Pharmacology Biochemistry & Behavior, Vol 16, pp 377–385, 1982 Printed in the U S A

Attention and Habituation: Catecholamine Interactions and Sex Differences

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Received 24 September 1981

CHEAL, M L *Attention and habituation Catecholamine interactions and sex differences* PHARMAC BIOCHEM BEHAV **16**(3) 377–385, 1982 —Norepinephrine (NE)-dopamine (DA) interactions in mediation of long-term habituation were tested using the stimulus-elicited investigation paradigm Systemic injections of clonidine hydrochloride (CLON), an α-adrenergic receptor agonist, given before apomorphine (APO) (1 mg/kg SC), a DA receptor agonist, prevented APO-induced disruption of the duration measure of habituation 24-hr later A lower dose was effective in female gerbils than that required for male gerbils suggesting that females are more sensitive to the effects of clonidine In addition, apomorphine with or without CLON (0 01 mg/kg), increased locomotor activity in male gerbils but reduced activity in female gerbils The same dose of CLON that prevented APO-induced disruption of 24-hr habituation did not reverse APO-induced reduction of investigation of an object or an odor, or APO-induced changes in activity Neither CLON nor desmethylimipramine, a blocker of NE reuptake, had any selective effects on investigation of novel stimuli or short-term habituation showing that relative NE-DA activity can be disrupted without interfering with the behavior However, CLON interfered with the frequency measure of long-term habituation in male gerbils but not in female gerbils at any dose used (0 01–0 3 mg/kg) The data suggest important sex dependent NE-DA interactions in mediation of long-term habituation

Habituation Investigation Gerbils Locomotor activity Clonidine Apomorphine Desmethylimipramine Catecholamine Dopamine Norepinephrine Sex Differences

NOREPINEPHRINE (NE) and dopamine (DA) pathways have been proposed to "play critical roles in numerous basic survival-related activities " [2] Evidence was cited of an interaction of these systems in motor activity, avoidance behavior, aggression, electrical self-stimulation of the brain, and eating behaviors Attention and habituation to novel stimuli can be thought of as "survival-related activities" because approaches to stimuli in the environment are a necessary part of localization of food, sexual partners, and possible predators The stimulus-elicited investigation paradigm [6,11] offers a unique experimental, ethological method to study the neural mediation of attention and habituation This paradigm provides multiple measures within the same animal Duration and frequency are recorded of (i) initial investigation of simple stimuli, a measure of awareness of stimuli, (ii) subsequent habituation (as defined by Thompson and Spencer [40]), reflecting selectivity of responding and a simple form of memory, and (iii) dishabituation, which occurs in this paradigm when the stimulus is moved, providing a control for adaptation or sedation The relative frequency and duration measures allow inferences as to the maintenance of attention [10] In addition, it is possible to test for both short-term and long-term habituation because normal animals can show habituation after intervals from 60 sec up to 4 weeks [11] Therefore, it is possible to give a drug treatment and either test for habituation immediately, or wait and test for memory (in the form of habituation) after the acute effects of the drug are past

I postulated earlier [8] that DA and NE may be involved in selective investigation of, and habituation to, novel stimuli Stimulating DA receptors with apomorphine (APO) resulted in a disruption of habituation to novel stimuli either within a 20 min test [7] or 24 hr later [8] If the animals were habituated prior to drug treatment, habituation continued after APO treatment, showing that retrieval of memory was intact The DA receptor blocker, pimozide, prevented the disruption of 24-hr habituation, indicating DA mediation of the apomorphine effect In contrast, amphetamine, except at very high doses, did not disrupt the behavior [5,8] One of several possible explanations for the difference in the effect of APO and amphetamine on this behavior was the influence of amphetamine, but not apomorphine, on NE [8]

To test the hypothesis of the necessity of relative NE-DA activity, investigation and habituation were observed in gerbils given systemic injections of NE agonists, clonidine (CLON) or desmethylimipramine (DMI), or of CLON plus APO If relative DA-NE activity are necessary for normal attention and habituation, then NE agonists should disrupt the behavior, but combining a NE agonist with a DA agonist should result in normal habituation Obviously this simplistic hypothesis is complicated by the intricacies of drug action, a problem that is addressed in the discussion The hypothesis was supported in an earlier report in which it was shown that a small dose of CLON blocked disruption of habituation by APO [9]

An additional hypothesis that was tested in this paper was

based on the growing body of data on the role of sex steroids in catecholamine function [14,19] To determine whether there were sex differences in response to drug treatment, the effect of sex on each behavioral measure was assessed

METHOD

Animals

Male and female Mongolian gerbils (*Meriones unguiculatus*) were born in the laboratory from stock primarily derived from Tumblebrook Farms, West Brookfield, MA After weaning, the gerbils were maintained in groups of 5–11 of the same sex in glass cages (31×61×32 cm) on a 14 10 light dark cycle with food and water continuously available At testing at approximately 15 wks of age, the gerbils weight varied from 44–102 g

Apparatus

Tests were conducted in a 30×45 cm semi-circular white plastic arena set into a glass aquarium The semi-circular design, chosen to make the entire arena within the gerbil s visual field [13,22], minimized competing stimuli A solid floor was used for the object test and an interchangeable floor with two or five holes was used for the odor test The apparatus was washed with water between animals in order to remove any concentrated areas of animal odor without introducing other, possibly distracting odors An illustration of the apparatus was published previously [6]

Procedure

To gentle the gerbils and to adapt them to the apparatus, they were handled daily for 9 or 14 days, placed in the apparatus in groups for ten minutes on each of the three days preceeding the tests, and separately given three adaptation trials prior to testing This preliminary adaptation is necessary in order to focus the investigatory behavior on the novel stimulus [6,11] The adaptation trials were conducted just as the test trials, but with no stimulus present in the arena The tests were conducted in the stimulus-elicited investigation paradigm [6,11] with 30–60 seconds between trials except where stated otherwise The drop door was opened, the gerbil ran in, the door was closed and then reopened 60 seconds later to allow the gerbil to run out If the gerbil did not readily run through the door, the experimenter gently directed him towards the opening

