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Noradrenergic Influences on Blocking: Interactions With Development[1]

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CAZA, P. A. *Noradrenergic influences on blocking: Interactions with development.* PHARMACOL BIOCHEM BEHAV **21**(1) 9–17, 1984.—Two experiments assessed the effects of various noradrenergic agents on selective attention. Selective attention was operationally defined as blocking of a conditioned odor aversion by prior conditioning to spatial cues. In Experiment 1, there was a trend for such blocking to increase developmentally. In addition, the administration of isoproterenol prior to training tended to facilitate the demonstration of blocking in adolescent rats and reduced it in adult subjects. In Experiment 2, administration of yohimbine or propranolol prior to training also reduced blocking in adult subjects; however, the concomitant administration of these two drugs restored blocking in adult rats. These results were interpreted to support a model relating an optimal level of noradrenergic activity in the dorsal bundle and selective attention.

Blocking Dorsal noradrenergic bundle Development Isoproterenol Propranolol Yohimbine Rats Selective attention

RESEARCH efforts directed towards identifying physiological substrate of attentional behavior have intensified in recent years. A model currently exists which suggests that the dorsal ascending noradrenergic bundle (DB) plays a critical role in selective attention and stimulus sampling processes [17,21]. Briefly, this model postulates that the DB filters stimuli impinging on an organism. Following neural activity in this tract, irrelevant or noninformative stimuli become ignored. Thus, this noradrenergic (NE) system effectively causes an organism to focus its attention on stimuli relevant for solving the task at hand. As such, activity in the DB is proposed to facilitate selective attention.

The behavioral alteration most often reported following 6-OHDA destruction of the DB is an increased resistance to extinction (e.g., [19, 20, 30]). In addition, a lesion of the DB has also been shown to increase the distractibility of rats in various tasks (e.g., [24]), impair the acquisition and reversal learning of visual discrimination tasks [18], and attenuate latent inhibition and blocking effects [15,22]. Such results appear best explained by a model which suggests a role for the DB in selective attention (see [21] for a discussion of various alternative theories).

Selective attention has been reported to improve ontogenetically [6]. The underlying mechanism(s) for such a developmental improvement has not yet been delineated. The DB is probably immature at birth in the rat. There is a developmental increase in the concentration, replenishment and secretion of NE [13,32]; however, both pre- and post-synaptic adrenoreceptors appear to be present and functional much earlier in the rat [8, 14, 31]. The majority of post-synaptic NE receptors in the neocortex and limbic regions are β-adrenergic [28] and there is ample evidence for corresponding presynaptic alpha-adrenergic receptors in these regions [29]. Thus, although the axonal fibers and receptors seems to mature relatively early in the rat, the lesser concentrations of NE in the young animals probably prevents the system from responding in a truly adult fashion.

There is a resemblance between the behavioral responses exhibited by young rat pups and those seen in DB-lesioned adult rats for a variety of tasks. For example, Amsel and his students have repeatedly found retarded extinction performance in continuously reinforced appetitive tasks for rat pups under 22 days of age (e.g., [1]). Reversal learning of a tactile discrimination task was poorer in 11–14 day old pups than in 15–18 day olds [3], and latent inhibition has been reported to be less in younger than in adult rats [25]. These data strongly suggest that infant rats may be deficient in selective attention processes. This putative deficit could be caused, at least partially, by an immature DB system.

It may be possible to attenuate some of the putative attentional deficits in young rats by pharmacologically stimulating the NE receptors of the DB. By stimulating these functional receptors, the system may respond in a more mature fashion, and possibly the young pups would display more adult-like behavioral responses. To assess the possibility of eliciting relatively mature behavioral responses from young pups following administration of a β-adrenergic agonist, a blocking task was used. In the classic demonstration of blocking, prior conditioning to stimulus A interfered with subsequent conditioning to stimulus B when presented in the compound AB [11]; it has been suggested that such blocking involves selective attention processes (e.g., [16]).

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The first experiment determined if blocking increases ontogenetically. Young (15 days), adolescent (30 days) and adult rats were selected to represent various stages of NE development, and also various stages of selective attention development. In addition, this first experiment assessed whether pharmacologically-induced NE receptor stimulation affects the occurrence of blocking. The β-adrenoreceptor agonist, isoproterenol, was administered to all subjects prior to conditioning. This treatment was hypothesized to improve the blocking performance of the young and the adolescent subjects.

EXPERIMENT 1

METHOD

Subjects

The subjects were 288 experimentally-naive, male and female, Sprague-Dawley derived albino rats. All subjects were born and bred in the SUNY Binghamton colony, and were maintained on a 16 hr light, 8 hr dark illumination cycle (light onset of 0600 hr). All were given food and water ad lib. At the time of training, the rats were classified as either young (15 days of age), adolescent (30 days) or adult (55–65 days); the day of partuition was considered to be Day 0. The 15 day old pups were housed with their parents and conspecifics in standard opague maternity cages partially filled with pine shavings. Adolescent subjects were weaned at Day 21 and housed with their littermates in similar opaque cages. Adult rats were also weaned at Day 21. Three to five similar sex conspecifics were housed in wire mesh cages until the day prior to training when they were transferred to opaque cages (4–7 per cage).

