Pharmacology Biochemistry & Behavior, Vol. 35, pp. 903–909. Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Scopolamine Differentially Disrupts the Behavior of Male and Female Wistar Rats in a Delayed Nonmatching to Position Procedure

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Received 9 December 1988

VAN HEST, A., J. STROET, F. VAN HAAREN AND M. FEENSTRA. *Scopolamine differentially disrupts the behavior of male and female Wistar rats in a delayed nonmatching to position procedure.* PHARMACOL BIOCHEM BEHAV 35(4) 903–909, 1990.—Evidence is available that pharmacological interference with the cholinergic system may disrupt behavior in experimental procedures designed to investigate learning and memory processes. Recently it has been suggested that the cholinergic system may be sexually dimorphic. The present experiment was designed to investigate whether or not manipulation of the cholinergic system differentially affected memory processes in both sexes. Male and female Wistar rats were exposed to a delayed nonmatching to position procedure and were challenged with increasing doses of scopolamine hydrobromide (a central and peripheral muscarinic receptor blocker) and scopolamine methyl bromide (which does not pass the blood-brain barrier). Response accuracy decreased in both sexes as the delay interval duration increased. Behavioral differences between saline-treated males and females were not observed. Response accuracy decreased dose-dependently after subjects were injected with scopolamine hydrobromide. Response accuracy also decreased after treatment with scopolamine methyl bromide, but to a smaller extent. Males showed less accurate responding after treatment with either drug than females. These results provide behavioral evidence for the hypothesis that cholinergic functioning may differ between the sexes.

Sex differences Cholinergic system Memory Delayed nonmatching to position procedure

IT has been suggested that the central cholinergic system may functionally be involved in memory processes. Reduced levels of choline acetyltransferase (CAT), the anabolic enzyme for acetylcholine (ACh), have been correlated with impaired memory during normal aging and dementia of the Alzheimer type in humans. It is assumed that the loss of CAT activity observed in Alzheimer's disease reflects a degeneration of the cholinergic neurons originating in the nucleus basalis of Meynert and the medial septal area (MSA), which provide the major cholinergic input to the amygdala and cortex, and the hippocampus respectively [(3), for a review]. Results of animal experiments have supported the cholinergic hypothesis of memory. It has now repeatedly been reported that the behavior of rats is disrupted after lesioning the nucleus basalis magnocellularis (NBM), a group of large, multipolar neurons in the ventro-medial globus pallidus, which appears to be the major source of ACh in the fronto-parietal cortex of the rat, and which is considered to be homologous to the primate nucleus basalis of Meynert (4, 19, 20, 22, 36). Others have shown that the behavior of rats in procedures designed to investigate memory function (passive avoidance, Morris water maze, radial maze, operant delayed response procedures) is impaired dose-dependently after subjects were challenged with pharmacological agents which directly interfere with cholinergic transmission [(39), for a review].

Evidence is available that the cholinergic system is organized in a sex-dependent way. Cholinergic enzyme activity in the MSA and the hippocampus reaches adult levels earlier in females than in males (24). CAT and AChE (the metabolic enzyme for ACh) activity is also higher in the adult female brain than in that of males, and is influenced by the female's estrous cycle. It has been

[1]This research was conducted while Annemieke van Hest was supported by a grant from the Netherlands Organization for Scientific Research (NWO 560-258-024) awarded to Frans van Haaren.

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hypothesized that sex differences in enzyme activity may be mediated by the female gonadal hormone estradiol (25–29, 31, 34). Furthermore, it has been shown that the total number of muscarinic binding sites for males and females varies across brain areas, but is generally higher in males than in females (50). Sex differences in muscarinic binding capacities have been reported to depend upon perinatal exposure to male gonadal hormones (1). Binding capacity in hypothalamic and amygdaloid tissue was increased after treatment with testosterone, whereas both estradiol and progesterone decreased the binding capacity. However, Miller (34) has reported that the binding capacity in striatal tissue is higher in females than in males. Age- and sex-dependent alterations in cholinergic enzyme activity have also been reported. CAT and AChE activity decrease with aging in both sexes, but differences between the sexes have been observed. Whereas enzyme activity in females decreases especially in the NBM, the largest decrease in males is observed in the MSA (30). It has also been shown that the density of muscarinic binding sites in the cerebral cortex, the hippocampus and the striatum decreases with age in both sexes. However, muscarinic receptor density in the brainstem of males increased with aging, whereas for females such increase was not observed (13).