For the object test, magnets held an orange cup on the wall of the arena Licking, sniffing, and biting of the cup by the gerbil were recorded by two observers, at least one of whom did not know the drug treatment Duration was timed either with a stopwatch or by manually operated switches that recorded directly on a Radio Shack computer For the odor test, soiled shavings taken from a cage of adult strange male gerbils were hidden under one of two or five holes in the floor The hole with odor was counterbalanced across animals The trials were filmed at 4 frames per second with a Super 8 movie camera Later, the number of frames in which the gerbil's nose was poked into each hole was recorded by an observer who knew neither the hole with the odor nor the drug treatment of the gerbil From the number of frames, the frequency and estimated duration of nose poking for each hole were obtained For two trials, the film was projected onto a grid to determine the number of times the gerbil's head crossed a line of the grid This count furnished a locomotor activity score [5]

TABLE 1

INJECTION AND TESTING SCHEDULE EXPERIMENT 2

Injections	Exposure Condition	Trials Day 1	Trials Day 2
CLON and NaCl*	Exposed	1	2–6
	Nonexposed	—	1–6
CLON and APO†	Exposed	1	2–6
	Nonexposed	—	1–6

*Clonidine hydrochloride (0 (n=44), 0 01 (n=16) 0 03 (n=29), 0 3 (n=16) mg/kg) 40 min preadaptation sodium chloride (0 9%) 10 min preadaptation

†Clonidine hydrochloride (0 (n=17), 0 01 (n=16) 0 03 (n=27), 0 3 (n=16) mg/kg) 40 min preadaptation, apomorphine hydrochloride (1 0 mg/kg), 10 min preadaptation

Experiment 1, Short intertrial intervals, clonidine or desmethylimipramine Gerbils were injected SC with a full range of doses of either clonidine hydrochloride (CLON) (Catapres®, Boehringer Ingelheim Ltd) or desmethylimipramine (DMI) (Geigy) 5 min or 40 min, respectively, prior to adaptation trials Times were chosen based on times when behavioral responses were found in rats, such as effects on avoidance behavior [25] Immediately following adaptation 5 trials were given with the cup on the left and then a trial with the cup on the right side of the arena The object test was followed immediately by 5 trials for responses to strange male odor hidden under one of 5 holes The adaptation and testing trials (object-elicited investigation and odor-elicited investigation) were concluded in approximately 35 min

Experiment 2, Long intertrial intervals, clonidine and apomorphine Gerbils were injected SC using a range of doses of CLON and either 1 mg/kg apomorphine hydrochloride (APO) (Merck), calculated as the salt, or saline This dose of apomorphine was chosen because it disrupted 24-hr habituation previously even though the gerbils spent 11–12 sec investigating the cup following drug injection [8] See Table 1 for the sequence of injections and trials Ten minutes after the second injection, the gerbils were given three adaptation trials Half of the gerbils in each drug group were then given one 60-sec trial with the cup on the left side of the arena The gerbils were returned to the home cage and tested again the next day The gerbils that had been exposed to the cup on Day 1 received 4 additional trials with the cup on the left on Day 2, and one trial with the cup on the right side of the arena Those gerbils that had not seen the cup on Day 1, received 5 trials with the cup on the left and one trial with the cup on the right on Day 2

Experiment 3 Investigation of odors clonidine and apomorphine Gerbils were injected with CLON (0 01 mg/kg) or saline Thirty min later half of each group were injected with APO (1 0 mg/kg) and half with saline This dose of CLON was used because it appeared to block the APO-induced disruption of 24-hr habituation in preliminary work [9], and because of sex differences found in Experiment 2 Twelve min after injection the 2-hole odor test was given One trial was given with no particular odor under the holes Then five trials were given with strange male odor under one hole

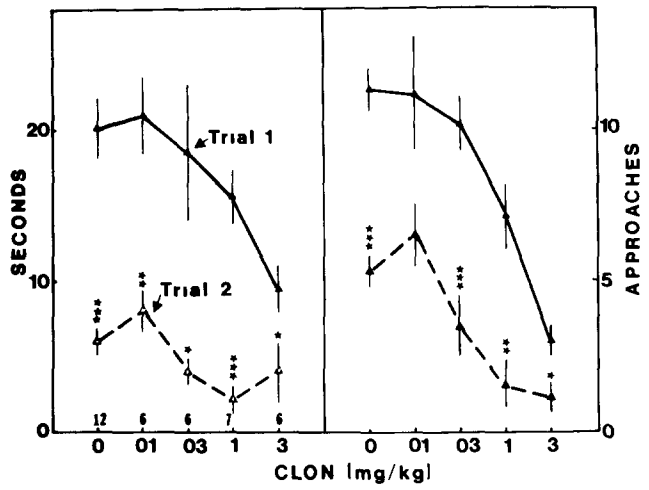

FIG 1 Experiment 1, object-elicited investigation, clonidine Dose/response characteristics of investigation of a cup by gerbils following injection of CLON on trials 1 (solid symbols) and 2 (open symbols) Mean duration (sec) and frequency (number of approaches), bars indicate SE Number of gerbils per group is indicated by small numbers above dose Significant decrease on Trial 2 indicated by *$p<0$ 05 **$p<0$ 01 ***$p<0$ 001

