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Differential Effects of Intraventricular Administration of 6-Hydroxydopamine on Behavior of Rats in Approach and Avoidance Procedures: Reversal of Avoidance Decrements by Diazepam[1]

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BEER, B. AND L. G. LENARD. *Differential effects of intraventricular administration of 6-hydroxydopamine on behavior of rats in approach and avoidance procedures: reversal of avoidance decrements by diazepam.* PHARMAC. BIOCHEM. BEHAV. 3(5) 879–886, 1975. – The administration of 6-hydroxydopamine (6-HD) and pargyline to rats produced similar selective decreases in responding during the conditioned stimulus (CS) on a discriminated avoidance test where the unconditioned stimulus (US) was shock and on an analogous conditioned approach test where the US was water. Approach behavior during the CS generally recovered, however, while avoidance decrements in the same rats remained for the duration of testing. This suggested that 6-HD-induced avoidance decrement was a result of two independent but interacting processes: (1) a decrease in conditioned behavior as reflected by the similar decrease in responding on both tests; and (2) a hyper-reaction to aversive stimuli that resulted in a tendency to selectively suppress avoidance behavior after the animal received shock. In support of this hypothesis, it was found that 6-HD-induced avoidance decrements could be reversed (1) by treatment with diazepam, a drug that releases suppressed responses; or (2) by delaying avoidance testing until conditioned responding had recovered, thus minimizing the interaction of the two processes

6-Hydroxydopamine Conditioned approach Catecholamines Diazepam Avoidance Response suppression

PERMANENT depletion of the brain catecholamines (CA) norepinephrine (NE) and dopamine (DA), after the intraventricular administration of 6-hydroxydopamine (6-HD), causes long-term decrements in conditioned avoidance responding, with little or no effect on escape behavior [6, 21, 22]. The great susceptibility of the avoidance response to disruption has also been noted after treatment with other pharmacologic agents that interfere with CA transmission in the central nervous system [5,18]. Dews and Morse [12] have suggested that this selective behavioral decrement may be the "salient characteristic" of neuroleptics, drugs that also have inhibitory influences on central CA mechanisms.

The survival of the escape response after 6-HD treatment indicates that the animal's failure to avoid is not simply the result of a physical inability to make the required response (jumping, shuttling, etc.). Selective decreases in responses similar to those observed in avoidance situations have never been reported after treatment with 6-HD when appetitive procedures have been employed. The use of appetitive procedures in studies of fixed ratio (FR) responding for food or water has shown that 6-HD causes either no change [17], a short-term decrease [31], an increase [28], or a long-term decrease [23] in response rates. Cooper *et al.* [7] reported no change in lever-pressing rates for rats on a continuous-reinforcement schedule in a T-maze goal-box,

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[2] Much of these data were used by L. G. Lenard to partially fulfill the requirements for the Ph.D. degree at Rutgers, The State University.

but large, and apparently long-term, decreases in responding were observed when the apparatus was changed to a double T-maze [16]. Schoenfeld and Uretsky [30] observed a long-term increase in lever-pressing rates for rats on a variable-interval schedule for food. Mason and Iversen [25] observed decreased responding in a situation that required a rat to push a ball through a cylinder. Thus, experiments involving 6-HD and behaviors maintained by positive reinforcement have not clarified the issue of selective decreases in responding.

One of the primary distinctions between avoidance procedures, for which selective drug effects have been reported, and appetitive procedures, for which they have not, is the presence of salient discriminative stimuli in discriminated-avoidance procedures. Furthermore, the animal in the avoidance situation has the opportunity to respond to either of two discriminative stimuli, the CS or shock. Studies with chlorpromazine (CPZ), a drug that has selective effects on avoidance [5], have shown that, after CPZ treatment, pigeons responding for food on multiple- or mixed-operant schedules tend to exhibit a blurring of the differences in typical response patterns between different components of the schedule [11,33]. Such results suggest that CPZ produces at least part of its behavioral effect through a diminution of the significance for the animal of those discriminative stimuli. It is noteworthy that, under these conditions, CPZ actually caused an increase in response rates during schedule components normally associated with low rates [33]. These data indicate that, given the proper stimulus conditions in an appetitive procedure, selective decreases in responding might be observed after treatment with 6-HD.