If the cholinergic system is functionally involved in memory, then one may expect that the sexual dimorphism of the system differentially affects the behavior of males and females in experimental procedures designed to investigate learning and memory. Such seems indeed to be the case. Males show better retention when memory is investigated in passive avoidance procedures (9). Males also perform better than females when memory is assessed in complex maze procedures (2, 8, 21, 37). In addition, it was shown that disruption of the cholinergic input to the fronto-parietal cortex by lesioning the globus pallidus disrupted one-way avoidance behavior in males, but not in females (6). Recent observations in our own laboratory have shown that response rates of males are disrupted to a greater extent than those of females when subjects were treated with different doses of scopolamine hydrobromide, a centrally acting cholinergic antagonist (43). These results combined, thus, seem to provide evidence for the notion that cholinergic processes may underlie behavioral differences between the sexes in memory processes. However, two major problems may arise if one is to accept such a notion. First, it is at present not at all clear what aspects of behavior are affected by anticholinergic treatment. Evidence is available that scopolamine disrupts behavior in delayed response procedures independently of the duration of the delay interval (15,16). Since it may reasonably be assumed that drug effects on memory increase as working load increases, it has been suggested that impaired performance after anticholinergic treatment results from drug effects on nonmnemonic processes such as attention, discrimination or stimulus encoding (5, 7, 10, 12, 14, 15, 32, 33, 35, 39, 42, 47). The observation that scopolamine treatment disrupts visual and auditory discrimination performance at no-delay conditions is in agreement with such a notion (43). Secondly, sex differences in cognitive abilities are usually not observed when memory is assessed in different delayed response procedures covering a wide range of delay interval durations (41, 45, 46). It was shown that males and females attain similar levels of response accuracy, even though males seemed to reach final accuracy levels faster than females. As such, it was suggested that male and female rats are equally capable of processing, storing and retrieving information.

The present experiment was designed to answer the following questions: 1) are cholinergic mechanisms involved in memory functioning, and 2) can *behavioral* evidence be obtained for the proposed sexual dimorphism of the cholinergic system? Male and female rats were exposed to a delayed nonmatching to position procedure. In such a procedure, subjects are required to behave under the discriminative control of stimuli which are no longer present at the time of response execution. The time between stimulus presentation and the opportunity to respond (delay interval) can be experimentally manipulated. With such a procedure, one would be able to differentiate between treatment effects on memory per se, and other, nonspecific effects. Effects on memory processes would be reflected in a differential decrease in response accuracy with increasing doses of the drug and increasing delay interval durations, whereas effects on other, nonmnemonic processes would be delay-independent. Subjects were required to initiate the delay interval by pressing a response lever. They were challenged with different doses of scopolamine hydrobromide (a central and peripheral nonspecific muscarinic receptor blocker) and scopolamine methyl bromide (a quaternary compound which does not readily cross the blood-brain barrier) to assess whether or not manipulation of the cholinergic system differentially affected memory processes in both sexes.

METHOD

Subjects

Eight male and eight female rats were obtained from Animal House, TNO (Zeist, The Netherlands), when they were eight weeks old. Upon arrival in the laboratory, subjects were housed in group cages (four same sex subjects to a cage) under a reversed light-dark cycle (lights on 6:30 p.m.–6:30 a.m.). One week after arrival, subjects were food deprived on a 23-hour food deprivation schedule resulting in a weight loss to approximately 85% of free-feeding body weight (17). Body weights averaged 384.3 ($\pm$ 18.8) gram for males and 235.5 ($\pm$ 12.0) gram for females. Water was always available in the homecages. At the start of the present experiment, all subjects were eight months old. One of the male subjects died during the experiment. His results were excluded from all analyses.

