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The Effects of Scopolamine and Methylscopolamine on Visual and Auditory Discriminations in Male and Female Wistar Rats

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VAN HAAREN, F. AND A. VAN HEST. *The effects of scopolamine and methylscopolamine on visual and auditory discriminations in male and female Wistar rats.* PHARMACOL BIOCHEM BEHAV **32**(3) 707–710, 1989.—The present experiment was designed to investigate whether or not the administration of scopolamine hydrobromide would differentially disrupt auditory or visual discrimination performance in male and female Wistar rats. Two groups of male and female Wistar rats were trained to discriminate between a continuous and intermittent visual stimulus, while two other groups were trained to discriminate between a continuous or intermittent auditory stimulus in a discrete-trial discrimination procedure. Once discrimination performance had stabilized, subjects were treated with different doses (0.125, 0.25, 0.50 or 1.0) of scopolamine hydrobromide or scopolamine methylbromide. Treatment effects were assessed with respect to discrimination performance, as well as with respect to the number of trials which were not completed. Scopolamine hydrobromide, but not scopolamine methylbromide, disrupted visual and auditory discrimination performance. The auditory discrimination was more seriously disrupted. However, both the administration of scopolamine hydrobromide and of scopolamine methylbromide increased the number of trials which were not completed suggesting that the accuracy of visual and auditory discriminations after drug treatment may have been influenced by other variables than drug effects on memory processes. Sex differences were not observed, neither with respect to discrimination performance, nor with respect to the number of trials which were not completed.

Visual and auditory discrimination Acetylcholine Scopolamine hydrobromide Scopolamine methylbromide Discrimination accuracy Male and female rats

A number of experiments have shown that short-term memory functions may effectively be disrupted by lesioning or pharmacological challenge of the central cholinergic system (2, 9, 11, 12, 21, 22, 24, 25, 28). However, it is presently not known which aspects of memory functioning are affected by interference with central cholinergic activity. It has been suggested that decreased performance in memory procedures may be mediated by effects on 1) attentional or discrimination processes, on 2) stimulus encoding processes or on 3) the time-dependent process of retention (4, 5, 27).

Previous experiments have shown that anticholinergic drugs can affect behavior in visual and auditory discrimination procedures. Accurate behavior in such procedures is mostly dependent upon intact reference memory functioning (3, 6, 8, 18, 19). Limited evidence is available to suggest that anticholinergic treatment may affect auditory discriminations more than visual discriminations. Moore, Goodell and Solomon (20) and Harvey, Gormezano and Cool-Hauser (7) have shown that the intensity of auditory, but not of visual conditioned stimuli in rabbit nictating membrane experiments had to be increased after treatment with scopolamine hydrobromide (SCOP), a muscarinic blocking agent.

Evidence is available to suggest that central cholinergic mechanisms may differ between the sexes. Sex differences have been reported with respect to choline acetyltransferase (CAT) and acetylcholine esterase (AChE) activity in the brains of male and female rats (13–16). Cholinergic enzyme activity reaches adult levels earlier in females than in males (17). Other investigators have shown that the total number of muscarinic binding sites is generally higher in males than in females and dependent upon perinatal exposure to male gonadal hormones (1). Recently it has

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been shown that the administration of SCOP may differentially decrease the response efficiency of male and female rats in an operant delayed spatial nonmatching to position task (van Hest, Stroet, van Haaren and Feenstra, submitted), while other experiments have also shown that response rates in fixed-consecutive-number procedures may be differentially affected (van Haaren, van Hest and van Hattum, in press).

The present experiment was designed to investigate whether or not the administration of SCOP would differentially interfere with the behavior of male and female Wistar rats in auditory and visual discrimination procedures, to assess the extent to which pharmacological challenge of the central cholinergic system disrupts discrimination processes.

METHOD

Subjects

Sixteen male and 16 female Wistar rats were housed in group cages (4 same-sex subjects to a cage) under a reversed light-dark cycle (lights on 6:30 p.m.–6:30 a.m.). They were food-deprived and fed daily to maintain bodyweight at approximately 85% corrected for growth throughout the experiment (10). Water was always available in the homecages. Subjects had previously participated in another experiment in which they had been trained to make either a visual or auditory discrimination (26). The present experiment started when subjects were approximately 7 months old.

Apparatus

The 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 Sonalert located 15 cm above each lever was then activated for 0.10 sec. A stimuluslight (green on the left- and red on the righthand side of the intelligence panel) was located 9 cm directly above each lever. The pellet retrieval unit, which was centered 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. Experimental contingencies and data acquisition procedures were programmed using SKED-11 (23).

Procedure

Preliminary training. Preliminary training was not necessary since subjects had previously been trained to discriminate between intermittently or continuously presented visual or auditory stimuli (26).