Another major difference between avoidance and appetitive paradigms is the presence of a shock component only in avoidance. The animal's response to very aversive stimuli is almost certainly relevant to its ability to avoid. Both NE and DA neurons in the brain have been shown to be active during stress [2, 9, 10]. Activity in NE neurons, in particular, seems to inhibit the release of ACTH during stress [14]. Lenard and Beer [20] have observed that rats treated with 6-HD show exaggerated responsivity (increased vocalization, piloerection, rigid posture, urination, and defecation) during shock as well as during the CS that has been paired with shock. This hyperemotional responsivity appears to interfere with the avoidance response and, thus, produces an increase in the suppression of that response. These data suggested that this reaction to aversive stimuli might be responsible for the long-term decrease in avoidance behavior that was observed in rats treated with 6-HD. If this were the case, it would be expected that treatment with 6-HD should have little or no effect on the performance of an appetitively motivated task.

One means of testing the significance of these stimulus and shock variables and the response of the 6-HD-treated animal to them might involve a positive reinforcement paradigm that was analogous to discriminated avoidance in terms of stimulus conditions, but lacked the shock component. Such a procedure has recently been developed by Migler [26]. In this conditioned approach procedure, a monkey was presented with a CS of fixed duration. If the animal made the required response (CR) during the CS, that response was reinforced with food (UCS). This was analogous to avoidance procedures in which a response during the CS is also reinforced (with shock avoidance). If the monkey failed to respond during the presentation of the CS, the food was presented and the animal was allowed to make a consummatory response (UCR). This is analogous to an escape response because if the animal fails to respond during the CS, the unconditioned stimulus (shock) is presented and the animal can then respond during that stimulus and be reinforced by its termination.

Migler's results in monkeys indicate that conditioned approach behavior is analogous to avoidance behavior. His animals made most of their responses during the early portion of the CS, but, when given CPZ, they frequently did not respond during the CS, but did respond during the UCS (i.e., they consumed the food). Diazepam (DZP), which has little or no effect on avoidance behavior, except at neurotoxic doses [29], also had no effect on conditioned approach behavior [26]. We have obtained similar results with CPZ, using a conditioned approach procedure designed for rats [20].

EXPERIMENT 1

This experiment was designed to examine the role of the UCS in the avoidance behavior of 6-HD-treated rats by comparing the behavior of such rats in both avoidance and approach procedures. Each rat was, therefore, trained on both a discriminated avoidance and a conditioned approach test, and was then given 6-HD intraventricularly. The performance of these rats in each procedure was then studied for an extended period.

METHOD

Animals

Male Sprague-Dawley (Holtzman) rats, 200–250 g, were housed in individual cages and were maintained on a 12 hr light-dark cycle. The rats were kept on a water-deprivation schedule that allowed them 18 ml of water per day, a maximum 8 ml of which could be earned during conditioned approach testing.

Procedure

Surgical procedure. Each rat was implanted with a permanent cannula (Plastic Products Co.) in either the left or right lateral ventricle. The details of the implantation procedure have been described elsewhere [21]. In brief, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal® – Abbott), 25 mg/kg, and chloral hydrate, 150 mg/kg. They were placed in the stereotaxic instrument (David Kopf Instruments), using earbars designed to avoid puncture of the eardrum. The coordinates for implantation into the lateral ventricle were AP = +7.0, Lat. = ±2.0, DV = +7.0, according to the atlas of Albe-Fessard *et al.* [1]. Training did not begin until at least 7 days after surgery.

Avoidance procedure. Avoidance behavior was measured in shelf-jump avoidance chambers based on a design by Tenen [32]. Shock (2.0 mA, provided by a BRS/Foringer SG-901 shock generator) was delivered to the grids at appropriate times via a shock-scrambler circuit.

Each test session consisted of 50 trials. A trial was initiated by the presentation of the CS (withdrawal of a black wall and consequent exposure of a shelf, with accompanying noise and vibration). If the rat jumped onto the shelf within 10 sec after the start of the CS, thus tripping a microswitch under the shelf, shock was avoided and that trial was terminated. A 20 sec intertrial interval (ITI) followed. For the first 10 sec of the ITI, the rat was

permitted to remain on the shelf, but was then gently pushed off the shelf by the moving wall, back onto the grids, where it was allowed 10 sec more before the start of the next trial. If the rat failed to jump onto the shelf within 10 sec after start of the CS, a series of shock pulses (0.5 sec in length, separated by a 2 sec shock-shock interval) was initiated. If the rat then jumped onto the shelf, it escaped from the shock, ending that trial and initiating an ITI, as described above. A maximum of 10 shocks was presented if no escape response occurred.