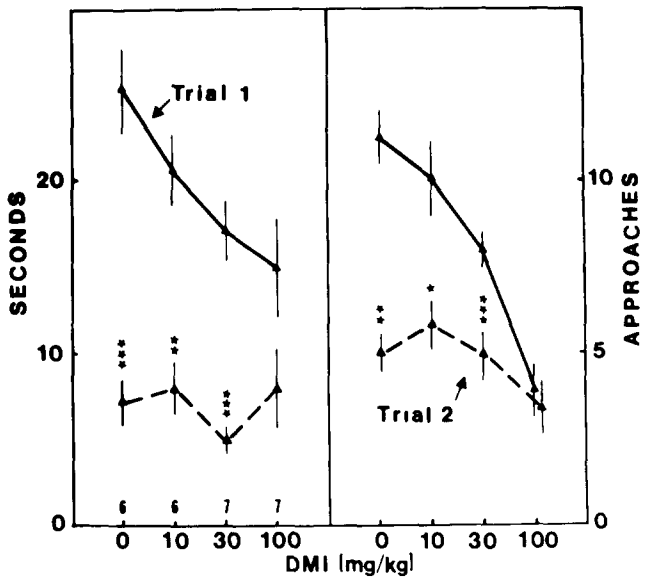

FIG 2 Experiment 1, object-elicited investigation, desmethylimipramine Dose/response characteristics of investigation of a cup by gerbils following injection of DMI Details as in Fig 1

Data Analysis

Analysis of variance with repeated measures was computed using Biomedical Data Program package (BMDP 2V) Each measure (duration, frequency, activity scores) was independently analyzed for each experiment On the first analyses, sex was entered as an independent variable If there were no significant effects of sex, the data were reanalyzed without sex as a variable All of the data for an experiment were first analyzed together and then additional comparisons were made to facilitate interpretation of the many significant interactions Only the significant F-scores used in the final analyses are presented here Posthoc t-tests were used to determine specific differences based on *a priori* hypotheses of habituation or odor preference Probabilities <0 05, 2-tailed test, were considered significant

RESULTS

Experiment 1, Short Intertrial Intervals

Object-elicited investigation clonidine Analysis of variance on the complete experiment was computed with sex and CLON treatment as grouping variables, and the 6 trials as repeated measures Significant main effects of CLON (duration $F(4,32)=4$ 89, $p<0$ 01, frequency $F(4,32)=27$ 71, $p<0$ 0001) and trial (duration $F(5,160)=76$ 48, $p<0$ 0001, frequency $F(5,160)=72$ 71, $p<0$ 0001) and a significant CLON × trial interaction (duration $F(20,160)=1$ 88, $p<0$ 02, frequency $F(20,160)=3$ 13, $p<0$ 0001) were revealed The effects of trial reflected habituation, typical with repeated trials in this paradigm [6,11] All groups significantly decreased responding over trials (Fig 1) Every group increased responding when the cup was moved on Trial 6 although the increase only reached significance for controls and the 0 01 mg/kg groups

CLON at low doses did not alter investigation, but at 0 3 mg/kg the responses were severely attenuated (Fig 1) This high dose attenuation of responding is not specific as it can be induced with any drug if a high enough dose is used A similar high dose effect has been reported previously for amphetamine [5,8], apomorphine [7,8], scopolamine, and physostigmine [10] The CLON × trial interaction was a result of a CLON effect only on Trials 1 (duration $F(4,32)=3$ 01, $p<0$ 05, frequency $F(4,32)=10$ 50, $p<0$ 0001), 2 (duration $F(4,32)=3$ 57, $p<0$ 02, frequency $F(4,32)=9$ 00 $p<0$ 0001), 3(frequency only $F(4,32)=7$ 41, $p<0$ 0002), 4 (duration $F(4,32)=2$ 92, $p<0$ 05, frequency $F(4,32)=6$ 56, $p<0$ 001), and 6 (duration $F(4,32)=3$ 15, $p<0$ 05, frequency $F(4,32)=15$ 58, $p<0$ 0001) It can be concluded that CLON did not interfere with selective responses to the cup except at the highest dose

Object-elicited investigation desmethylimipramine Analysis of variance, computed as above, revealed similar effects of DMI (Fig 2) Significant main effects were found for DMI on the frequency measure, $(F(3,22)=9$ 88, $p<0$ 001) and in the same direction for duration, $(F(3,22)=2$ 82, $p=0$ 0624) There were significant trial (duration $F(5,110)=59$ 74, $p<0$ 001, frequency $F(5,110)=44$ 21, $p<0$ 0001) and DMI × trial interaction (duration $F(15,110)=2$ 34, $p<0$ 01, frequency $F(15,110)=4$ 04, $p<0$ 0001) All groups decreased responding on Trial 2 (Figure 2) and increased responding when the cup was moved on Trial 6

There was a dose dependent attenuation of responses The DMI effect was significant on Trials 1 (duration $F(3,22)=3$ 63, $p<0$ 01, frequency $F(3,22)=16$ 93, $p<0$ 0001) and 6 (duration $F(3,22)=4$ 56, $p<0$ 02, frequency $F(3,22)=8$ 70, $p<0$ 001) With DMI, as with CLON, it can be concluded that there is no specific disruption of investigation

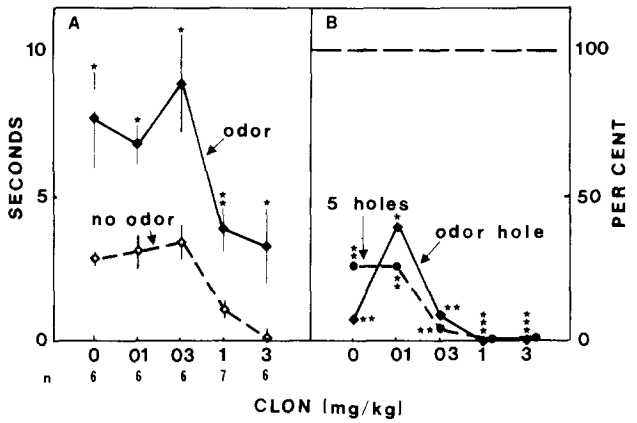

FIG 3 Experiment 1, odor-elicited investigation clonidine Dose/response characteristics of the duration (sec) of investigation of holes by gerbils following injection of CLON (A) Odor hole on Trial 1 (closed symbols) compared to mean of nonodor holes on Trail 1 (open symbols) Stars indicate significantly more investigation at odor hole Number of gerbils per group is indicated by small numbers below dose (B) Habituation of nose poking For clarity the data for Trial 5 are plotted as a percentage of the response on Trial 1 (Trial 1 odor hole=100%, compared to Trial 5 odor hole, Trial 1 5-holes=100%, compared to Trial 5 5-holes) Stars indicate significantly less than Trial 1, matched t-tests on the raw data