Apparatus

The conditioning apparatus was a two-chambered, 46.0×13.0×15.0 cm shuttlebox equipped with a gridded floor and kept in a dimly lit room. Both sides were fitted with colored plastic inserts. The insert for the shock side was black and that for the safe side, white. The two sides of the shuttlebox were separated by a guillotine door which was also colored appropriately. A 7.5 volt (120 W) flashing light was present in the shock side of the shuttlebox.

Noncontingent footshocks were delivered in a clear 37.0×10.0×14.0 cm rectangular box also equipped with a gridded floor. A Grason-Stadler shock generator delivered scrambled shock to the grid floors of the shuttlebox and noncontingent footshock apparatus.

Odor preference tests were conducted in a clear 44.0×14.0×15.0 cm rectangular box fitted with a wire mesh floor. This test apparatus was divided into two sections. In each section, cotton scented with an odor was placed under the last 10 cm of the mesh floor closest to the end of the apparatus. The ends of this box were fitted with perforated plastic walls, behind which cotton scented with an odor was placed. Thus, two different odors were localized at opposite ends of the test apparatus. The odors were used during conditioning and testing were lemon oil (Humco Laboratories, 1.5 cc), peppermint oil (Humco Laboratories, 1.0 cc) and alcohol-based banana (Virginia Dare, 1.5 cc).

(−)-Isoproterenol hydrochloride (Sigma Chemicals) was prepared daily in a saline vehicle. The drug was injected in a 5 cc/kg volume for the 15 and 30 day old subjects and in a 1 cc/kg volume for the adult subjects. Comparable volumes of 0.9% physiological saline were administered to control subjects.

Procedure

At each age, 48 subjects were randomly assigned to one of two conditioning groups: NCFS-BLO or BL-BLO, where the abbreviation to the left of the hyphen refers to the Phase I training and that to the right, to the Phase II training. Training procedures are discussed in detail below. Subjects were then further assigned to one of three drug conditions: 0 mg/kg (saline), 0.5 mg/kg or 2.0 mg/kg (−)-isoproterenol HCl. There was a total of 6 treatment conditions at each age: 2 (Conditioning Groups) × 3 (Drug Doses) with eight subjects per condition. Care was taken to assign equal numbers of male and female subjects to each of these treatment conditions.

Conditioning and testing occurred over a two-day period. On Day 1, designated as Phase I, all subjects received 10 conditioning trials. For the NCFS-BLO group, Phase I conditioning consisted of ten, 3 sec, 1.0 mA footshocks which were delivered in the noncontingent footshock apparatus. These footshocks were spaced approximately 50 sec apart and were not made contingent with any explicit cue presentation. For subjects in the BL-BLO group, Phase I conditioning consisted of ten pairings of the black shock side/flashing light cue (hereafter termed spatial cue) and a 3 sec, 1.0 mA footshock. Each conditioning trial followed the sequence of 20 sec in the CS− (white side of the shuttlebox without flashing light cue), 10 sec in the CS+ (spatial cue) the last three of which were accompanied by a 1.0 mA footshock, and 20 additional sec in the CS−. Trials were massed.

Twenty-four hours after Phase I conditioning, subjects received Phase II conditioning. During Phase II, only five conditioning trials were given. For NCFS-BLO and BL-BLO subjects, the spatial cue was compounded with an odor cue; subjects were given five pairings of the spatial/odor cue compound and footshock. Each Phase II trial followed the sequence: 20 sec in the CS− (white side of shuttlebox without flashing light + banana odor), 10 sec in the CS+ (spatial cue + lemon odor) the last 3 of which were accompanied by a 1.0 mA footshock, and 20 additional sec in the CS−. Again, trials were massed.

Ten minutes prior to both Phase I and Phase II conditioning, each subject was given a subcutaneous injection of its appropriate drug. In between injection and conditioning, the subject was isolated in an opaque cage partially filled with clean pine shavings. Upon completion of Phase II conditioning and until testing, subjects were placed with already-conditioned conspecifics in an opaque cage partially filled with clean pine shavings. This separation was to prevent non-conditioned subjects from being exposed to any residual odor which may have remained on the fur of conditioned subjects. For the 15 day old pups, the holding cages were placed on top of a heating pad which maintained the ambient temperature at 33±1°C.

Three hours following Phase II conditioning, all subjects received two tests. The first test assessed the magnitude of a conditioned aversion to the lemon odor. For this test, the subject was placed in the center of the odor-testing apparatus and the amount of time spent over each odor half was recorded for 5 min (300 sec). The two odors localized at either end of the testing apparatus were lemon (CS+ odor) and peppermint (novel odor). Approximately 2 hr after the odor test, the subject was given a second test to assess the degree

of conditioned fear to the spatial cue. This test was essentially an escape test in which the subject was placed into the CS+ side (black + flashing light) of the shuttlebox, the door lowered and the amount of time to cross to the CS− side (white + no flashing light) was recorded. No footshock was administered during this test. After entry into the CS− side, the guillotine door was raised and the rat was detained approximately 5 sec. Ten massed escape test trials were given; there was a maximum trial escape latency of 60 sec. If the subject did not cross over within 60 sec, it was gently nudged across and a latency of 60 sec was recorded.