Conditioned approach procedure. Conditioned approach testing took place in sound-attenuated test chambers (Lehigh Valley Electronics) that contained a house light, a speaker for the delivery of auditory stimuli and white noise, a dipper for the delivery of 0.1 ml of water, a lever, and a white light above the lever. A drinkometer (Grason-Stadler) was connected between the grids and the dipper so that consummatory responses could be detected.

Conditioned approach training. The rats were deprived of water for 24 hr preceding the first training session and were then maintained on the deprivation schedule previously described. During an initial training session with the lever absent, the water dipper was presented on an FT30 sec schedule. The UCS consisted of the presentation of a dipperful of water accompanied by a brief (0.25 sec) tone and the illumination of the white light above the site where the lever would later be located. This light remained lit and the dipper remained available until 10 sec after the rat's first contact with the dipper, after which the dipper was withdrawn and the light was extinguished. For the second session, a lever was installed and the presentation of the water dipper was made contingent upon a lever press. In addition, a CS (clicker) was presented to signal the availability of the reinforcer for each lever press. The CS was terminated with the lever response and was re-instituted at the withdrawal of the dipper.

During the third and fourth sessions, the CS was presented for a maximum of 30 sec. If a lever press occurred during this period, the CS was immediately terminated and the water dipper was made available for 10 sec. If no lever press occurred during the CS, the CS was terminated after 30 sec and the dipper was made available for 10 sec after the rat's first contact with the dipper.

On all occasions, an ITI of 20 sec followed the withdrawal of the dipper. During the fifth session and each succeeding session, the CS was presented for a maximum of 10 sec, but all other variables remained the same as described.

If no response occurred during the 10 sec CS or 20 sec UCS, the dipper was withdrawn and, after a 20 sec ITI, a new trial was initiated. A trial was extended beyond the 30 sec limit if the rat started a consummatory response with less than 10 sec remaining in the trial. Such a response started a timer that gave the rat 10 sec to drink, no matter how much time would normally have remained in that trial. Each session consisted of 50 trials.

General procedure. The rats were trained and tested for approach and avoidance behavior 7 days per week, according to the following schedule: Day 1 – Avoidance; Days 2–6 – Approach; Days 7–8 – Avoidance; Days 9–10 – Approach; Days 11–12 – Avoidance; Days 13–17 – Approach.

At the conclusion of training, all 19 rats received an intraperitoneal injection of pargyline (Eutonyl® – Abbott), 50 mg/kg. Thirteen rats each received an intraventricular injection of 6-HD (Regis), 250 μg dissolved in 20 μl of 0.9 percent saline + 0.05 percent ascorbic acid, 30 min after the pargyline treatment; the remaining 6 rats received 20 μl of the saline-ascorbic acid vehicle. A second intraventricular injection of 6-HD, 250 μg, or of vehicle was given 24 hr later, without pargyline pretreatment. Doses were calculated as the weight of the salt. The procedure for intraventricular injection has been described in detail elsewhere [21].

Testing resumed 5 days after the second intraventricular injection.

For the remainder of the experiment, the rats were tested on a schedule that included 4 daily sessions of approach alternating with a single session of avoidance.

Biochemical assay procedure. At the conclusion of the experiment, the rats were decapitated, and their brains were excised and immediately frozen on dry ice. NE and DA levels were measured fluorometrically by the trihydroxyindole procedure [3]. Whole brains were homogenized in 4 volumes of fresh 0.4N perchloric acid at 0–4° [3]. The homogenized samples were centrifuged for 20 min at 9000 rpm. The CA's were adsorbed onto alumina at pH 8.5, then eluted into 0.1N acetic acid and oxidized according to the method described by Chang [4]. After oxidation, the samples were read for NE on a spectrophotofluorometer (Aminco-Bowman) at excitation wavelength 385 mμ, emission wavelength 485 mμ. The samples were stored overnight under a fluorescent light and read the next day for DA at 320/380 mμ.

RESULTS

Figure 1 depicts the mean percent responses made during the CS on both the avoidance and approach procedures. Vehicle + pargyline treatment did not change the pattern of responding in either motivating condition, but 6-HD + pargyline resulted in decreased responding during the CS in both situations. Ten of the 13 6-HD-treated rats showed decreases in approach responding lasting from 2 to more than 6 weeks. The approach behavior of 3 of these 10 rats never returned to baseline. The remaining 3 rats showed no change in approach behavior after 6-HD treatment. Thus, 10 of 13 rats either showed no decrement in approach behavior or showed decrements that eventually disappeared. Although most rats showed decreased responding during the CS, only rarely did any rat fail to consume the water when it was presented.

