation (Tsai et al., 1994a). Thus, such differences in copulatory activity between these two groups may be caused by other age-related changes in the neural and/or endocrine system regulating sexual behavior rather than by monoamine levels in the NAcc.

Among the monoamines, DA has facilitative effects on sexual motivation, copulatory proficiency, and genital reflex (Hull et al., 2004; Melis and Argiolas, 1995). DA in the mesolimbic tract activates numerous motivated behaviors, including copulation (Hull et al., 1990, 1991; Moses et al., 1995), while this monoamine in the medial preoptic area controls genital reflexes, copulatory patterns, and specifically sexual motivation (Edwards and Einhorn, 1986; Paredes et al., 1993; Pfaus and Phillips, 1991; Warner et al., 1991). On the contrary, 5-HT is primarily regarded as inhibitory (Ahlenius et al., 1971; Albinsson et al., 1996; Fernandez-Guasti et al., 1992; Kondo and Yamanouchi, 1997; Malmnas and Meyerson, 1971; Mitler et al., 1972; Verma et al., 1989; Waldinger et al., 1998), although stimulation of 5-HT_{2C} receptors increases erections and inhibits ejaculation (Millan et al., 1997; Steers and de Groat, 1989), whereas stimulation of 5-HT_{1A} receptors has the opposite effects (Fernandez-Guasti et al., 1992; Matuszewich et al., 1999; Rodriguez-Manzo and Fernandez-Guasti, 1994). The steady-state levels of metabolite/monoamine ratio can reflect the functional activity of monoaminergic neurons better than changes in concentration of amines. Increased brain levels of the monoamine metabolite 5-HIAA have been reported to indicate enhanced serotonergic nerve activity (Shannon et al., 1986). NAcc 5-HT levels in the NC group were significantly higher than those in both the MIE and MI rats; however, as shown in Table 2, there were no statistical differences in the 5-HIAA/5-HT ratio either between the three middle-aged groups or between the young and MIE rats. Thus, these results suggest that serotonergic activity in the NAcc may be not associated with male sexual performance in the two age groups.

Lesion of the locus coeruleus or systemic administration of the NE synthesis inhibitor, diethyl-dithiocarbamate inhibited copulation in one study (McIntosh and Barfield, 1984), but not in a more recent one (Fernandez-Guasti and Rodriguez-Manzo, 1997). Inhibiting α_2-receptors with yohimbine enhances sexual behavior (Clark et al., 1984; Smith et al., 1987) and yohimbine is also able to reverse sexual satiety (Rodriguez-Manzo and Fernandez-Guasti, 1994, 1995; Rodriguez-Manzo, 1999). However, its effects are blocked by the DA antagonist haloperidol, thus suggesting that its effects are mediated via the DA system (Rodriguez-Manzo, 1999).

Although the NAcc is mainly innervated by dopaminergic neurons from the ventral tegmental area, it also contains both serotonergic and noradrenergic terminals (Feldman and Quenzer, 1984). Since increased DA and DOPAC release in the NAcc of male rats has been shown to occur after exposure to estrous females, indicating a sexual arousal response (Damsma et al., 1992; Pfaus et al., 1990; Pleim et al., 1990; Wang et al., 1995), any changes in NAcc tissue levels of DA might affect sexual motivation in male rats. However, it is unlikely that the NAcc mediates sexual arousal in male rats entirely through the dopaminergic system. The roles that 5-HT and NE in the NAcc play in male sexual behavior and whether they interact in regulating sexual performance in male rats remain to be elucidated. As shown in Figs. 1A and 2C, although the tissue levels of both DA and NE in the NAcc of MI rats were lower than those in the MIE group, the difference was not statistically significant. However, NC rats showing little sniffing or pursuing of females or any copulatory behavior had significantly lower tissue levels of both DA (Fig. 1A) and NE (Fig. 2C) and significantly higher tissue levels of 5-HT (Fig. 2A) in the NAcc than the MIE and MI groups did, indicating that a critical level of NAcc monoamines may be a prerequisite for male sexual performance in rats. Although a significant increase in DA release in the NAcc has been shown when sexual motivation is elicited in male rats (Damsma et al., 1992; Fumero et al., 1994; Mas et al., 1990; Pfaus et al., 1990; Pfaus and Phillips, 1991; Pleim et al., 1990), it is not known whether release of 5-HT or NE in the NAcc is affected during sexual arousal.