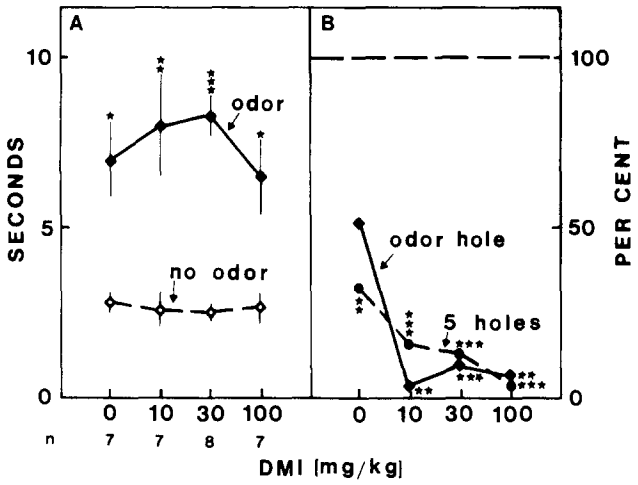

FIG 4 Experiment 1, odor-elicited investigation, desmethylimipramine Dose/response characteristics of duration (sec) of investigation of holes by gerbils following injection of DMI Details as in Fig 3

Odor-elicited investigation clonidine On the first trial all groups of gerbils spent significantly more time at the odor hole than at the holes that had no specific odor (Figure 3A) Preference for the odor disappeared with habituation on subsequent trials

The responses to the odor hole were analyzed for sex, CLON, and trials effects There were significant main effects of CLON (duration $F(4,26)=5\ 28$, $p<0\ 01$, frequency $F(4,26)=13\ 56$, $p<0\ 0001$) and trial (duration $F(4,104)=25\ 60$, $p<0\ 0001$, frequency $F(4,104)=81\ 59$, $p<0\ 0001$) and a trial × CLON interaction (duration, $F(16,104)=1\ 87$, $p<0\ 05$, frequency $F(16,104)=3\ 95$, $p<0\ 0001$) CLON effect was significant for the duration measure on Trials 1 and 2 (Trial 1 $F(4,26)=3\ 99$, $p<0\ 02$, Trial 2 $F(4,26)=4\ 24$, $p<0\ 01$) and on all trials for the frequency measure (Trial 1 $F(4,26)=12\ 86$, $p<0\ 0001$, Trial 2, $F(4,26)=7\ 24$, $p<0\ 001$, Trial 3, $F(4,26)=5\ 43$, $p<0\ 01$, Trial 4, $F(4,26)=2\ 78$, $p<0\ 05$, Trial 5, $F(4,26)=5\ 71$, $p<0\ 01$) reflecting attenuation of responding with increasing dose (Fig 3) The trial effect reflected habituation There was a decrease over trials for each group, both for investigation of the odor hole and for all 5 holes Habituation in this test is slower than in the object test [11] Therefore, Trial 5 is compared with Trial 1 in Fig 3B

For the activity score, there were significant main effects of CLON, ($F(4,26)=89\ 23$, $p<0\ 0001$) and trial, ($F(4,26)=33\ 67$, $p<0\ 0001$), which reflected a monotonic decrease in activity with increasing dose, and a decrease in activity on Trial 2 at each dose Thus, in spite of a decrease in total investigation of the holes and a decrease in locomotor activity with increasing doses, CLON did not interfere with preference for the odor or with habituation

Odor-elicited investigation desmethylimipramine Gerbils given DMI spent significantly more time at the odor hole than at the nonodor holes in the first trial (Fig 4A) On subsequent trials, habituation occurred and preference was no longer shown

There was a weak overall effect of DMI on odor investigation (duration $F(3,21)=3\ 64$, $p=0\ 05$, frequency $F(3,25)=4\ 07$, $p<0\ 02$), and a strong trial effect (duration $F(4,100)=72\ 64$, $p<0\ 001$, frequency $F(4,100)=51\ 04$, $p<0\ 0001$) Separate analysis by trial showed an effect of DMI on the duration measure on Trial 5, $F(3,25)=6\ 08$, $p<0\ 01$ The amount of responding for the odor hole and for all 5 holes decreased by Trial 5 even at the highest dose (Fig 4B)

For the activity score, analysis of variance revealed a significant effect of DMI, $F(3,25)=11\ 83$, $p<0\ 0001$ trial, $F(1,25)=140\ 03$, $p<0\ 0001$, and a trial × DMI interaction, $F(3,25)=12\ 40$, $p<0\ 0001$ There was a significant decrease in activity on Trial 2 by controls and 10 or 30 mg/kg DMI groups (matched t-tests, $t(6$ or $7)=8\ 55$, $17\ 20$, $7\ 38$, $p<0\ 001$) The gerbils that received 100 mg/kg had a low activity score on Trial 1 which did not decrease on Trial 2, $t(6)=0\ 67$, NS As with CLON, DMI did not selectively interfere with preference for the odor or with habituation

Experiment 2, Long Intertrial Intervals, Clonidine and Apomorphine

Overall effects Data were analyzed for sex, CLON, APO, and exposure condition as grouping variables, and six trials as repeated measures Significant main effects were revealed for each variable, the first four of which will be discussed below Trial effects (duration $F(5,745)=220\ 83$, $p<0\ 0001$, frequency $F(5,745)=188\ 48$, $p<0\ 0001$) again reflected habituation and dishabituation All groups decreased responding over trials Every group increased responding when the cup was moved on Trial 6 The only possibly meaningful effect on Trials 3 to 6 was a main effect of CLON Responding was increased in some groups on some trials,