Whether or not their approach behavior was changed, all 13 6-HD-treated rats showed avoidance decrements that lasted the entire test period (about 6 weeks). As can be seen in Fig. 1, the mean percent responses during the CS was nearly identical for approach and avoidance behavior in the first session after 6-HD treatment. Such a progressive decrease in CS responding was not observed during conditioned approach testing; instead, responding improved daily after an initial decrement, reaching 90 percent of baseline after 6 weeks.

The 3 rats showing no change in approach behavior generally had less severe avoidance decrements than those showing disruptions in approach behavior. These 3 rats avoided at a mean of 51 percent during the first 3 sessions after treatment, as compared with 36 percent for the 10 rats showing decreased approach responding. Moreover, these 3 rats avoided more frequently (68 percent) during their last 3 sessions than did the rats with approach decrements (32 percent).

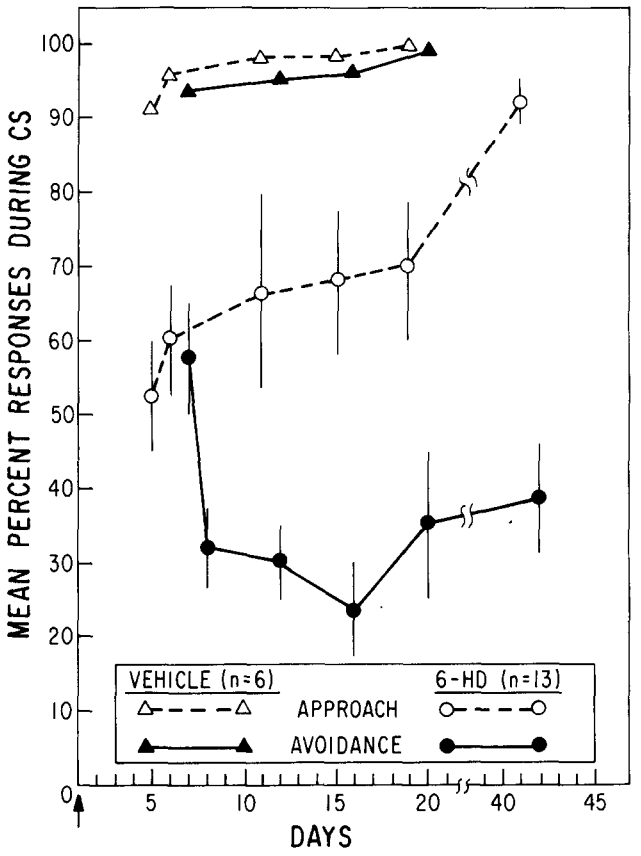

FIG. 1. Effects of treatment with 6-HD and pargyline on behavior in both approach and avoidance procedures. Each point represents the mean ± S.E.M. Arrow indicates injection of 6-HD.

Figure 2 shows the mean inter-response time (IRT) for all animals on both approach and avoidance procedures. The first cell in each instance (labelled Control) shows the distribution of responses for the last approach session and the last avoidance session prior to the injection of 6-HD. At this time, responses almost always occurred early in the CS interval, and the patterns of occurrence were virtually identical for both approach and avoidance. During the first tests after treatment of the rats with 6-HD and pargyline, both the avoidance and approach behaviors showed a decrease in short IRT's (responses made during the CS) and an increase in long IRT'S (responses made during the UCS). These changes in the distribution of responses were similar in both the avoidance and approach conditions. After approximately 2 weeks of testing, avoidance (CS) responding was slightly decreased. The IRT's for the avoidance tests remained almost constant throughout the 6 weeks of testing. The IRT's for the approach tests changed gradually during the test period and, by the sixth week after administration of 6-HD, were similar to the control values.

Results of the Biochemical Assay

Mean NE and DA levels for vehicle-treated rats were 505.8 ± 44.7 and 1029.6 ± 79.2 ng/g, respectively. Mean NE and DA levels for 6-HD treated rats were 43.4 ± 7.6 and 274.0 ± 33.9 ng/g, respectively. Thus, in rats treated with 6-HD, NE and DA were depleted by 91 and 73 percent, respectively.

