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Learning-Induced Changes in D_2 Receptors of Rat Brain Are Sexually Dimorphic

SAKIRE PÖĞÜN,[1] LÜTFIYE KANIT AND BERRIN E. OKUR

Ege University School of Medicine, Department of Physiology, Bornova, Izmir 35100, Turkey

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PÖĞÜN, S., L. KANIT AND B. E. OKUR. *Learning-induced changes in D_2 receptors of rat brain are sexually dimorphic.* PHARMACOL BIOCHEM BEHAV **43**(1) 71–75, 1992.—Pharmacological agents known to stimulate monoamine systems improve memory, and destruction of the dopaminergic systems or dopamine depletion lead to impairments in various learning-related tasks. These reported effects of the central dopaminergic system imply the involvement of D_2 receptors. The aim of the present study was to investigate changes in [^{3}H]spiroperidol binding in seven areas of rat brain following informal and active avoidance learning. Littermate male and female rats were reared until 3 months of age in standard colony conditions and treated as active controls or in enriched environmental conditions and exposed to pole-jump active avoidance trials. Female rats acquired avoidance behavior more rapidly than males. Among the brain regions, only the hippocampus showed significant variations in D_2 receptor binding between the groups; sex differences and learning–sex interaction were observed in the corpus striatum. There was an inverse correlation between learning performance and hippocampal D_2 receptor binding. Our results show that learning affects hippocampal D_2 receptors in a sexually dimorphic pattern.

Learning Spiroperidol D_2-Receptors Sexual Dimorphism Hippocampus

LESION studies, direct pharmacological manipulations of dopamine (DA) activity, and self-stimulation studies provide evidence for the involvement of central dopaminergic neurotransmission in learning and memory. The role of DA receptor subtypes in learning and memory and the possible interaction between D_1 and D_2 receptors on behavior are presently being investigated by many laboratories. Activation of the D_2 receptor has been implicated in DA-mediated locomotion (3), stereotypy (2), and reward processes (14). Administration of spiroperidol (SPI) has been shown to impair the acquisition of a water Y-maze discrimination (24). Packard and White (21) recently pointed out the D_2 receptor involvement in memory-improving properties of DA agonists in the hippocampus and caudate.

Sex hormones affect the hippocampus, an area of the brain that is importantly involved in learning and memory processes. There is controversy on research with regard to possible sex differences in learning tasks. Female rats are better in active avoidance, but this may be reflecting differences in activity levels rather than cognitive capacities. Sex differences in some transmitter systems have been shown (15). In addition to the sexually dimorphic pattern of brain development and chemistry, brain responses to various experiences, including learning, may show sexual dimorphism.

The aim of the present study was to investigate changes in D_2 receptors following learning trials in various regions of rat brain, elucidate any related sexual dimorphisms, and correlate any observed changes in D_2 receptor binding with performance.

METHOD

Animals

Littermate Sprague-Dawley rats were assigned to differential conditions at weaning (25 days).

Differential Environmental Conditions

Rats were placed in enriched and standard colony conditions, providing differential opportunities for informal learning (25–125 days). Males and females were housed separately.

Active Avoidance Learning

An auditory stimulus was followed by a foot-shock. The rat had to jump onto a pole to avoid it.

- *Correct response*: The rat jumps onto the pole after the auditory stimulus and avoids the shock.

[1] To whom requests for reprints should be addressed.

- *Escape response*: The rat jumps onto the pole after the shock.
- *Response failure*: The rat does not jump onto the pole even after the shock.

The pole was removed in the active control group.

Experimental Protocol

Enriched Males and Females (EM and EF). Following 100 days in the enriched environment, rats were given active avoidance learning trials for 5 days (15 trials/day).

Active Control Males and Females (ACM and ACF). Following 100 days in standard colony conditions, rats were taken to the active avoidance chamber with the pole removed for 5 days (15 trials/day). They received the auditory signal and the shock in the same pattern with the experimental group.

At the termination of the experiments, rats were decapitated and brains were removed, dissected on ice, and weighed.