Experimental procedure. One group of male rats (n = 8) and one group of female rats (n = 8) was exposed to the auditory discrimination procedure, another group of male rats (n = 8) and another group of female rats (n = 8) was exposed to the visual discrimination procedure. The visual and auditory discrimination procedures were identical, except for the presentation of the different stimuli. Either an intermittent or continuous stimulus was presented after the expiration of an intertrial-interval (ITI) of 40 sec, immediately followed by the insertion of the two levers into the experimental chamber. The levers remained available for 5 sec. A press on the left lever was immediately followed by the presentation of a food pellet if the intermittent stimulus had been presented. Food was presented following a press on the right lever if the continuous stimulus had been presented. The light in the pellet retrieval unit was illuminated for 1.25 sec during pellet delivery. Evidently, food was not presented when subjects pressed the wrong lever. A press on either one lever always resulted in the retraction of both levers from the experimental chamber and the initiation of a new ITI. The levers were also retracted from the chamber and a new ITI was initiated when subjects did not press the levers during the 5 sec that they were available. The continuous visual stimulus consisted of the illumination of the houselight and the two stimulus lights above the levers for 5 sec. The intermittent visual stimulus consisted of the presentation of the same stimuli on a 0.25 sec on/off cycle. The presentation of the continuous auditory stimulus consisted of the activation of the Sonalerts for 2.5 sec. The Sonalerts were activated on a 0.25 sec on/off cycle during the presentation of the intermittent auditory stimulus. Sessions were run five days a week (Monday through Friday) and terminated after 45 min or when subjects had correctly completed 40 trials, whichever came first.

Drug treatment. Subjects were injected intraperitoneally (IP) with vehicle solution (physiological saline, 1 ml/kg) for 6 sessions. Thereafter they were treated with different doses of scopolamine hydrobromide (SCOP, Sigma, 0.125, 0.25, 0.50 or 1.0 mg/ml/kg) or scopolamine methylbromide (METHSCOP, Sigma, 0.125, 0.25, 0.50 or 1.0 mg/ml/kg). Injections always occurred 15 min before the start of each session. All subjects twice received each dose of each drug. Drugs were administered on Tuesdays and Fridays, vehicle injections were administered on all other days of the week.

RESULTS

Figures 1 and 2 show the percentage of trials which were correctly completed (Fig. 1), as well as the percentage of trials not completed (Fig. 2) during the administration of both SCOP (lefthand panels) and METHSCOP (righthand panels). The percentage of correctly completed trials was calculated by dividing the number of correctly completed trials by the number of correctly and not-correctly completed trials. The percentage of trials not-completed was calculated by dividing the number of not-completed trials by the total number of trials which were presented.

All data were arc-sine transformed to increase homogeneity of variance (29) before analysis of variance in which the factors Sex, Stimulus, Drug, and Dose were analyzed. Drug and Dose were considered to be repeated measures within subjects. Vehicle control values were obtained by averaging across vehicle control sessions which preceded and which were interspersed between drug sessions. During vehicle sessions there were no differences between males and females in the percentage of correctly completed trials during both the presentation of the visual and auditory stimuli [Sex, $F(1,28) = 2.65$, n.s.; Stimulus, $F(1,28) = 4.11$, n.s.]. The percentage of correct responses after the presentation of the visual stimulus averaged 82.0 (s.d. 8.9) for females and 87.0 (s.d. 8.4) for males. The percentage of correct responses after the presentation of the auditory stimulus averaged 86.0 (s.d. 6.4) for females and 91.0 (s.d. 5.8) for males. Differences between males and females or between the presentation of visual and auditory stimuli were also not observed when the percentage of trials not-completed were analyzed under vehicle conditions [Sex,

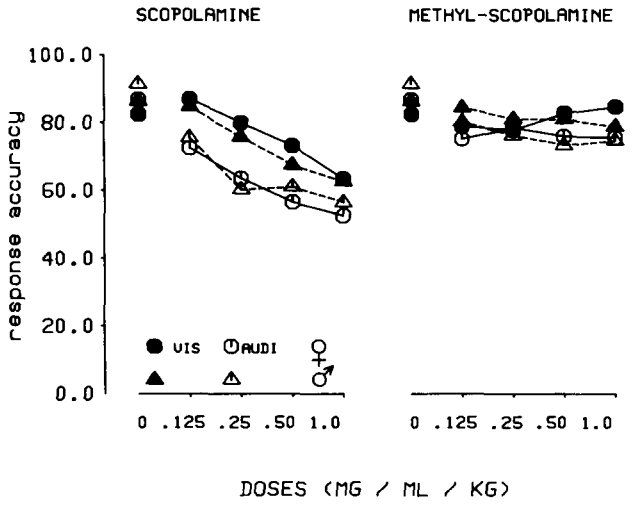

FIG. 1. The effects of different doses of scopolamine hydrobromide (left panel) and scopolamine methylbromide (right panel) on visual and auditory discrmination accuracy in male and female Wistar rats.

$F(1,28) = 1.58$ and Stimulus, $F(1,28) = 3.36$, n.s., respectively]. The percentage of trials not completed during the visual discrimination procedure averaged 0.30 (s.d. 0.30) and 1.0 (s.d. 0.90) for females and males respectively. During the auditory discrimination procedure, males and females did not complete 0.40 (s.d. 0.50) and 0.50 (s.d. 0.40) trials, respectively.