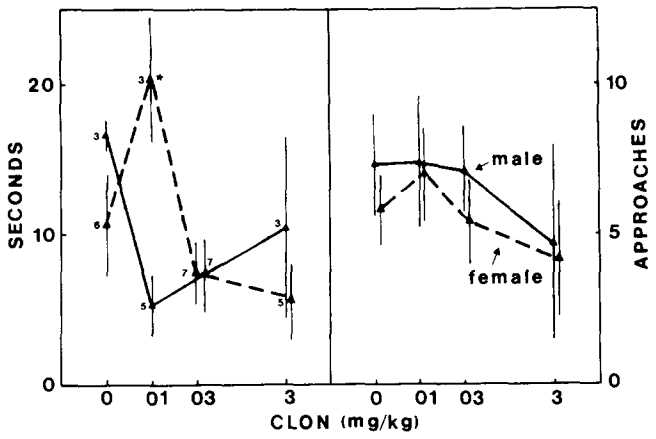

FIG 5 Experiment 2, Trial 1 clonidine and apomorphine Dose/response characteristics of investigation of a cup by male (solid symbols) and female (open symbols) gerbils following injections of CLON and 1 mg/kg APO Mean duration (sec) and frequency (number of approaches), bars indicate SE The number of gerbils per group is indicated by the small number next to each symbol *$p<0$ 05, male vs female, Student t-test

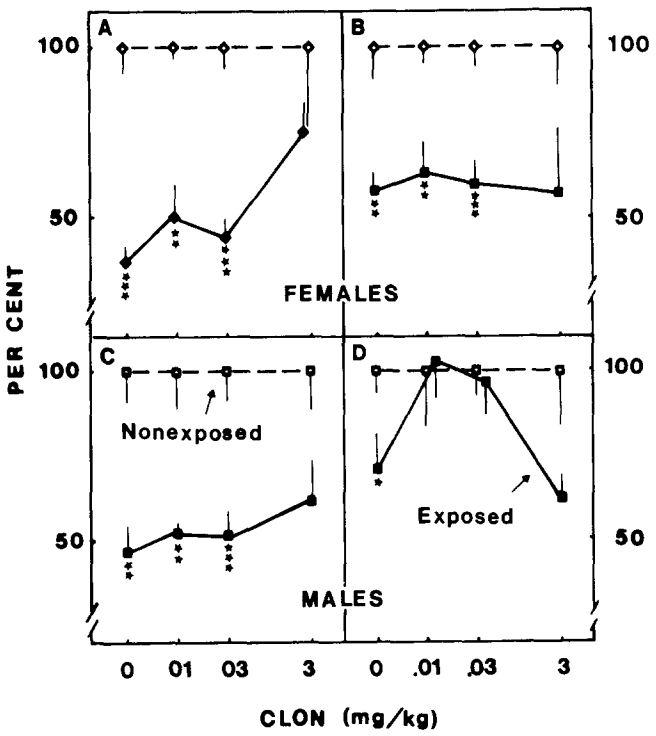

FIG 6 Experiment 2, habituation, clonidine Dose/response characteristics of investigation of a cup by female (A and B) and male (C and D) gerbils 24-hr after injection of CLON and saline Responses of gerbils that were exposed to the cup on Day 1 are presented as the percentage of responses of gerbils that had not seen the cup previously Duration (A and C) and frequency (B and D) Stars indicate significantly less investigation by Exposed gerbils than Nonexposed Student t-tests on the raw data

but there was no consistent pattern as to treatment, either drug or exposure

Acute drug effects, Day 1 Following drug injections on Day 1, there were significant main effects of CLON (duration $F(3,78)=7$ 86, $p<0$ 0001, frequency $F(3,87)=8$ 82, $p<0$ 0001) and APO (duration $F(1,78)=19$ 64, $p<0$ 0001, frequency $F(1,87)=12$ 16, $p<0$ 001), and significant interactions of CLON × APO (duration $F(3,78)=5$ 36 $p<0$ 01, frequency $F(3,87)=2$ 94, $p<0$ 05), and sex × CLON (duration only $F(3,78)=3$ 41, $p<0$ 05) CLON results on Trial 1 replicate the results of Experiment 1 and, therefore, are not graphed The responses did not vary as a function of sex and only the 0 3 mg/kg dose caused a severe attenuation of responding APO alone caused a small, significant decrease in responding in comparison to saline-treated (compare 0 mg/kg in Fig 5 with 0 mg/kg Trial 1 in Fig 1) CLON with APO did not decrease the number of responses more than APO alone, they were not additive in effect In the duration measure, the sex × CLON interaction was seen only in APO-treated gerbils As shown in Fig 5, there was considerable variability in the responses of all of these animals They were extremely jumpy and hard to handle However, female gerbils that received 0 01 mg/kg CLON and APO responded with approximately the same duration as gerbils without drug or with low doses of CLON (Figure 1), although the frequency was reduced Females given 0 01 mg/kg CLON and APO spent significantly more time investigating the cup than males, $t(6)=3$ 29, $p<0$ 05 There were no other significant differences between males and females on the first trial

Twenty four hour habituation, Day 2 For the duration measure, there was a main effect of exposure condition, $F(1,149)=94$ 94, $p<0$ 0001, and interactions of sex × CLON, $F(3,149)=3$ 74, $p<0$ 02, CLON × APO, $F(3,149)=7$ 69, $p<0$ 0001, and APO × exposure condition, $F(1,149)=7$ 39, $p<0$ 01 On the other hand, the frequency measure reflects main effects of sex, $F(1,150)=7$ 34, $p<0$ 01, CLON, $F(3,150)=3$ 77, $p<0$ 02, and exposure condition, $F(1,150)=29$ 14, $p<0$ 0001, and an interaction between APO and exposure condition, $F(1,150)=6$ 63, $p<0$ 02 These differences are displayed graphically in Fig 6 (CLON) and 7 (CLON + APO) CLON- (0 01 and 0 03 mg/kg) and control-injected gerbils (males and females) exposed to the object on Day 1 responded for shorter durations on Day 2 than those with the same treatment who were not exposed to the cup (Fig 6A and C), reflecting memory of the cup In the frequency measure, control-injected gerbils and CLON-injected female gerbils responded significantly less if they were exposed to the cup on Day 1 (Fig 6B and D) Exposed male gerbils given CLON did not respond significantly differently from Nonexposed