DA Receptor Binding (8)

Brain regions were homogenized in 100 vol 50 mM Tris-HCl (pH = 7.7) and centrifuged at 50,000 × *g* for 10 min twice. The resulting pellet was resuspended in Tris-buffer, ions, and ascorbate and used in 1 h for binding assays.

Incubation tubes, in triplicate, received [^{3}H]SPI (0.4 nM final concentration), Tris-buffer, ions, and ascorbate and tissue homogenate; 1 μM (+)butaclamol was used to define nonspecific binding. Incubation was carried out at 37°C for 40 min and terminated by vacuum filtration on GF/B filters followed by three 5-ml washes of ice-cold Tris-buffer. Bound radioactivity on the filters was counted after addition of omnifluor in toluene at 40% efficiency using a liquid scintillation counter.

Statistical Evaluation

Statpack and Epistat statistics programs were used.

RESULTS

Performance

One-way analysis of variance (ANOVA) revealed significant differences in performance between the 5 days of trials for both sexes regarding correct responses, correct + escape responses, and response failures ($p < 0.000001$ for all groups).

Female rats performed better than males in active avoidance learning trials. Figures 1A and 1B show learning curves with regard to correct responses or response failures; significant differences were observed on days 4 and 5. Similar results were obtained when escape responses were evaluated with correct responses.

D_2 Receptor Binding

[^{3}H]SPI binding in seven brain regions for all groups studied are shown in Fig. 2. One-way ANOVA revealed significant differences between groups only in the hippocampus ($p < 0.005$). Two-way ANOVA showed that the effect of learning on [^{3}H]SPI binding in the hippocampus was significant ($p < 0.025$). Two-way ANOVA also showed sex-related effects on [^{3}H]SPI binding in the corpus striatum ($p < 0.026$) and to a lesser extent in the hippocampus ($p < 0.058$). Significant interaction between learning and sex was also observed in the corpus striatum ($p < 0.003$).

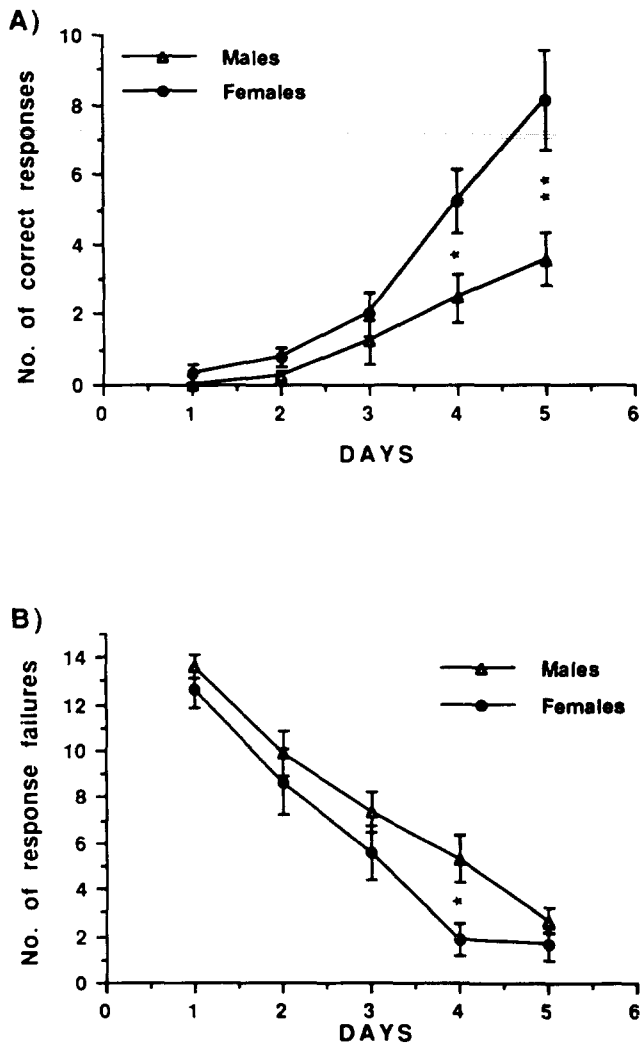

FIG. 1. Learning curves of male and female rats with regard to A) correct responses and B) response failures. The points represent mean ± SEM; $n = 13$ for males and $n = 9$ for females. *$p < 0.02$, **$p < 0.006$ (t-test).