In addition to the NCFS-BLO and BL-BLO groups, 48 subjects in each age group were randomly assigned to one of two control conditions: NCFS-O or BL-O/NC, and one of three drug conditions: 0 mg/kg, 0.5 mg/kg or 2.0 mg/kg isoproterenol. Subjects in the NCFS-O condition provided a baseline measure of odor conditioning, and those in the BL-O/NC group provided a baseline measure of spatial cue conditioning. In addition, potential effects of isoproterenol on escape latency were evaluated by examining escape test data from NCFS-O subjects, and possible effects of isoproterenol on unconditioned odor preferences were assessed by examing odor test data from BL-O/NC subjects.

Training for both control groups was divided into two phases, and all subjects received a SC injection of their appropriate drug ten min prior to both Phase I and Phase II training. For NCFS-O subjects, Phase I training was identical to that for NCFS-BLO subjects. Phase II training for these control subjects consisted of five conditioning trials with the odors and footshock. Each trial followed the sequence: 20 sec in the CS− (banana odor), 10 sec in the CS+ (lemon odor) the last three of which were accompanied by a 1.0 mA footshock, and 20 additional sec in the CS−. The colored shuttlebox inserts and the flashing light were not present in Phase II conditioning for NCFS-O subjects. Thus, subjects in this control group did not receive conditioning to the spatial cue.

Phase I training for the BL-O/NC group was identical to that for subjects in the BL-BLO group. Phase II training for the BL-O/NC control group consisted of five unpaired exposures to the odors and footshock. Subjects in this control group received five, 3 sec, 1.0 mA footshocks spaced approximately 50 sec apart. Thirty to sixty min later, these subjects received five exposures to the banana and lemon odors in the same temporal sequence as rats conditioned with these odors: 20 sec in the banana odor, 10 sec in the lemon odor and 20 additional sec in the banana odor (repeated 5 times). For BL-O/NC subjects, the odors were located beneath two wire mesh cages fitted with plastic tops. Thus, these control subjects were not given explicit conditioning to the odor stimuli.

Three hours after Phase II training, subjects in these two control conditions were given the odor preference and the escape tests.

RESULTS

Odor Conditioning

The degree of odor conditioning is reflected by the difference in the amount of time a subject spends on the novel peppermint half of the testing apparatus and the amount of time it spends on the lemon half. The more time spent on the peppermint half relative to the lemon half, the greater the conditioned fear to the odor. Because of the great variability associated with a simple time measure, a transformation of the data involving a z-score calculation was performed for all subjects using the odor test score. The z-score calculation has the advantage of reducing the standard deviation to unity while leaving the shape of the original data distribution unaltered. By using this particular transformation, significant differences can be assessed more readily. The following formula was used to compute these standardized preference scores: (Individual subject's time over lemon half − treatment group's mean time over peppermint half)/treatment group's standard deviation for time over peppermint half. These normalized preference scores were used in the various Analyses of Variance (ANOVA) described below. It should be noted that the more *negative* a score is, the *stronger* the conditioned aversion to the lemon odor. A positive normalized score represents a preference rather than an aversion to the lemon odor.

TABLE 1

MEANS AND STANDARD ERRORS OF THE MEAN FOR NORMALIZED PREFERENCE SCORES IN BL-O/NC GROUPS

	Ages		
Drug Treatment	15 days	30 days	Adults
Saline	0.25 ± 0.40	−0.54 ± 0.35	0.05 ± 0.35
0.5 mg Iso	−0.41 ± 0.36	−1.13 ± 0.35	−1.51 ± 0.35
2.0 mg Iso	2.42 ± 0.56	−1.30 ± 0.35	−0.85 ± 0.43

Baseline preference for the lemon odor in the adolescent and adult BL-O/NC subjects did not change following treatment with isoproterenol; following administration of the 2.0 mg/kg dose, however, 15 day old pups displayed a greater lemon preference than did their saline-injected counterparts, Age×Drug Dose interaction, $F(4,63)=6.06$, $p<0.01$. Table 1 presents the mean and standard errors of the mean for the standardized preference scores in the BL-O/NC conditions.

Blocking

Blocking refers to poorer conditioning to stimulus B in the compound AB when there has been prior conditioning with stimulus A alone. In the present experiment, blocking is operationally defined as weaker odor conditioning in group BL-BLO relative to group NCFS-BLO. To statistically assess blocking, these two experimental groups were compared in a 3 (Age) × 2 (Conditioning Group) × 3 (Drug Dose) ANOVA using the standardized preference scores described above. Significant main effects of Age, $F(2,126)=11.57$, $p<0.01$; Conditioning Group, $F(1,126)=40.01$, $p<0.001$; Drug Dose, $F(2,126)=29.85$, $p<0.01$; as well as Age × Conditioning Group, $F(2,126)=18.25$, $p<0.001$; and Age × Drug Dose, $F(4,126)=24.66$, $p<0.001$; and Age × Conditioning Group × Drug Dose, $F(4,126)=31.21$, $p<0.001$, interactions were revealed. To assess the validity of the predictions concerning drug effects on blocking at each age, planned comparisons involving the 2 Conditioning Groups and 3 Drug Doses at each of the three ages were calculated separately. At each age, significant Conditioning Group × Drug Dose interactions were found: 15 days, $F(2,126)=5.57$, $p<0.05$; 30