Exposed and Nonexposed gerbils who were treated with APO alone did not differ significantly (Fig 7, 0 mg/kg CLON) CLON prevented the disruption of the duration measure by APO at 01 and 03 mg/kg in female gerbils (Fig 7A) The weaker effect of APO in male gerbils required a higher dose of CLON (0 03 and 0 3 mg/kg) to significantly reverse the effect (Fig 7C) CLON, at 0 3 mg/kg, either alone or with APO disrupted habituation in female, but not in male gerbils APO disrupted the decrease in approaches by Exposed gerbils on Day 2 and CLON did not reverse the effects in either male or female gerbils (Fig 7 B and D)

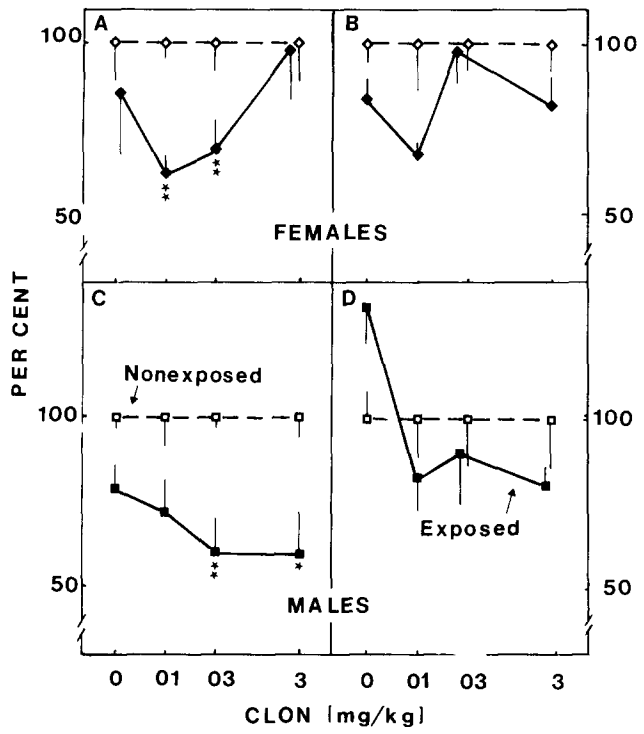

FIG 7 Experiment 2, habituation, clonidine and apomorphine Dose/response characteristics of investigation of a cup by female (A and B) and male (C and D) gerbils 24-hr after injection of CLON and 1 mg/kg APO Duration (A and C) and frequency (B and D) Details as in Fig 6

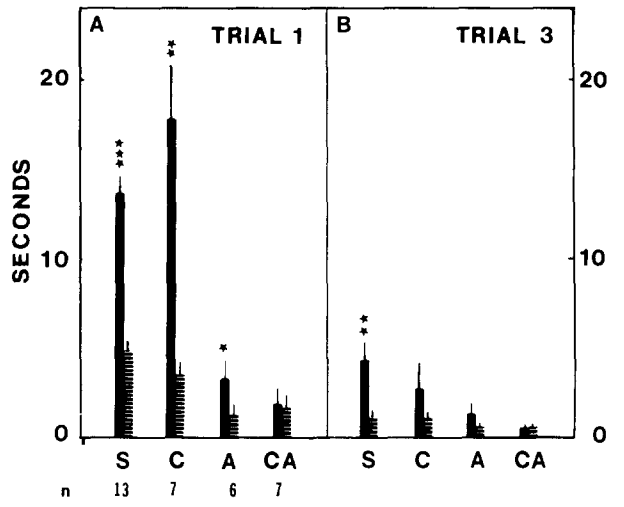

FIG 8 Experiment 3, odor-elicited investigation, clonidine and apomorphine (A) Mean (SE) duration of poking the nose into a hole with odor of strange male bedding (solid bars) or the mean of the nonodor holes (striped bars) on Trial 1 by gerbils following injections of saline (S), 0 01 mg/kg CLON and saline (C) saline and 1 mg/kg APO (A), or CLON and APO (CA) (B) Mean duration of responses on Trial 3 Habituation is reflected by the decrease in responding in comparison to Trial 1 Stars indicate significantly longer investigation of odor hole than nonodor holes

To examine whether there was a relationship between body weight and sex differences, a correlation coefficient was computed between weight and the number of approaches on Trial 2 for Exposed gerbils that received 0 01 and 0 03 mg/kg CLON and no APO These groups showed large sex differences in the number of approaches to the cup The small positive correlation (Pearson $r(21) = 277$) was not significant

Experiment 3, Odor-elicited Investigation, Clonidine and Apomorphine

Investigation of odors The control and CLON injected groups responded significantly more to the hole with odor than to the hole without on the first trial (Fig 8A) APO-treated gerbils spent somewhat more time at the odor hole but did not go to that hole more frequently than to the hole without a specific odor When given CLON and APO, the gerbils showed no preference for the odor

The analysis of variance on the investigation of the odor hole revealed no strong sex effects There were significant main effects of APO (duration $F(1,29) = 25\ 16$, $p < 0\ 0001$, frequency $F(1,29) = 12\ 19$, $p < 0\ 01$) and trial (duration $F(5,145) = 16\ 32$, $p < 0\ 0001$, frequency $F(5,145) = 16\ 10$, $p < 0\ 0001$), and a trial × APO interaction (duration $F(5,145) = 11\ 16$, $p < 0\ 0001$, frequency $F(5,145) = 12\ 11$, $p < 0\ 0001$) The APO effect was statistically significant on