Correlation Between Performance and [^{3}H]SPI Binding

A significant inverse correlation was observed between [^{3}H]SPI binding in the hippocampus and performance on day 5 of learning trials when data from male and female rats were handled together ($p < 0.0008$, Pearson's correlation coefficient) (Fig. 3).

DISCUSSION

There is a substantial body of experimental evidence concerning the role of hippocampus in learning and memory (6). Our results show that, of the brain regions studied, only hippocampal D_2 receptors were affected by learning trials; there was increased [^{3}H]SPI binding in male rats reared in enriched environmental conditions and exposed to active avoidance

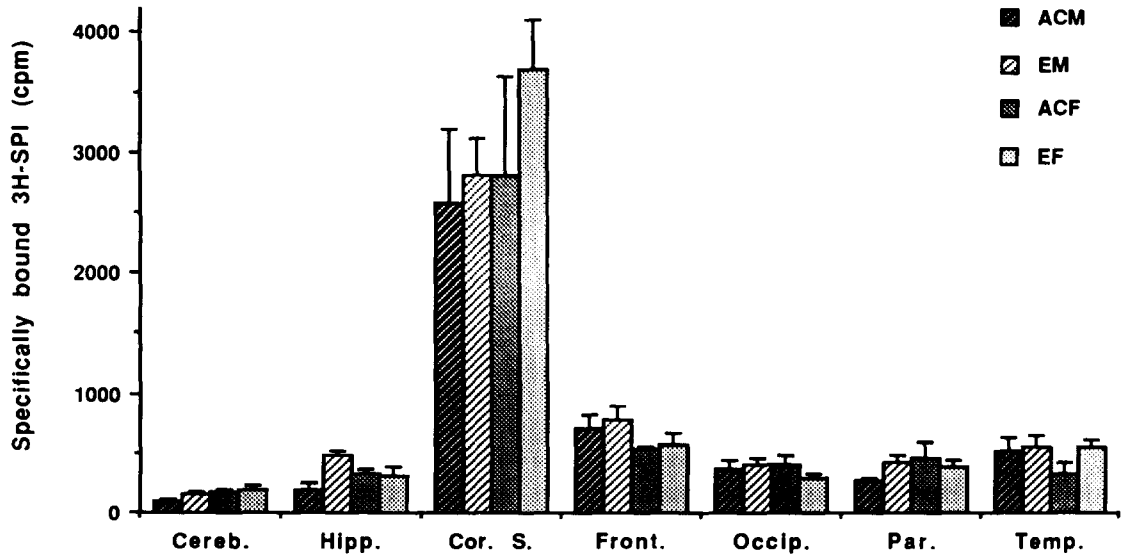

FIG. 2. [^{3}H]SPI binding in various regions of rat brain. The bars represent mean ± SEM for the four groups studied. The results of ANOVA are given in the Results section. *t*-test for ACM-ACF, $p < 0.036$ in the cerebellum; EM-EF, $p < 0.031$; and ACM-EM, $p < 0.0001$ in the hippocampus.

learning trials. Females did not respond in the same pattern. Despite an increase in D_2 receptor binding with learning in male rats, an inverse correlation was observed between learning performance and [^{3}H]SPI binding. Female rats performed better than males in active avoidance learning and no significant difference was observed between female rats' D_2 receptor binding. Memory-improving properties of DA agonists on tasks sensitive to both hippocampal and caudate lesions are mediated by the D_2 receptor (21). Administration of SPI impairs acquisition of a discrimination task in male rats (24).

The negative correlation observed between learning performance and D_2 receptor binding is hard to explain if the dopaminergic system is considered alone. If the availability of DA is assumed to be inversely related to D_2 receptor number via receptor downregulation, then it can be concluded that lower D_2 binding reflects higher levels of DA and results in better performance. In our study, we looked only at specific [^{3}H]SPI binding in various brain regions; saturation studies will give a better understanding of the dopamine D_2 receptors with regard to B_{max} and K_d values.