Apparatus

Experiments took place in eight, locally constructed rat chambers (34 cm wide, 33 cm long and 37 cm high). The side walls and intelligence panel were made of black Perspex. The front door of the chamber was made of translucent Plexiglas. The floor consisted of 26 grids, spaced 1.3 cm apart. Two retractable rodent levers (2.5 cm long, 2.8 cm wide and 0.75 cm thick, when extended) were located symmetrically to the side of the pellet retrieval unit. The levers required a force in excess of 0.20 N to be operated. A stimulus light (green on the left- and red on the right-hand side of the intelligence panel) was located 9 cm directly above each lever. The pellet retrieval unit, which was located in between the two levers, could be illuminated by a white light. A houselight was mounted in the middle of the intelligence panel 3 cm from the ceiling of the chamber. All experimental chambers were enclosed in a sound-attenuated, ventilated cabinet; the front door of this cabinet was also made of translucent Plexiglas. The chambers were connected to a PDP 11-73 microcomputer (Digital Equipment Corporation, Maynard, MA), located in an adjacent room, through a locally constructed RSX 232 interface. Experimental contingencies and data acquisition procedures were programmed using SKED-11 (38), obtained from State Systems Inc. (Kalamazoo, MI).

Procedure

Preliminary training. Lever press training was not necessary since all subjects previously participated in another experiment

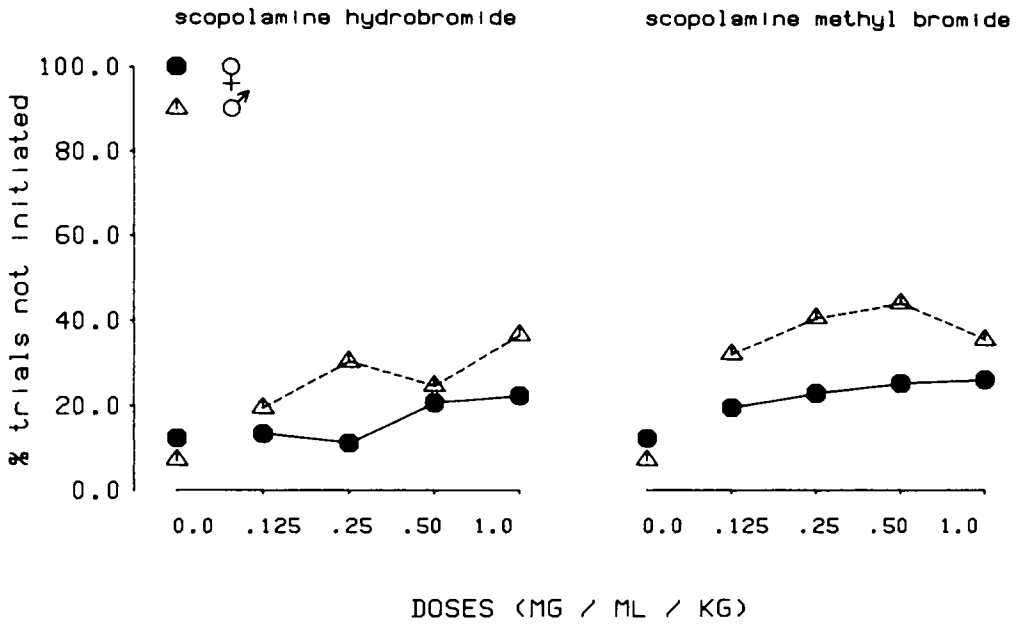

FIG. 1. The effects of increasing doses of scopolamine hydrobromide (left part of the figure) and scopolamine methyl bromide (right part of the figure) on the percentage of not-initiated trials for male (triangles) and female (circles) Wistar rats exposed to a delayed nonmatching to position procedure. The percentage of not-initiated trials was calculated to be the number of trials without an initial response divided by the total number of trials.

designed to investigate the behavior of males and females in spatial matching and nonmatching procedures (41).