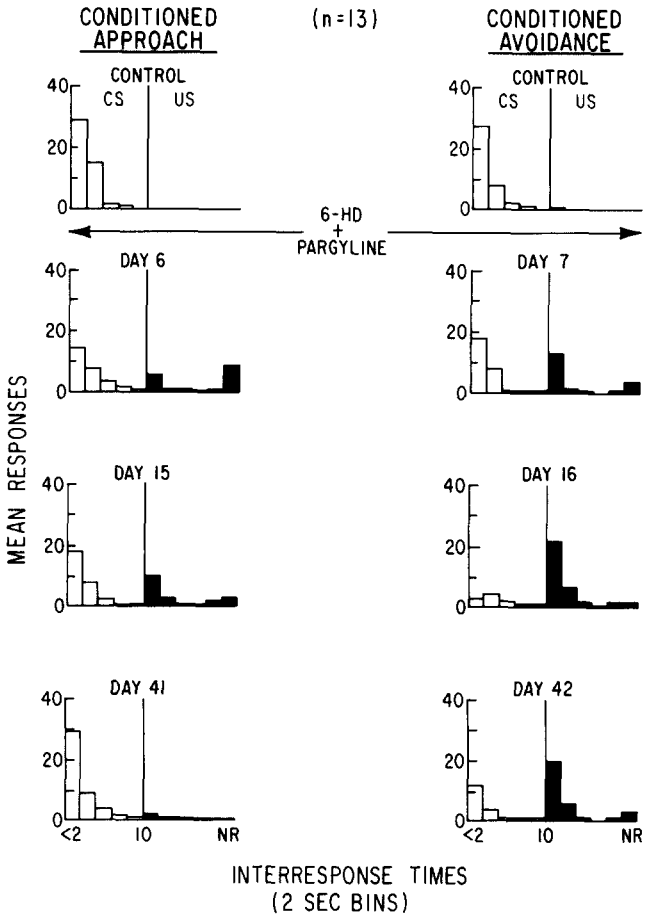

FIG. 2. Effect of treatment with 6-HD and pargyline on interresponse times (IRT) of rats on conditioned approach and conditioned avoidance procedures. Each histogram represents the mean IRT's of 13 rats for a single session. Control session is the last session before 6-HD treatment.

DISCUSSION

Substantial depletion of NE and DA in the brain by the intraventricular administration of 6-HD resulted in analogous decreases in conditioned responding on tests involving either positive or negative reinforcement. In the avoidance situation, this treatment resulted in failure to avoid shock, but not in failure to escape it. In the approach situation, this treatment resulted in failure to press a lever to obtain water, but not a failure to consume the water when it was presented. A somewhat similar result has been reported by Fibiger *et al.* [13], who tested rats that had been depleted of striatal DA for the acquisition of a shuttle-box approach response for food. Their 6-HD-treated rats failed to learn to approach the food during the presentation of a CS, but ate when they were placed near the food dish.

Despite this commonality of the effects of 6-HD on approach and avoidance behavior, the decrease in avoidance responding appears to be the more complex phenomenon. Although all 13 6-HD-treated rats showed decrements in avoidance behavior that lasted for the duration of the experiment, 3 of these rats never showed approach decrements; of the remaining 10 rats with approach decrements, 7 eventually recovered and attained pretreatment baselines.

The avoidance responding of the 6-HD-treated rats decreased progressively during the first 4 sessions after treatment; many of these rats exhibited the typical signs of the fear reaction that has been described elsewhere [21]. It has been suggested [21] that 6-HD-treated rats in an avoidance situation apparently learned to freeze during the presentation of a CS that was associated with shock and, thus, were unable to perform an avoidance response that was incompatible with immobility. The primary difference between the discriminated avoidance and the conditioned approach procedures lies in the nature of the UCS in each procedure. In the conditioned approach situation, the CS was associated with water, not with shock, and the rats never learned to suppress lever responses. In the absence of this suppression, responding during the CS almost always returned to normal levels.

The data from the present experiment suggest an explanation of the avoidance decrement in terms of two apparently independent, but interacting, factors. First, 6-HD-induced depletion of CA may have resulted in a decrease in conditioned responding, as demonstrated by decrements of the same character and magnitude in both approach and avoidance behaviors in the early sessions after administration of 6-HD. If this decrease in conditioned responding had been the only cause of the avoidance decrement, however, it would have been expected that the avoidance behavior of these rats would have recovered in much the same manner that approach behavior did. In fact, the avoidance decrements are almost always permanent. Thus, the animal's response to shock may have been an additional factor that had the effect of prolonging and increasing the severity of the decrement in avoidance responding.