Since the tissue levels of DA in the NAcc of NC rats were markedly lower than those in MIE rats, it is possible that NAcc DA levels in NC rats may be too low to release sufficient DA to reach the threshold level for initiating sexual motivation, and the animals consequently fail to display copulatory behavior. Because NAcc DA release in NC rats was not directly measured in the present study, we cannot say whether a lack or low level of DA release in the NAcc was responsible for the absence of sexual behavior in these animals. It is generally accepted that male copulatory behavior is mainly regulated by the medial preoptic area (Pehek et al., 1988; Stefanick and Davidson, 1987), while male sexual motivation is primarily controlled by the NAcc; with a contribution from the medial preoptic area (Everitt, 1990; Pfaus and Phillips, 1991; Pfaus and Everitt, 1995). Before male rats display copulatory behavior, sexual motivation must be initiated. Thus, the failure of NC rats to show any copulatory activity might be due to deficits in DA release mechanisms in the NAcc for sexual arousal; however, impairment of copulatory mechanisms per se in NC rats cannot be excluded.

The effects of age on levels of DA and its metabolites in the NAcc are still controversial. For example, it has been reported that DA and DOPAC levels and DOPA accumulation in the NAcc do not decrease with age in male rats (Demarest et al., 1980). A decrease in DA, DOPAC, and HVA levels in the mesolimbic system, including the NAcc, has been observed with age, whereas levels of 5-HT, 5-HIAA, and NE did not (Moretti et al., 1987). In contrast, DOPA accumulation in the NAcc 30 min after treatment with 3-hydroxybenzylhydrazine, an amino acid decarboxylase inhibitor, occurred at a slower rate in aged rats than in mature male rats, although no statistical differences in NAcc DA levels were seen between the old and mature groups (Watanabe, 1987). In addition, it has been found that tissue levels of various monoamines in the limbic system did not vary with age (Ponzio et al., 1984). DA turnover in the striatum is not affected by aging, as no differences in the various DA metabolite/DA ratios were seen between different age groups of rats ranging from 5–6 months to 27–28 months (Friedemann and Gerhardt, 1992). Our results obtained in young and MIE rats agree with these studies. However, a significant difference in NAcc DA levels was seen in the present study between MIE and NC rats of the same age. In addition, as shown in Table 1, there were significant differences in the DA metabolite/DA ratios in the NAcc between NC rats and the other two middle-aged groups. This difference is likely caused by the fact that the old animals used in earlier studies were not screened for behavior before sacrifice. Since variation in physiological functions between individuals is greater in older animals than in young adult animals, it may not be appropriate to compare young and old animals simply based on age. Thus, without any behavioral or physiological observation as an index, differences in monoamine levels between animals might have been masked by averaging. This also suggests that monoamine levels in the NAcc are not altered simply by aging, but may be more closely related to certain physiological functions, such as the ability to perform sexual behavior in male rats. Nevertheless, our findings still indicate that, during the aging process, some type of age-related change in the NAcc might influence the synthesis and metabolism of monoamines and result in changes in the levels of various monoamines in the NAcc. Subsequently, this change might inhibit the initiation of sexual motivation and orientation towards, and mounting of, females.

Although the NC rats failed to display any sexual behavior during the behavior test, this does not exclude the possibility that some might have exhibited mating behavior if the testing period had been prolonged. Similarly, we cannot exclude the possibility that some MI rats might have shown ejaculation if a longer testing period had been used.

In summary, our results indicate that changes in tissue levels of monoamines in the NAcc might correlate with changes in sexual motivation and may be indirectly related to male copulatory behavior in middle-aged male rats. In addition, our findings also suggest that the middle-aged male rat with a differential spontaneous decline in ability to perform different sexual behaviors provides a good model for studying the neural control of male sexual behavior.

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