Delayed nonmatching to position. All subjects were exposed to a discrete trial delayed nonmatching to position procedure. Each trial consisted of 1) the presentation of one of two response levers, selected at random, 2) the delay interval and 3) the presentation of both response levers after the expiration of the delay interval. The response levers were retracted from the experimental chamber immediately following a response, or after 5 sec had elapsed, whichever came first. Trials were presented on a fixed time (FT) 5-sec schedule. At the start of each session, the houselight was illuminated. After the expiration of the FT 5-sec interval, one of two response levers was randomly selected and was inserted into the experimental chamber while the stimulus light directly above the lever was illuminated. A response on the lever (initial response) started the delay interval during which the lever was retracted and the stimulus light was extinguished. After the expiration of the delay interval, both levers were extended and the stimulus lights were illuminated. Subjects were then required to make a response to one of both levers (choice response). A response on the same lever which was pressed to initiate the delay interval (incorrect response) resulted in a 5-sec time-out (TO) during which both levers were retracted and the houselight and stimulus lights were extinguished. After the TO had expired, the houselight was again illuminated and the next intertrial interval was started. A response on the lever not pressed at the beginning of the trial (correct nonmatching response) produced a food pellet (45 mg Bio-Serve food pellet). The delivery of the food pellet was accompanied by illumination of the light in the pellet retrieval unit for 1.25 sec. The next intertrial interval was started after reinforcement presentation. Failure to respond during the 5-sec lever presentation, both during initial as well as during choice presentations, resulted in the initiation of the next intertrial interval. Subjects were exposed to four different delay intervals within each experimental session: 5, 10, 20 and 40 sec. The order of presentation of the different delay intervals was randomized such that not more than three presentations of the same delay interval could be consecutively presented. Each delay interval was presented 15 times during each session. Sessions ended after 60 trials had been presented, or after 45 minutes, whichever came first. Sessions were run five days a week (Monday through Friday), during the subject's dark hours.

Drug regimen. All subjects received daily injections with vehicle solution (0.9% NaCl in distilled water, 1 ml/kg) for 6 days prior to the first injection with scopolamine. All subjects twice received each of four doses scopolamine hydrobromide (Sigma, 0.125, 0.25, 0.50 and 1.0 mg/ml/kg), and scopolamine methyl bromide (Sigma, 0.125, 0.25, 0.50 and 1.0 mg/ml/kg) freshly dissolved in vehicle solution. The order of administration of different doses of scopolamine was randomized over subjects. All injections were given intraperitoneally (IP), 15 min prior to testing. Subjects were injected with scopolamine on Tuesdays and Fridays, while vehicle injections were given on the other days of the week.

RESULTS

Vehicle Control Values

Vehicle control values for males and females were calculated by averaging over the total number of vehicle control sessions, both prior to (6 sessions), and interspersed between (24 sessions), drug sessions. All data were arc-sine square-root transformed (49) to increase homogeneity of variance. Vehicle control values for the percentage of not-initiated trials (the number of trials without an initial response, divided by the total number of trials) were subjected to a one-way analysis of variance (ANOVA) with the factor Sex. Differences between males and females in the percent-