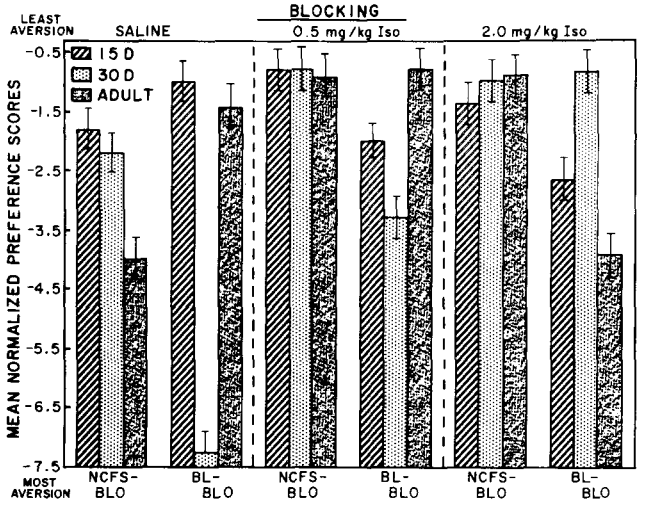

FIG. 1. Age and drug influences on blocking of a conditioned odor aversion. Blocking is operationalized as less negative mean normalized preference scores in the BL-BLO group relative to the NCFS-BLO group. Lines above bars indicate standard errors of the mean for the normalized preference scores.

days, $F(2,126)=32.69$, $p<0.001$; adults, $F(2,126)=30.84$, $p<0.001$. To better understand the nature of these interactions, multiple comparisons using the Newman-Keuls test ($p<0.05$) were conducted on each age group. An examination of Fig. 1, which plots the mean standardized preferences for the various experimental groups, would be useful for the following discussion.

Blocking: 15 Days

Pups at this age did not display blocking either under normal conditions (i.e., saline injections) or after isoproterenol administration. No significant differences in the extent of odor conditioning between NCFS-BLO and BL-BLO groups were observed after any of the three drug treatments. Isoproterenol, however, did influence the strength of odor conditioning for subjects in the BL-BLO groups. Pups receiving 2.0 mg/kg isoproterenol had significantly stronger aversions (more negative difference score) than did their saline-injected counterparts. No difference in aversion magnitudes were observed in the NCFS-BLO groups after isoproterenol administration.

Blocking: 30 Days

Saline-treated adolescent rats demonstrated the opposite of blocking; odor conditioning was *stronger* in BL-BLO than in NCFS-BLO subjects. This difference was attenuated by injections of either 0.5 or 2.0 mg/kg isoproterenol. Adolescent subjects in the BL-BLO groups receiving either drug dose had weaker odor aversions than their saline-injected counterparts; this effect showed a dose-related response. The strength of odor aversions were not altered by isoproterenol in the NCFS-BLO subjects.

The decreased odor aversions demonstrated by BL-BLO subjects treated with isoproterenol suggests that this drug may have increased blocking in these adolescent subjects; however, this conclusion is not unequivocal. At no drug dose did BL-BLO subjects display less aversion than NCFS-BLO subjects. A potential difference in aversion strength between NCFS-BLO and BL-BLO subjects reflective of blocking may have been obscured by ceiling odor preferences. Aversion magnitudes for the adolescent NCFS-BLO and BL-BLO pups given 2.0 mg/kg isoproterenol did not significantly differ from baseline unconditioned odor preference for similarily-injected BL-O/NC subjects, $F<1$.

Blocking: Adults

Blocking was observed in adult subjects treated with saline; less aversion was evident in BL/BLO than in NCFS-BLO subjects. The strength of odor conditioning in NCFS-BLO subjects was decreased by treatment with 0.5 and 2.0 mg/kg isoproterenol. Furthermore, administration of 2.0 mg/kg isoproterenol strengthened odor aversions for BL-BLO subjects. In the 2.0 mg/kg dose condition, adult BL-BLO subjects had stronger odor aversions than did NCFS-BLO subjects. It appears, therefore, that isoproterenol administered to adult rats attenuates or abolishes blocking.

Blocking: Summary

The degree of blocking using this paradigm increased ontogenetically. Young and adolescent rats failed to display blocking, while adult rats did. Administration of isoproterenol did not alter the degree of blocking in the 15 day old pups; however, this β-adrenergic agonist tended to ameliorate blocking in adolescent pups and attenuated it in adult animals. The alteration in the degree of blocking by isoproterenol in adolescent and adult rats was not due to drug-related changes in baseline odor preferences as normalized preference scores from subjects in the BL-O/NC groups did not differ as a function of isoproterenol administration at these two ages.

Escape Test

Trial latencies were transformed into reciprocal (1/time) latencies to reduce variability. These reciprocal latencies were then summed over two adjacent trials, and the mean latency for the trial block was calculated. Thus, reciprocal latency data from the escape test was reduced to 5 trial blocks.

To determine if isoproterenol influenced the latency of subjects to cross from the CS+ to the CS− side of the shuttlebox, a 3 (Age) × 3 (Drug Dose) × 5 (Trial Blocks) repeated measures ANOVA was computed using the mean reciprocal latencies from the NCFS-O subjects. This analysis indicated nonsignificant main effects of Age, $F(2,63)=2.38$, $p<0.05$, and Drug Dose, $F<1$, as well as a nonsignificant Age × Drug Dose interaction, $F(4,63)=1.16$, $p>0.05$. Subjects in the NCFS-O condition did, however, increase their amount of time to cross to the CS− side over trials, $F(4,252)=6.07$, $p<0.01$.