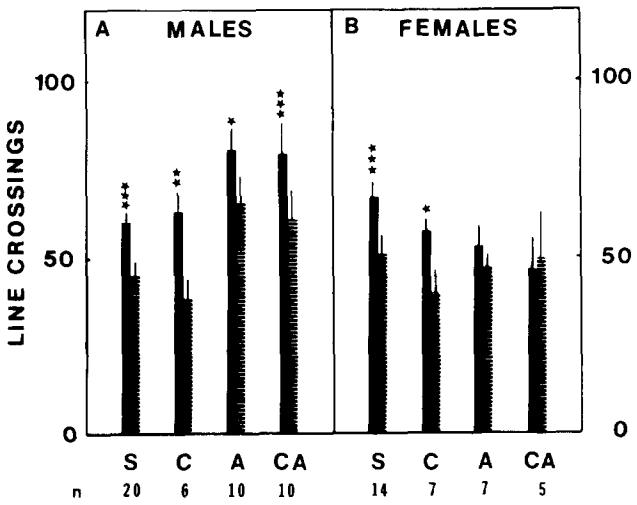

FIG 9 Experiment 3, activity score, clonidine and apomorphine Mean (SE) number of line crossings on Trial 1 (solid bars) and Trial 2 (striped bars) by male (A) and female (B) gerbils following injections of saline (S), 0 01 mg/kg CLON and saline (C), saline and 1 mg/kg APO (A), or CLON and APO (CA) Stars indicate significantly more line crossings on Trial 1 than on Trial 2

the first four trials for duration and on the first two trials for frequency The trial effect reflects habituation as in Experiment 1 (Fig 8B)

Activity score A strong sex effect, $F(1,25) = 23\ 11$, $p < 0\ 0001$, was found in the activity score of the 33 gerbils in

this experiment The sex effect did not appear to be due to weight differences between the sexes because there was no significant effect of weight when weight replaced sex as a grouping variable in the analysis, $F(2,21)=0$ 18 Because the size of the groups were relatively small when separated into sex groups, an additional 14 gerbils were tested for activity and data from these 47 gerbils were combined with data from Experiment 1 and from an earlier study [7] in which the same methods were used The larger group facilitated interpretation of the effects Analysis of variance revealed main effects of sex, $F(1,71)=6$ 89, $p<0$ 01, and trial, $F(1,71)=81$ 62, $p<0$ 0001, and interactions of trail × sex, $F(1,71)=8$ 69, $p<0$ 01, trial × APO, $F(1,71)=8$ 94, $p<0$ 01, and trial × sex × APO, $F(1,71)=4$ 27, $p<0$ 05 As in the other tests, the trial effect is the result of habituation There was a lower mean score on Trial 2 in all groups except female gerbils given both CLON and APO (Fig 9) APO increased the activity of male gerbils with CLON, $t(28)=2$ 112, $p<0$ 05, or without, $t(28)=3$ 337, $p<0$ 01 In contrast, APO reduced the activity score of female gerbils with CLON, $t(17)=2$ 215, $p<0$ 05, or without, $t(19)=2$ 119, $p<0$ 05

The changes in activity could be related to induction of different stereotypies in female and male gerbils However, this dose of APO does not consistently induce stereotypy Continuous sniffing and stereotypic route, the primary stereotypies induced by APO in gerbils [12], do not necessarily alter the line crossing activity score Both behaviors were observed in some of the APO-treated male and female gerbils In addition, two female gerbils (one in the APO group and one in CLON-APO group) spent much of the time chewing, a behavior incompatible with general activity The chewing may have been induced by APO, although chewing is common in normal gerbils also Although the activity scores of these two females were the lowest in their groups, they do not alone account for the lower scores of female gerbils

To determine if sex effects were due to weight differences between male and female gerbils, the data were analyzed by weight instead of sex There was no significant main effect of weight, $F(1,71)=0$ 04, $p=0$ 851

DISCUSSION

Norepinephrine Effects

Normal relative amounts of activity in NE and DA systems are not necessary for investigation of novel stimuli and normal habituation as shown by the comparatively normal pattern of responding in gerbils with acute changes in NE activity due to injections of either CLON or DMI This was true whether the stimuli presented were objects, or odors hidden under holes in the floor Only nonspecific effects of high doses of the drugs attenuated behavior Because high doses of many drugs decrease responding in this paradigm, they cannot be specific For instance, I have already reported similar effects of amphetamine [5,8], apomorphine [7,8], scopolamine, and physostigmine [10] The high dose effects on behavior may be confounded by sedation or toxicity, and will not be discussed in detail

Although CLON did not disrupt investigation or habituation after drug treatment, it affected one measure of habituation 24-hr after treatment, but only in male gerbils (Fig 6) Whereas, habituation was reflected in both duration and frequency measures in female gerbils, only the duration measure reflected habituation in males This can not be related to the frequency of approaching the cup on Day 1 as there were no sex differences on Day 1, nor does it appear to be a difference in sensitivity The effect of increased frequency in male gerbils does not appear to be related to activity levels as low doses of CLON did not affect the activity measure in either male or female gerbils (Fig 9) These results suggest that CLON-treated male gerbils had residual effects that resulted in difficulty in maintaining attention [10]

It is, of course, simplistic to think that the effects of CLON or DMI on these behaviors are solely an effect in stimulating NE receptors DMI increases NE at the synapse by inhibiting NE uptake [24] However, it has also been shown to increase serotonin turnover as measured by the metabolite 5-hydroxyindoleacetic acid (5-HIAA) [29] Mechanism of CLON activity are not thoroughly understood, but it is known that CLON can act in numerous ways Its principle action is to stimulate α-adrenergic receptors [4], but it is also thought to stimulate epinephrine [3], and histamine H_2 receptors [33] It increases serotonin and reverses increases in 5-HIAA induced with DA agonists [27] In addition, it appears to stimulate α-adrenergic autoreceptors causing a decrease in NE activity [31] as evidenced by a decrease in the NE metabolite, MHPG [39], inhibition of NE-elicited cAMP [36], and inhibition of firing of NE neurons in the locus coeruleus [38]

It is also possible that the inhibitory and excitatory actions of CLON are related to dose Some behavioral effects of CLON are found at very low doses, whereas others require much higher doses For instance, as low as 0 01 mg/kg CLON has affected the response of male gerbils (Fig 6), decreased responses on DRL schedules [41], reversed morphine withdrawal [37], decreased amphetamine-induced motor activity [36], and reduced potentiated acoustic startle in rats [16] On the other hand, hypothermia [42], hypotension [31], decreased locomotor activity of gerbils (Experiment 1), and decreased efficiency in operant avoidance [31] or 2-way avoidance [25], required 10–30 times as much CLON Some attempts have been made to separate pre- vs post-synaptic action by the differential blocking properties of NE-antagonists [28, 31, 42]