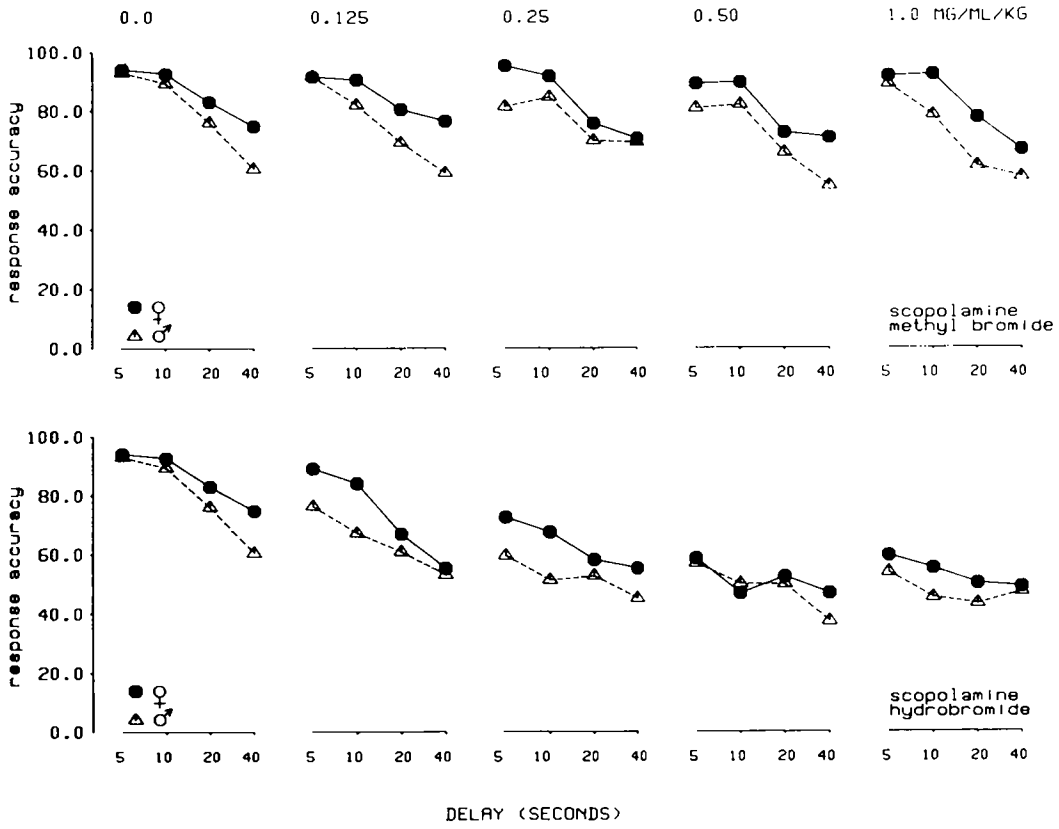

FIG. 2. The effects of increasing doses of scopolamine hydrobromide (lower part of the figure) and scopolamine methyl bromide (upper part of the figure) on response accuracy for male (triangles) and female (circles) Wistar rats exposed to a delayed nonmatching to position procedure. Response accuracy was calculated to be the number of correct choice responses divided by the total number of choice responses.

age of not-initiated trials were not observed, $F(1,13)=2.97$, $p>0.05$. Response accuracy (the number of correct choice responses divided by the total number of choice responses) was subjected to a two-way analysis of variance with the main factors Sex and Delay Interval Duration, the latter being a repeated measure. Response accuracy decreased as a function of delay interval duration, $F(3,39)=70.57$, $p<0.01$. Sex differences were not observed, $F(1,13)=2.41$, $p>0.05$. Significant Sex by Delay interaction effects were also not observed, $F(3,39)=2.82$, $p>0.05$.

Drug Effects

Figure 1 shows the effects of increasing doses of scopolamine hydrobromide and scopolamine methyl bromide on the percentage of trials which were not initiated. ANOVA with the factors Sex, Drug and Dose, the latter two being repeated measures, revealed that the percentage of not-initiated trials increased dose-dependently, $F(3,39)=4.86$, $p<0.01$. Subjects initiated fewer trials after they were challenged with scopolamine methyl bromide than after treatment with scopolamine hydrobromide, $F(1,13)=10.84$, $p<0.01$. An overall Sex effect confirmed that males initiated fewer trials after treatment with scopolamine than females, $F(1,13)=5.67$, $p<0.05$. Significant interaction effects were not observed.