An analysis examining the mean reciprocal latencies across trial blocks for the NCFS-BLO, BL-BLO and BL-O/NC groups yielded significant main effects of Age, $F(2,189)=7.31$, $p<0.01$, and Conditioning Groups, $F(2,189)=10.02$, $p<0.001$. However, isoproterenol did not significantly alter the escape latencies of subjects in these groups $F(2,189)=1.13$, $p>0.05$. Posthoc analyses using the Newman-Keuls test ($p<0.05$) revealed that adolescent pups crossed to the CS− side quicker than did 15 day old subjects or adults. These latter two age groups did not reliably differ

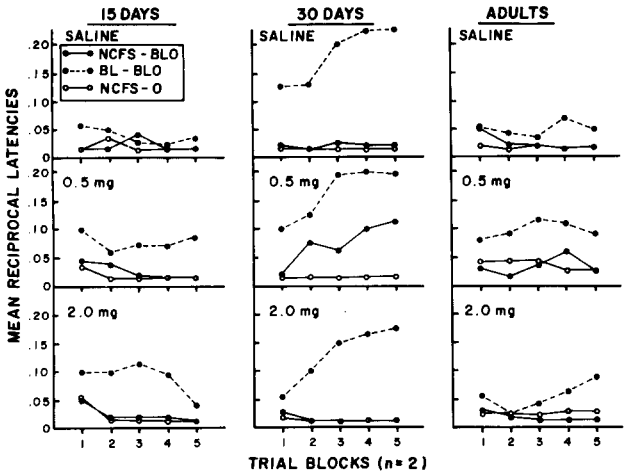

FIG. 2. Mean reciprocal escape latencies as a function of age, drug dose, and conditioning history. The degree of conditioning to the spatial cue is operationalized by the magnitude of the reciprocal latency; the greater the reciprocal latency, the greater the conditioned fear to the spatial cue.

in their escape latencies. Escape latencies were rank ordered according to the number of conditioning trials with the spatial cues; BL-BLO subjects were significantly faster than BL-O/NC subjects who were, in turn, significantly faster than NCFS-BLO subjects. Figure 2 reveals the mean reciprocal latencies for the NCFS-O, NCFS-BLO, BL-O/NC and BL-BLO groups at each age and drug dose.

The repeated measures ANOVA also yielded a significant Age×Trial Blocks interaction, $F(8,756)=4.29$, $p<0.01$. As seen in Fig. 2, adolescent pups increased their spped to escape over trials while the young and adolescent subject were fairly stable across trial blocks.

Escape Test: Summary

Conditioning to the spatial cue was demonstrable using the escape test. Subjects in the BL-BLO condition escaped reliably faster than those in the BL-O/NC or NCFS-BLO conditions. Although this difference was evident at all three ages, the adolescent pups had considerably quicker escape latencies overall. Isoproterenol had no influence on escape latencies for either the conditioned or unconditioned (NCFS-O) subjects.

DISCUSSION

The results of this first experiment indicate that (1) blocking of a conditioned odor aversion by spatial cues increases ontogenetically, and (2) the β-adrenoreceptor agonist, isoproterenol, alters this blocking. The directionality of this alteration appears to be contingent upon the baseline, nondrugged behavior of the animal. For example, 2.0 mg/kg isoproterenol tended to increase blocking in 30 day-old pups, but disrupted it in adults. In general, the results of this experiment can be summarized by the following statement: when selective attention is weak (operationalized by no to little blocking), isoproterenol has a facilitory effect; conversely when selective attention is strong, isoproterenol has a deleterious effect. Stated more simply, there appears to be an optimal level of NE activity for selective attention.

To facilitate discussion of the data, it will be assumed that subcutaneous administration of isoproterenol to subjects in this experiment influenced NE activity in the DB. By stimulating postsynaptic receptors of the DB in immature rats, NE activity in this tract should be increased and, according to Mason's model, selective attention (blocking) should also be increased in these animals. Noradrenergic activity in the DB of adult rats should already be sufficient for selective attention, and further stimulation of these postsynaptic receptors should serve to overstimulate the system.

In this experiment, selective attention tended to be improved in adolescent rats following administration of isoproterenol. Blocking was not evident in saline-treated adolescent pups. In fact, 30 day old subjects given prior training with the spatial cue (BL-BLO) demonstrated stronger conditioning to the odor cue when it was presented in compound with the spatial cue than did subjects without such prior conditioning (NCFS-BLO). Administration of either 0.5 or 2.0 mg/kg isoproterenol to these pups attenuated the magnitude of conditioned odor aversions displayed by subjects in the BL-BLO group. Following treatment with 2.0 mg/kg isoproterenol, no difference was observed in the degree of conditioned odor aversions for subjects in NCFS-BLO or BL-BLO groups. Because normalized preference scores from subjects in these two treatment conditions did not differ from those in nonconditioned subjects (BL-O/NC), a difference between BL-BLO and NCFS-BLO subjects reflective of blocking was probably obscured by ceiling odor preferences. It appears, therefore, that isoproterenol tends to increase selective attention in adolescent pups, presumably by stimulating the postsynaptic receptors of the DB.