These two factors might have interacted in the following manner: As a result of the decrease in conditioned responding in the early sessions after 6-HD treatment, the rats received shocks when they failed to avoid. The over-reactivity induced by the shocks and the CS that was paired with them increased the probability that the rats would freeze upon presentation of the CS, and this response came to be predominant in the absence of a viable avoidance response. The more the rats failed to avoid, the more shock they received and the more likely they were to freeze, because with each pairing of the CS and shock the CS became even more aversive. Even after normal behavior would have been expected to return, as indicated by the recovery of approach responding, avoidance responding continued to be suppressed, because the rats had by now learned a new response that was incompatible with avoidance.

EXPERIMENT 2

The long-term avoidance decrease after the intraventricular injection of 6-HD appears to be caused by the interaction of a decrease in conditioned responding and a learned response-suppression as discussed in Experiment 1. One way of releasing responses that have been suppressed by shock in other procedures has been through the administration of diazepam (DZP) [15, 24, 27]. If response suppression were a major factor in the avoidance decrement, it would then be expected that DZP would restore avoidance behavior in 6-HD-treated rats. This response-suppression hypothesis was tested in the following experiment in two ways: (1) animals from Experiment 1 that were already showing decrements in avoidance, but not in approach, received DZP prior to each of two avoidance sessions; (2) another group of rats that had been trained for approach and avoidance received DZP prior to each avoidance session after 6-HD treatment, in an attempt to prevent the animals from learning to suppress.

Finally, the results of Experiment 1 also suggested that eliminating the rat's exposure to shock during the period of decreased conditioned responding might minimize the interaction between nonresponding and hyper-reactivity to aversive stimuli. Accordingly, a third group of rats was trained for both approach and avoidance. After the injection of 6-HD, approach testing was resumed, but avoidance testing was delayed for about 4 weeks, at which time approach behavior had returned to pre-6-HD levels for all animals.

METHOD

All procedures for maintenance, surgery, and drug administration were the same as those described in Experiment 1.

Treatment Groups

Group 1 (n = 8) consisted of 6-HD-treated rats taken from Experiment 1. All had large avoidance decrements, but their approach behavior had returned to normal by the sixth avoidance session. Each rat received an intraperitoneal injection of DZP (Valium® – Hoffman-La Roche), 3 mg/kg (suspended in water and Tween 80) 30 min prior to their sixth and eighth avoidance sessions. All other conditions were the same as described above.

Group 2 (n = 3) consisted of rats that were trained in conditioned approach and avoidance as described above, except that approach and avoidance sessions alternated throughout training and testing after the administration of 6-HD. The rats were trained until 90 percent of their responses occurred during the CS; they were then given the standard 6-HD-pargyline treatment. On the fifth day after treatment, testing was resumed with an approach session. DZP (3 mg/kg) was administered intraperitoneally 30 min prior to avoidance Sessions 1–12. Saline was administered intraperitoneally 30 min prior to avoidance Sessions 13–16.

Group 3 (n = 4) consisted of rats that were trained in the same manner and to the same criterion as the rats in Group 2. On the fifth day after treatment with 6-HD and pargyline, approach testing only was resumed on a daily basis. Avoidance testing was resumed on the 29th day after 6-HD treatment, and was alternated with approach tests thereafter.

RESULTS

DZP increased the frequency of avoidance responses when it was given to rats in Group 1 prior to avoidance Sessions 6 and 8 (Fig. 3). The DZP performance levels seemed to depend, at least partially, on the No Treatment baseline. Of the 4 rats with post-6-HD avoidance baselines above 50 percent all avoided at pretreatment levels after DZP. Those rats with lower baselines made a substantial, but incomplete, recovery after the injection of DZP. Moreover, those rats that had previously been observed to overreact with an apparent fear response during the CS did not do so during DZP sessions.

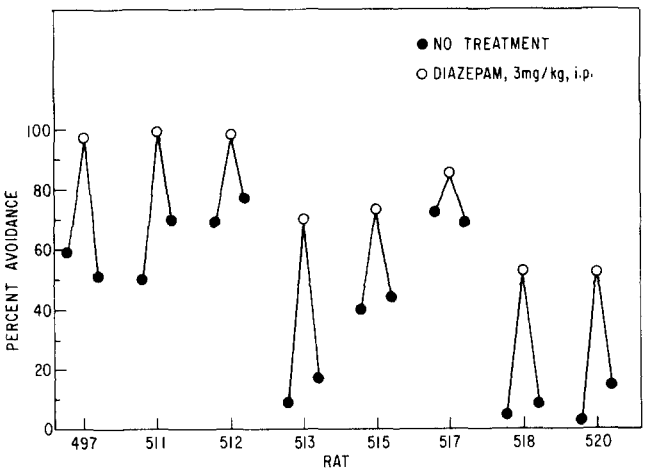

FIG. 3. Recovery of avoidance performance in rats treated with 6-HD and pargyline after diazepam administration. Each point represents the mean of two sessions. No Treatment sessions occurred immediately prior to, or immediately after, Diazepam sessions.