Figure 2 shows the effects of increasing doses of scopolamine hydrobromide and scopolamine methyl bromide on the response accuracy, which was analyzed by means of ANOVA including the factors Sex, Drug, Dose and Delay Interval Duration, the latter three being repeated measures. Response accuracy decreased as a function of the delay interval duration, $F(3,39)=70.66$, $p<0.01$. Response accuracy decreased dose-dependently in both sexes, $F(3,39)=19.79$, $p<0.01$, but males made more errors than females after they were challenged with either drug, $F(1,13)=8.19$, $p<0.05$. Response accuracy was more severely disrupted after treatment with scopolamine hydrobromide than after treatment with scopolamine methyl bromide, Drug, $F(1,13)=73.87$, Drug by Dose, $F(3,39)=6.80$, both $p<0.01$. The effects of scopolamine hydrobromide and scopolamine methyl bromide on response accuracy were then analyzed separately since all the interaction effects were statistically significant [Drug by Delay, $F(3,39)=8.61$, Drug by Dose by Delay, $F(9,117)=2.90$, Sex by Drug by Dose by Delay, $F(9,117)=2.13$, all $p<0.05$].

Scopolamine Hydrobromide

ANOVA with the factors Sex, Dose and Delay Interval Duration revealed that response accuracy after treatment with scopolamine hydrobromide decreased as a function of the dose and the duration of the delay interval, Delay, $F(3,39)=56.72$, Dose, $F(4,52)=64.69$, Delay by Dose, $F(12,156)=4.28$, all $p<0.01$. Males responded less accurately than females, $F(1,13)=6.79$, $p<0.05$.

Scopolamine Methyl Bromide

ANOVA similar to the one described above revealed that

response accuracy decreased as the dose and/or the duration of the delay interval was increased, Dose, $F(4,52) = 3.80$, Delay, $F(3,39) = 53.08$, both $p<0.01$. The behavior of males and females was differentially affected by treatment with scopolamine methyl bromide, Sex, $F(1,13) = 4.74$, Sex by Dose by Delay, $F(12,156) = 2.59$, both $p<0.05$. Further analysis showed a significant Delay by Dose interaction effect for males, $F(12,72) = 2.86$, $p<0.01$, but not for females, $F(12,84) = 1.28$, n.s.

DISCUSSION

The use of delayed alternation procedures as a measure of memory for recent events has often been criticized because of the fact that rats can mediate the correct response by facing toward the correct response lever until it becomes available, instead of remembering which lever was pressed to initiate the delay interval. Response accuracy in the present experiment decreased as a function of the retention interval. One would not predict a decrease in accuracy with increasing memory load from the point of view of mediating response strategies. In addition, comparable levels of response accuracy were obtained when we exposed subjects to a slightly modified delayed matching procedure in which subjects were required to engage in nosepoke activity in the foodtray during the delay interval to insure that they would not orient towards the response lever during the retention interval (41). These results combined make it seem very likely that indeed memory functioning is challenged in the present experiment. Differences in response accuracy between saline-injected males and females were not observed in the present experiment, suggesting that males and females possess basically identical memory capacities. As such, these results confirm and extend results from other experiments in which differences between the sexes in cognitive abilities were also not observed (44–46).

Response accuracy decreased after treatment with scopolamine hydrobromide. It has often been argued that scopolamine affects behavior in memory procedures by interfering with nonmnemonic processes. Viscardi and Heise (47) investigated the effects of scopolamine hydrobromide in an operant delayed response procedure and reported that scopolamine also impaired the behavior of rats at the no-delay interval condition. In addition, it has repeatedly been observed that the scopolamine-induced disruption of response accuracy is independent of the duration of the delay interval (15, 16, 40, 42). It can be argued that it is reasonable to expect that the disruptive effects of drugs which are assumed to interfere with memory processes increase as memory load increases. Statistically significant dose by delay interaction effects were observed in the present experiment, thus suggesting that scopolamine acts upon mnemonic processes. However, as can be seen in the figures, these interaction effects are not due to an increased effect of scopolamine on the response accuracy at the longer delay interval durations, but rather result from the fact that vehicle control values of accurate responding at the long delay interval durations were just above chance level. As such, the results of this and other experiments suggest that the scopolamine-induced decrease in response accuracy as observed in memory procedures results, at least partly, from the effects of scopolamine upon nonmnemonic processes such as stimulus discrimination or attention (15, 16, 22, 42, 47) or encoding and retrieval (11, 36, 39, 40).