The drug doses used in this experiment did not affect blocking in the 15 day old subjects. It is possible that the doses of isoproterenol used were insufficient, and that by increasing the dose, blocking may be enhanced in these young animals similar to that in the adolescent pups.

Selective attention was not magnified by stimulation with isoproterenol in adult rats. Rather, administration of this drug attenuated blocking these subjects, and the 2.0 mg/kg dose actually abolished blocking in this experiment. These data suggest that past an optimal point, further stimulation of NE activity in the DB results in the disruption of selective attention and a degradation of behavior indicative of this process.

The drug-related results from the adolescent and adult rats strongly suggest an inverted U-shape relationship between noradrenergic activity in the DB and selective attention. Too little activity (i.e., immature rats) or too much activity (i.e., adult rats given isoproterenol) results in poor focused attention, operationalized in the present experiment as decreased blocking.

Although significant conditioning to the spatial cues was evidenced at all three ages in this experiment, isoproterenol had no significant impact on behavior in the escape test. Why this drug did not modify the degree of spatial cue conditioning in a fashion similar to its effect on blocking is not clear. Perhaps the escape test used in this experiment was too insensitive to yield the interactions necessary for concluding differential drug effects on attention, and therefore, the magnitude of conditioning. One possible reason for this apparent insensitivity is that the escape test always followed the odor preference test, and thus was subject to any extinction which may have resulted from the odor test.

Whatever the reason for its failure to affect escape test

behavior in this experiment, it is clear that isoproterenol modifies blocking. The explanation offered thus far for this drug's effect on blocking behavior has rested upon the assumptions that (1) blocking involves some measure of selective attention, (2) selective attention is influenced by activity in the DB, and (3) isoproterenol directly affects neural pathways mediating such attentional processes. The first two assumptions, while not unequivocally proven, have been substantiated by other experimenters. The last assumption, however, is not directly supported by any data. Although there is evidence that isoproterenol crosses the blood-brain barrier [5], no data exists demonstrating that isoproterenol injected SC stimulates β-adrenoreceptors associated with the DB. It is feasible, therefore, that the effects of isoproterenol in the present experiment are attributable to its action on systems or processes other than the DB. Isoproterenol may have affected behavior in this experiment by (a) altering the perception of the UCS intensity, (b) altering the perception of the CS, (c) altering activity levels during the test, (d) directly influencing the associability of the CS and UCS, or (e) influencing the probability of state-dependent learning. These alternate explanations must be dismissed before any conclusions can be reached concerning the effects of isoproterenol on blocking behavior in this experiment.

A number of isoproterenol's physiological effects are similar to those observed following administration of epinephrine [5]. Because epinephrine is secreted in stress-provoking situations (such as the shock-motivated task used in the present experiment; [33]), injections of isoproterenol may have intensified the perceived aversiveness of the UCS in this experiment. A direct relationship has been reported between the intensity of the UCS and the strength of conditioning [2,26]; therefore, if isoproterenol increased the perceived aversiveness of the shock UCS, stronger conditioning should be evidenced by the drug-treated subjects relative to those treated with saline. Stronger conditioning to the spatial cues in Phase I could yield greater blocking to the odor cue in Phase II. Subjects given isoproterenol should show greater blocking than those given saline. This was somewhat true for the 30 day old pups in this experiment; however, adult subjects had reduced blocking following isoproterenol treatment. The results of Experiment 1, therefore, do not readily conform to an explanation involving isoproterenol-induced alterations in UCS intensity.

Likewise proposing that isoproterenol altered perception of the odor or spatial CS's in this experiment is unlikely. If isoproterenol altered subject's perception of either these two conditioning stimuli, test behavior should have changed as a function of drug dose in the unconditioned subjects. Baseline odor preferences in the BL-O/NC groups did not vary as a function of isoproterenol dose in either the adolescent or the adult rats, and isoproterenol did not influence the latency to escape from the spatial cues in the NCFS-O subjects.

Isoproterenol has been shown to depress locomotor activity in rats when administered intraventricularly [12]; thus it is feasible that the results of the present experiment are due to a drug-induced activity change. Isoproterenol, however, did not effect latencies in the escape test, and data from the odor test do not conform to a simple pattern of drug-induced depression of locomotion. Drug-related activity changes at the time of test is not adequate explanation of the present data.

Because catecholamines have been implicated as important for both acquisition (e.g., [23]) and retention (e.g., [9]) processes, it is possible that isoproterenol effected changes in the associability of the CS and UCS in Experiment 1. To adequately explain the present data, it must be proposed that (1) an inverted U-shape function exists between NE activity and CS-UCS associability, and (2) the ability to form an association between the CS and UCS improves ontogenetically. While there is no data to refute these two proposals, less information is available to substantiate these hypotheses than there is to support similar hypotheses concerning selective attention.