In spite of these complexities, it is safe to conclude that altering NE action had no selective effects on investigation, habituation, or locomotor activity following injection Only 24-hr later, the frequency measure, but not the duration measure was increased in male gerbils One possible explanation for the normality of responses following NE stimulation may be the counteraction of effects on two different NE systems [26] It was shown in male rats that decreasing forebrain NE content by lesion of the locus coeruleus caused a decrease in locomotor activity, whereas decreasing NE forebrain content by lesion of the ventral NE bundle caused an increase in locomotor activity

Norepinephrine-Dopamine Interactions

As hypothesized, it was possible to prevent the disruption of habituation 24-hr after injection of APO by concurrently giving the appropriate dose of CLON Although these findings suggest an interaction of DA and NE systems in mediating investigation of novel stimuli and subsequent habituation, the effects could be serendipitous As discussed earlier, CLON has numerous effects on neuropharmacology and the present set of experiments do not differentiate between these effects Nonetheless, the data are consistent with the hypothesis that DA and NE play critical roles in survival-related activities[2]

In addition, it was found that different doses of clonidine

were required to prevent the apomorphine effect dependent on sex The sex differences in drug effects on the duration of investigation 24-hr after injection suggest that female gerbils are more sensitive to the reversing effects of clonidine than are male gerbils (Fig 7A and C) The lower dose (0 01 mg/kg) prevented apomorphine from disrupting memory of the cup in females and the higher dose (0 3 mg/kg) was completely disruptive whether given alone or with apomorphine

It appears that sex differences in effective doses are not merely related ot the amount of responding following the drugs on Day 1 for two reasons (1) The percent decrease used to determine habituation is based on the responses of gerbils that received the same drug treatment but were not exposed to the cup on Day 1, providing a within sex, within drug treatment comparison (2) A high duration of responding on Day 1 is not necessary for habituation on Day 2 This was demonstrated by the 0 03 mg/kg CLON plus APO groups On Day 1, males and females responded for only approximately 7 sec, comapred to 20 sec for saline-treated gerbils (Fig 5) Both males and females had significantly shorter durations of responding on Day 2 than Nonexposed gerbils (Fig 7a and C) There was no significant habituation reflected in the frequency measure for either male or female gerbils even at doses where habituation was clearly seen in the duration measure This again suggests residual drug effects that disrupted mechanisms that maintain attention to the stimulus

Day 1 responses may be related to changes in activity level induced by the drugs Subjectively, the combination of CLON and APO resulted in very large startle responses with low thresholds in male and female gerbils as reported in female [1] and male mice [20] Male gerbils moved around the arena more rapidly after APO (1 mg/kg) or after APO and CLON (0 01 mg/kg) than they did after saline, whereas female gerbils moved more slowly (Fig 9) Thus, clonidine did not affect apomorphine-induced increase in locomotor activity to either attenuate or potentiate it This contrasts with the potentiation of apomorphine-induced locomotor activity in mice [1,20] and the attenuation of amphetamine-induced activity in male rats [36] The sex difference in activity could also reflect a difference in sensitivity level as only one dose of APO is reported Although activity scores are not available for gerbils receiving 0 03 mg/kg CLON plus APO, our subjective notes state that male and female gerbils had low threshold startle responses Female gerbils given 0 3 mg/kg CLON and APO also had low threshold startle responses whereas sedation was noted in some of the males We have seen previously that sedative effects of other drugs decreased responding the next day [8,10]

The increase in locomotor activity in male gerbils following APO in this experiment was not reported previously in apomorphine-treated gerbils [7] However, in that experiment, at 1 0 mg/kg apomorphine (n=8), the mean activity score for males was larger than females but did not differ statistically, nor did the combined mean for males and females statistically exceed the mean for controls (n=52) In comparison, female rats had lower activity scores after injections of 0 1 mg/kg, and fewer nose pokes after 0 1 or 1 mg/kg APO than male rats, but the findings were confounded by repeated injections and by sex differnences in the control data [23]

Unfortunately, the majority of the vast literature on behavioral effects of drugs has not included sex differences There is a growing body of data, however, that suggest that sex differences are very important in CA mediated behaviors, such as amphetamine- or apomorphine-induced steroetypy [32,34], locomotor activity after 6-OHDA DA lesions [15], amphetamine-induced locomotor activity [17,35], and amphetamine-induced rotation [30] Estrogen treatment in ovariectomized female, intact male, and prepuberal female rats changes behaviors induced by DA agonists [14,21] and modulates DA agonist effects on other neurotransmitters [18], thus indcating that sex differences may be related to the inhibitory and facilitatory action of estrogen on CA neurons [19] It is tempting to speculate that the sex differences found in the present report are related to natural estrogens in female gerbils, but only additional research will answer that question

To summarize, the same doses of CLON that prevented APO-induced disruption of 24-hr habituation did not reverse the APO-induced reduction in object investigation, the APO-induced reduction in odor investigation and preference, nor the APO-induced changes in locomotor activity Thus, catecholamine interactions in mediation of 24-hr habituation are different than those involved in mediation of behaviors during the acute phase of drug action

ACKNOWLEDGEMENTS

This research would not have been completed without the able technical assistance of E Bartzikus M Chery, J Cohen R Fitzgibbons, D Genovario D Hills, A Horne, G Koumantzelis C Layton, K O Brien D Reisert, S Scalese and R Shumsky, typing of the manuscript by B Panza, and the generous donation of drugs by Dr R Baldessarini, Boehringer Ingelheim Ltd, and Merck Sharp & Dohme The research was supported by the Scottish Rite Schizophrenia Research Program, N M J U S A and by the Biomedical Research Support Program D R R N I H

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