Further support for the notion that the effects of anticholinergic treatment on response accuracy must have been mediated by other, nonmnemonic processes is provided by the observation that response accuracy also decreased dose-dependently when subjects were challenged with increasing doses of the peripherally acting muscarinic receptor blocker scopolamine methyl bromide. A decrease in response accuracy after rats have been challenged with scopolamine methyl bromide has previously not been reported. This may be due to the fact that usually only low (0.125 mg/kg) doses of scopolamine methyl bromide are given in order to control for peripheral effects of increasing doses of scopolamine hydrobromide. It could be argued that parts of the higher doses of the quaternary compound scopolamine methyl bromide do cross the blood-brain barrier. This possibility seems, however, not very likely since we could not detect any scopolamine in samples of homogenized brain tissue obtained from rats which were intraperitoneally injected with 1.0 mg/kg scopolamine methyl bromide 30 minutes prior to decapitation (van Hest, in preparation).

In addition, the results of the present experiment showed that the proposed sexual dimorphism of the cholinergic system may also be observed at the behavioral level. Males initiated fewer trials and showed less accurate responding than females when subjects were challenged with scopolamine hydrobromide or methyl bromide. These results thus suggest that the behavior of males may be more sensitive to manipulation of the cholinergic system. Similar observations were made by Beatty and Siders (6), who reported a sex-dependent disruption of one-way avoidance behavior after lesions of the globus pallidus, males being more sensitive to the disruptive effects than females. It has previously been suggested that behavior may be differentially vulnerable to cholinergic manipulation as a function of the level of training (36). This suggestion obviously does not explain the behavioral differences between the sexes as observed in the present experiment since vehicle control values did not differ between the sexes. It can also be argued that sex differences in food motivation underlie behavioral differences between scopolamine-treated males and females in memory procedures. It has previously been observed that scopolamine treatment affects food motivation in appetitively motivated procedures (van Haaren and Heinsbroek, submitted). Male and female rats consumed fewer pellets and showed less food approach behavior after they were challenged with 1.0 mg/kg scopolamine hydrobromide or scopolamine methyl bromide. Differences between males and females were, however, not observed, making it less likely that sex differences in motivational variables contribute to sex differences as observed in the present experiment.

The precise mechanisms which underlie behavioral differences between the sexes in response to cholinergic manipulation remain as of yet unclear. It could be argued that the observed sex differences might at least partly be mediated by the higher binding capacity of muscarinic binding sites in males as compared to females (1,50). It could, however, also be the case that these behavioral differences result from the well-known sex differences in drug metabolism in rats (18). Definitive interpretations regarding the mechanisms underlying the observed behavioral differences between the sexes must await further studies.

In summary, the results of the present experiment have shown that delayed matching procedures are suitable procedures for measuring memory capacities. Response accuracy decreased with increasing memory load. The sexes attained similar accuracy levels. Pharmacological manipulation of the cholinergic system disrupted ongoing behavior of male and female rats exposed to a delayed nonmatching procedure. The effects of anticholinergic treatment were, however, independent of the delay interval duration. Therefore, it is as of yet not clear whether the cholinergic system is critically involved in memory processes per se, or whether the effects on memory parameters are secondary to the effects on other nonmnemonic processes such as attention or stimulus discrimination, encoding or retrieval. Behavioral differences between the sexes were observed when male and female rats were challenged with increasing doses of scopolamine hydrobromide and scopolamine methyl bromide, suggesting that the cholinergic system may be sex-dependently organized. The precise

mechanisms which mediate sex differences in cholinergic functioning can only be assessed after exhaustive neuropharmacological, neurochemical and behavioral studies have been conducted. Such studies will significantly contribute to a better understanding of the complex interactions between gonadal hormones and neurotransmitter systems, and may be of utmost importance for the development of new memory-enhancing drugs.

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