When DZP was administered to Group 2 prior to each of the first 12 avoidance sessions, the usual severe avoidance decrement was largely eliminated. Figure 4 shows the performance levels for the 3 rats during the first 4 avoidance sessions, which were preceded by injection of DZP, and the last 4 avoidance sessions, which were preceded by injection of saline. Also shown are the first 4 and last 4 approach sessions for these animals. For the sake of comparison, data from nine rats from Experiment 1 (No Diazepam), which were selected because their conditioned approach baselines were within the range of the rats in the present experiment, are also shown in this figure. The No Diazepam group showed a sharp decrease in avoidance behavior during their first 4 sessions. The DZP-treated rats (Nos. 521, 527, and 528) in the present experiment showed only slight decreases during a comparable period. In fact, approach and avoidance levels were nearly identical for all 3 rats during the entire DZP phase of the experiment. This similarity between approach and avoidance levels was seen only during the first session for the No Diazepam group, a time at which response suppression had not yet been learned.

When DZP treatment was discontinued, 2 of the 3 rats showed the typical 6-HD-induced avoidance decrement, a progressive decrease in avoidance responding in the next 4 saline sessions. Of particular interest is Rat 528, which avoided on 100 percent of the trials during its first saline session, 48 hr after the last DZP injection. Beginning with the fourteenth trial of the second saline session, however, and continuing with each succeeding session, this rat's avoidance performance deteriorated, and it received more and more shocks. By the fourth saline session, Rat 528 avoided on only 12 percent of the trials.

The delay in avoidance testing for Group 3 for 29 days resulted in a smaller decrease in avoidance behavior than was found for animals given No Delay in testing (9 rats from Experiment 1) (Fig. 5). The Delay group avoided better during their first avoidance session (85 percent) than the No Delay group (68 percent); the level of avoidance for the Delay group, was always higher than the No Delay

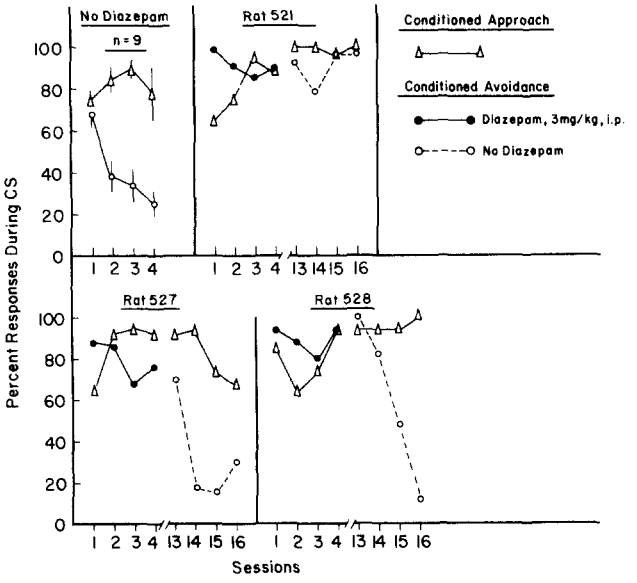

FIG. 4. Effect on avoidance behavior of the injection of diazepam prior to each of the first 4 avoidance sessions of rats treated with 6-HD and pargyline. Each point in the No Diazepam group represents the mean ± S.E.M. of 9 rats.

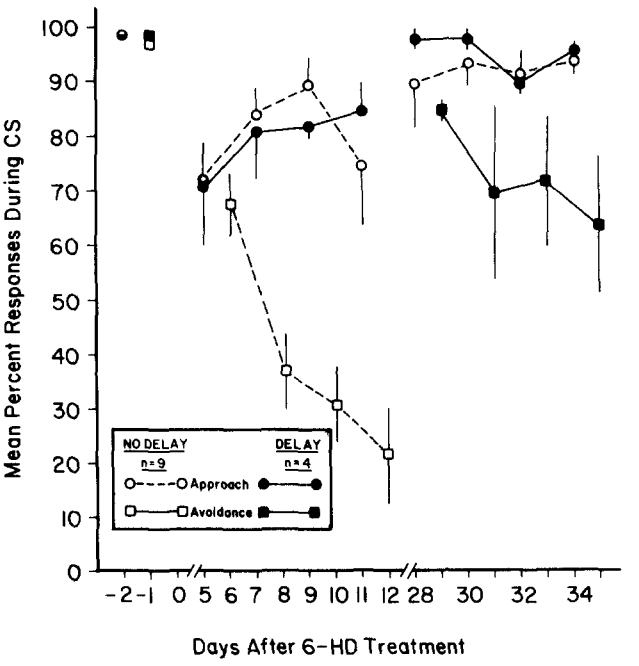

FIG. 5. Effect of delaying avoidance testing on the avoidance performance of rats treated with 6-HD and pargyline. Each point represents the mean ± S.E.M.