Yet another potential explanation for the effects of isoproterenol in the current experiment is the possibility of state-dependent learning. State-dependent learning refers to the empirical findings that learning acquired in a particular state shows better transfer when the organism is in the same state. In the present experiment, subjects were trained following injection of isoproterenol and tested in a drug-free state. Some dissociation, therefore, may have occurred at the time of test, and poorer test performance should be expected for isoproterenol-treated subjects. Adult rats given isoproterenol did display less blocking than saline-injected subjects, but younger subjects showed, if anything, better blocking following isoproterenol treatment. In view of the finding that younger rats may be more susceptible to the detrimental dissociation of learning when switched from a drug to a drug-free state [4], the improved performance in the younger subjects following a drug-no drug transfer argues against a state-dependent interpretation of the present data.

The most viable explanation of the results from Experiment 1 is one which proposed isoproterenol-induced changes in selective attention. Such changes could be mediated by stimulation of the postsynaptic NE receptors associated with the DB. The present data suggest that an optimal level of NE activity in the DB is necessary for selective attention. These results further suggest that selective attention in rats improves developmentally, and that attentional behavior in immature pups benefits from peripherally administered isoproterenol.

EXPERIMENT 2

The results of Experiment 1 suggest that an optimal level of NE activity results in selective attention, and increases or decreases in this activity produce attention that is less than focused. Accordingly, any pharmacological agent which affects NE activity in the DB should influence selective attention, and thus, blocking. For example, by blocking postsynaptic adrenoreceptors in the DB, β-adrenoreceptor antagonists should reduce NE activity and behaviorally, result in less focused attention in adult rats. Thus, propranolol when given to adult rats in the present paradigm should disrupt blocking. Another agent which should influence NE activity in the DB is yohimbine, an alpha-adrenoreceptor antagonist. Yohimbine's effects are largely restricted to the $alpha_2$ or presynaptic receptors [27]. Agents which block the $alpha_2$ adrenoreceptors should inhibit the feedback system regulating NE activity and more NE should be released from the presynaptic terminals. In adult rats, this should result in a greater than optimal level of NE stimulation for selective attention. Thus, drugs such as yohimbine are also predicted to reduce blocking.

Because of this opposing directionality of the proposed drug effects, it is predicted that administration of yohimbine concomitantly with propranolol should return NE activity to near optimal levels, and selective attention should be re-

stored. Although blocking is predicted to be disrupted in adult rats following treatment with either propranolol or yohimbine alone, blocking should recur when these two drugs are given in combination.

To test these predictions, adult rats were assigned to receive either yohimbine, propranolol or propranolol + yohimbine prior to training in both Phases I and II. As in the first experiment, the effects of these drug treatments on blocking of a conditioned odor aversion were assessed. The escape test was not given because of the relatively poor performance of adult subjects in this test in Experiment 1.

METHOD

Subjects

Sixty-four experimentally naive, male and female adult (65–80 days), Sprague-Dawley derived rats were used as subjects. They were housed and maintained as in Experiment 1.

Apparatus

The training and testing apparati were identical to those used in Experiment 1. The drugs utilized in this experiment were yohimbine hydrochloride and propranolol hydrochloride (Sigma Chemicals). Yohimbine was dissolved in distilled water and propranolol, in a 0.9% physiological saline solution; both drug solutions were prepared daily. Based on pilot studies in which the effects of various drug doses on blocking were examined, a 1.0 mg/kg dose of yohimbine and a 20.0 mg/kg dose of propranolol were chosen. Both drugs were injected intraperitoneally (IP): yohimbine in a 1.0 cc/kg volume and propranolol in a 2.0 cc/kg volume.

Procedure

Subjects were randomly assigned to one of four drug conditions: saline, yohimbine, propranolol or propranolol + yohimbine. Within each drug condition, subjects were further assigned to either NCFS-BLO or BL-BLO conditioning groups. There was a total of 8 groups (2 Conditioning Groups × 4 Drug Treatments) with an equal number of male and female subjects in each.

Prior to both Phase I and Phase II conditioning, subjects were given two drug injections. Twenty minutes prior to both Phases, subjects received an IP injection of either saline (saline and yohimbine treatment conditions) or propranolol (propranolol and propranolol + yohimbine treatment conditions). Ten minutes later they were given and IP injection of either saline (saline and propranolol treatment conditions) or yohimbine (yohimbine and propranolol + yohimbine treatment conditions). Phase I and Phase II training was conducted 10 min after this second injection. Conditioning and testing procedures for the NCFS-BLO and BL-BLO groups were identical to those in Experiment 1.

RESULTS

Three subjects were discarded due to equipment failure; therefore, odor preference test data from only 61 subjects were analyzed (at least 7 subjects were present in each Drug Treatment × Conditioning Group cell). The data were transformed into standardized preference scores using the formula outlined in Experiment 1.

A 2 (Conditioning Group) × 4 (Drug Treatment) ANOVA conducted on these standardized scores indicated significant main effects of Conditioning Group, $F(1,53)=55.92$, $p<0.001$, Drug Treatment, $F(3,53)=25.22$, $p<0.001$, as well as significant interaction between these two factors, $F(3,53)=24.09$, $p<0.001$. It is obvious from Fig. 3 that blocking was evident in saline-treated subjects; NCFS-BLO subjects displayed greater odor aversions than did BL-BLO subjects. Treatment with either yohimbine or propranolol eliminated this blocking, while treatment with both drugs concomitantly reinstated blocking.