The administration of SCOP disrupted visual and auditory discriminations in male and female rats, while such disruption was not observed after administration of METHSCOP [Drug, $F(1,28) = 35.28$, $p<0.001$]. Auditory discriminations were more disrupted than visual discriminations by SCOP, but not by METHSCOP [Stimulus, $F(1,28) = 7.23$, $p<0.01$, Stimulus by Drug, $F(1,28) = 4.34$, $p<0.05$]. Discrimination accuracy decreased dose-dependently after administration of SCOP, but not after administration of

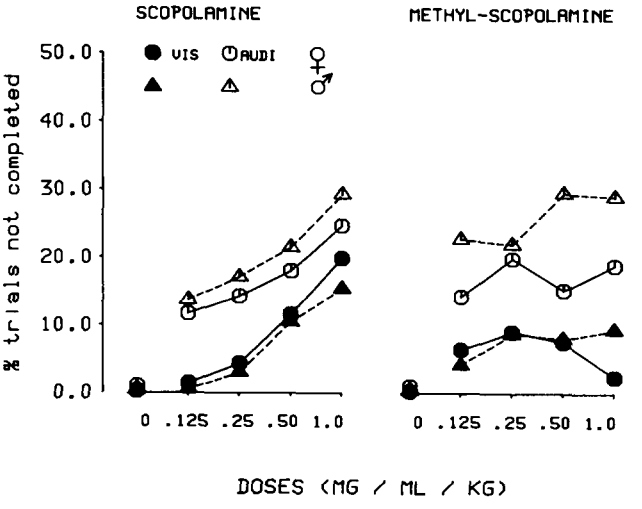

FIG. 2. The effects of different doses of scopoalmine hydrobromide (left panel) and scopolamine methylbromide (right panel) on the percentage of trials which were presented, but not completed during the visual and auditory discrimination procedure in male and female Wistar rats.

METHSCOP [Drug by Dose, $F(3,84) = 19.83$, $p<0.001$]. Sex differences were not observed [Sex, $F(1,28) = 0.01$, n.s.], nor were any other interactions.

Administration of SCOP and METHSCOP both increased the number of not-completed trials. The number of trials not-completed increased more during the auditory than during the visual discrimination [Stimulus, $F(1,28) = 7.18$, $p<0.01$]. The percentage of trials not-completed increased with increasing doses of both SCOP and METHSCOP [Dose, $F(3,84) = 19.56$, $p<0.001$]. The increase in the percentage of trials not-completed was larger after the administration of SCOP [Drug by Dose, $F(3,84) = 11.83$, $p<0.001$]. Sex differences were not observed [Sex, $F(1,28) = 0.33$, n.s.].

DISCUSSION

Different groups of male and female Wistar rats were trained to discriminate between different auditory or visual stimuli in a discrete-trial experimental procedure. Male and female rats were equally able to discriminate between the two different stimuli during vehicle sessions. The groups of rats which were required to discriminate between the two visual stimuli behaved as efficiently as the groups of rats required to discriminate between the two auditory stimuli. However, pharmacological challenge of the central cholinergic system through systemic administration of SCOP disrupted auditory and visual discriminations in male and female Wistar rats. The auditory discrimination was more seriously disrupted than the visual discrimination, both when discrimination performance and the number of trials not-completed were analyzed. Visual and auditory discriminations were not dose-dependently disrupted after administration of METHSCOP, which mostly acts at the peripheral cholinergic receptors. However, both the administration of SCOP and METHSCOP increased the number of trials which were not completed. The increase in the number of trials which were not completed was larger during exposure to the auditory discrimination procedure than during exposure to the visual discrimination procedure. The increase in the number of trials which were not completed observed during administration of SCOP and METHSCOP suggests that pharmacological challenge of the cholinergic system may not only affect long- or short-term memory processes, but also other, possibly attentional, variables. Behavioral differences between the sexes were not observed, suggesting that behavioral differences between male and female rats observed in other experimental procedures after treatment with SCOP and METHSCOP are probably not due to differences in the processing of sensory information (van Haaren *et al.*, submitted; van Hest *et al.*, submitted).

The results of the present experiment support observations by others (3, 6, 7, 18–20) that anticholinergic treatment may seriously disrupt visual and auditory discriminations and that auditory discriminations are more likely to be affected than visual discriminations. It has previously been suggested that the more serious disruption of auditory discrimination performance may be due to the fact that important cholinergic pathways are known to exist in those parts of the brain which are involved in the processing of auditory information (20). The results of the present experiment extend previous observations by indicating that at least some of the effects of anticholinergic treatment on discrimination performance may be related to interference by other than long-term memory processes.

The present experiment has shown that pharmacological challenge of the central cholinergic system may seriously disrupt discrimination performance. Adequate discrimination of visual or auditory stimuli is a prerequisite in many procedures in which the effects of anticholinergic treatment on memory functioning are

assessed. If anticholinergic treatment interferes with accurate discrimination performance, one may question the validity of the effects of anticholinergic treatment in procedures designed to evaluate memory functioning. It thus seems relevant to always include measures of discrimination in experimental procedures employed to evaluate the effects of pharmacological challenge of the central cholinergic system on memory functioning.

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