group even though both groups were injected at the same time. Conditioned approach performance for both groups was virtually identical. Both groups showed approach decrements at the resumption of testing but, by the time avoidance testing was initiated, all animals were pressing the lever normally.

Results of the Biochemical Assay

The mean NE and DA levels for the rats from Groups 2 and 3 were 52.4 ± 11.1 and 76.4 ± 32.8 μg, respectively. Thus, in rats in these groups, NE and DA were depleted by 90 and 93 percent, respectively, as compared with the control levels mentioned in the results of Experiment 1.

DISCUSSION

Previous data had indicated that the decrement in avoidance behavior after the intraventricular administration of 6-HD might be, at least partially, a function of increased suppression of responding. The improvement of avoidance after DZP injections strongly supports this hypothesis. DZP facilitated avoidance both when it was given to rats with previously established avoidance decrements (Group 1) and to rats before each test for avoidance after the administration of 6-HD (Group 2). Under the latter conditions, DZP largely eliminated that portion of the usual avoidance decrement that seems to be a function of response suppression. Despite several shocks during most sessions, the large progressive decrease in responding did not appear in these rats until DZP had been withdrawn during the last four sessions. The Group 2 rats did show small progressive decreases in avoiding during the first four sessions but, in terms of the number of responses during the CS, the approach and avoidance behaviors of Group 2 rats were very similar. If DZP were eliminating only part of a compound decrement, it would not have been expected that the entire decrement would be abolished, because the general behavioral decrement, as shown by below normal conditioned approach responding, was still present. Thus, it is not surprising that these animals showed small decreases in approach and avoidance during the first few sessions after DZP.

The absence of a suppression factor during DZP treatment was shown very dramatically by the data from Rat 528, which did not receive its first shock of the saline phase of the experiment until the fourteenth trial of the second saline session. This shock seems to have been enough to cause an increase in response suppression, however, because the rat then failed to avoid again on the next trial, and the frequency of avoidance responses declined sharply thereafter, as freezing and the other symptoms of the typical hyper-reaction to aversive stimuli began to appear.

Delaying avoidance testing of 6-HD-treated rats (Group 3) until conditioned approach behavior had returned resulted in a mean decrease in avoidance that was substantially smaller than that seen when avoidance testing was begun before approach behavior had recovered. Similarly, Laverty and Arnott [19] have reported that 6-HD-induced avoidance decrements were less severe if testing was delayed for 10 days after treatment than when testing began immediately after treatment. Unpublished data from our laboratory also indicate that, the longer one waits after 6-HD-treatment before resuming testing, the less severe the resulting avoidance decrements are likely to be.

The difference in avoidance behavior between the Delay and No Delay groups could not have resulted from a greater decrease in conditioned responding in the No Delay group; these animals had been specifically selected for comparison because their approach levels were not different from those of the Delay group either at the beginning or the end of testing. Therefore, the most likely explanation for the different avoidance decrements was that testing in one group began 5 days after 6-HD treatment, but was delayed an additional 24 days in the other.

It might be argued that the avoidance decrement was lessened after the long delay not because the decrease in conditioned responding had been eliminated, but because the tendency to freeze had simply decreased with time. If this were true, however, animals that had been tested continually after 6-HD injections would be expected to recover with time. Such recovery rarely occurs. Alleviation of the decrement appears to occur only when animals are not tested in the interim and, thus, are not permitted to experience shock in their CA-depleted state until recovery of conditioned responding has taken place.

It can be concluded from these data that destroying CA neurons in the brain results in two kinds of behavioral changes: (1) there is a specific decrease in responding during conditioned stimuli (seen in both approach and avoidance); and (2) 6-HD animals are hyper-reactive to highly aversive stimuli, and there is a great likelihood that these animals will learn to freeze during the presentation of a CS that had been paired with shock. They are, therefore, unable to perform an avoidance response that is incompatible with this immobility.

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