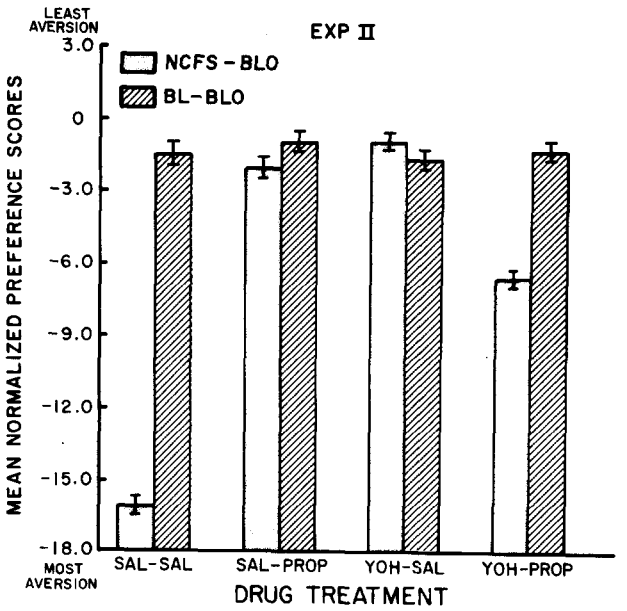

FIG. 3. Mean normalized preference scores for adult rats in the NCFS-BLO and BL-BLO groups. Lines above bars indicate standard errors of the mean.

A Newman-Keuls posthoc analysis ($p<0.05$) yielded no significant difference between the standardized preference scores from any of the BL-BLO groups or from the NCFS-BLO groups given yohimbine or propranolol treatment. In addition, the degree of conditioned aversion to lemon was less for propranolol + yohimbine NCFS-BLO subjects than for saline-treated NCFS-BLO subjects. The degree of conditioned aversion for the former NCFS-BLO group was significantly greater than for that from the remaining six groups

In summary, the results of the second experiment indicate that the blocking observed in adult rats given saline injections was abolished by treatment with either the alpha$_2$-adrenoreceptor antagonist, yohimbine, or the β-adrenoreceptor antagonist, propranolol. Blocking was restored following concomitant treatment with both drugs.

DISCUSSION

As predicted, blocking was disrupted in adult rats treated with either propranolol or yohimbine prior to training. Also as hypothesized, administration of both these agents prior to training reversed their individual effects and blocking was restored. These results support an inverted U-shape relationship between NE activity in the DB and selective attention.

The blocking observed in the propranolol + yohimbine condition was of a significantly lesser magnitude than that which was observed in the saline-treated groups. Although odor aversions from the two BL-BLO groups did not differ, aversion strength was weaker for NCFS-BLO subjects given

the drug combination than for those given saline. This difference may suggest that the doses of propranolol and yohimbine necessary to yield odor aversions equivalent to those in saline-treated NCFS-BLO subjects were not used.

It is improbable that the observed influences of yohimbine and propranolol on blocking are related to the peripheral effects of these two drugs. Yohimbine, like isoproterenol, has sympathomimetic activity; propranolol is devoid of such activity [5]. It is unlikely that the similar actions of yohimbine and propranolol on blocking are related to their very different peripheral actions. Rather, the behavioral effects of these drugs in this experiment appear best understood within the framework linking selective attention with NE activity in the DB.

GENERAL DISCUSSION

These two experiments have demonstrated that selective attention, as represented by blocking of a conditioned odor aversion, changes as a function of age and NE stimulation. Using a paradigm in which an odor stimulus was presented in compound with a previously conditioned spatial cue, young (15 days) and adolescent (30 days) rats failed to exhibit blocking; adult rats demonstrated blocking in this paradigm. The lack of blocking in the 15 and 30 day old pups was not due to a failure to condition to the spatial or odor cues, but instead, the age-related pattern of blocking suggests that rat pups are deficient in selective attention processes and these processes improve ontogenetically.

Selective attention in the adolescent pups was somewhat facilitated by treatment with the β-adrenoreceptor agonist, isoproterenol. Following administration of 2.0 mg/kg isoproterenol during training, there was a tendency towards blocking in adolescent pups. Adult rats receiving the same treatment had decreased blocking. Thus, increasing NE activity by stimulating postsynaptic β-adrenoreceptors tended to improve selective attention in adolescent rats and impaired it in adult subjects. This pattern of results indicates that an optimal level of NE activity is required for selective attention. Additional support for this relationship was provided in Experiment 2. In this experiment, administration of either yohimbine, an alpha$_2$-adrenoreceptor antagonist, or propranolol, a β-adrenoreceptor antagonist, abolished blocking in adult rats; the two drugs in combination, however, reinstated it. Such an inverted U-shape function involving an attentional variable is not novel. Manipulation of factors such as activiation and noise level has also yielded similar functions (e.g., [7,10]). It may be that these factors are also influencing NE activity.

Because the DB has been implicated in selective attention processes, it is proposed that the various psychopharmacological agents used in these experiments exerted their effects by influencing NE activity in the DB. Such a model relating selective attention to an optimal level of NE activity in the DB is clearly amenable to future research. Its reserach potential is enhanced by its ability to provide a useful heuristic for dealing with ontogenetic changes in learning and memory, and by its ability to predict possible treatments for the alleviation of learning and memory deficits.

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