Previous studies have implicated both striatal (23) and septohippocampal (5,7,12) cholinergic function in memory. In the caudate, interaction between DA and acetylcholine (ACh) has been studied extensively (17). Recent evidence suggests

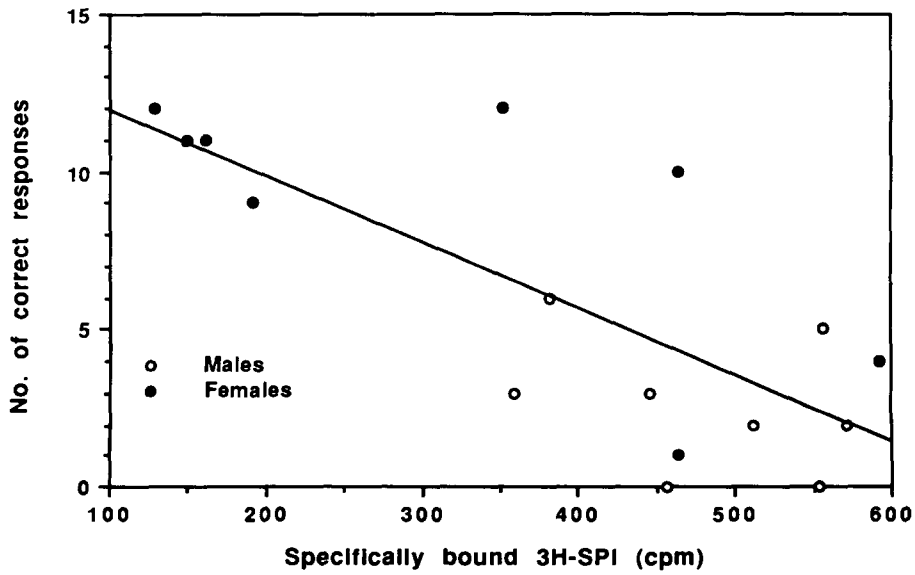

FIG. 3. Correlation between performance and hippocampal [^{3}H]SPI binding. $p < 0.003$.

that DA receptor modulation of ACh is D_2 subtype in the striatum (11,13,25,27). This may also be the case in the hippocampus (21). Other studies also point out to the ACh-DA link in memory processes (4,16,18,19). In view of these findings, it is clear that the DAergic neurotransmission cannot account for all the observed effects. Previous work from our laboratory has also shown sexually dimorphic effects of learning on central cholinergic systems under similar experimental conditions. Increased cholinergic activity was observed in the corpus striatum as a result of learning experiences, and male rats had higher muscarinic cholinergic receptor binding in the hippocampus compared to females (22).

Disruption of brain catecholamines by lesion or drugs can have dramatic effects on active avoidance behavior (20). These effects are due to a reduction in brain DA; DAergic pathways may be involved specifically in motor learning (26). In our study, we reversed the procedure almost totally and it was therefore not surprising to see the effects of learning on D_2 receptor binding, especially in the hippocampus and corpus striatum. Neonatal DA depletion also results in poor motor performance and maze learning in rats (1). Release of DA from neurons of the nigrostriatal bundle can promote memory consolidation and manipulation of cholinergic function in the caudate can improve or disrupt retention; DA release may act on a cholinergic substrate in the caudate to improve certain kinds of memory (28).

Our results show that there is sexual dimorphism with regard to both basal levels (active control groups) and learning-induced changes in D_2 receptor binding in the corpus striatum and hippocampus in rats. Crowley et al. (9) found higher DA levels in the caudate and arcuate nuclei and median eminence of males and Demarest et al. (10) in the tuberoinfundibular system of females.

CONCLUSION

Hippocampal D_2 receptors are involved in active avoidance learning in rats.

There is sexual dimorphism in D_2 receptors in the corpus striatum and hippocampus mediating in active avoidance learning.

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