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Modification of Avoidance Behavior in 6-Hydroxydopamine-Treated Rats by Stimulation of Central Noradrenergic and Dopaminergic Receptors[1]

LANE G. LENARD[2] AND BERNARD BEER

The Squibb Institute for Medical Research
Princeton NJ 08540

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LENARD, L. G. AND B. BEER. *Modification of avoidance behavior in 6-hydroxydopamine-treated rats by stimulation of central noradrenergic and dopaminergic receptors.* PHARMAC. BIOCHEM. BEHAV. 3(5) 887–893, 1975. – Rats showing reliable decrements in conditioned avoidance behavior after the intraventricular administration of 6-hydroxydopamine (6-HD) with pargyline pretreatment were given various dopaminergic and noradrenergic agonists. Intraventricular injections of DA or L-NE or intraperitoneal injections of apomorphine or L-DOPA reversed the avoidance decrements, often restoring performance to pre-6-HD-treatment levels. Furthermore, these agonists all produced behavior characteristic of activity in dopaminergic neurons. Clonidine, a noradrenergic agonist, also reversed avoidance decrements, but did not produce behavior characteristic of stimulation of dopaminergic neurons in the brain. Pretreatment with spiroperidol, a dopaminergic receptor blocker, prevented the recovery induced by all agonists, although clonidine-induced recovery was affected least. The results are discussed in terms of possible separate roles for dopaminergic and noradrenergic neurons in the brain in avoidance behavior.

6-Hydroxydopamine Norepinephrine L-DOPA Catecholamines Dopamine Clonidine Avoidance Stereotyped behavior Spiroperidol Apomorphine

ALTHOUGH it appears certain that brain catecholamines (CA) have an important function in avoidance behavior, the relative roles of the CAs norepinephrine (NE) and dopamine (DA), have not been clearly differentiated. Nevertheless, the major effects of CAs on avoidance behavior have been consistently related to the functioning of dopaminergic, rather than noradrenergic, neurons in the brain.

Much of the early evidence on this issue came from the work of Seiden and his associates. They found that reserpine-induced disruption of avoidance behavior in mice, rats, and cats could be reversed by the subsequent administration of a precursor of CA, L-DOPA [24, 25, 26]. They further showed that L-DOPA was converted almost exclusively to DA in the brains of reserpine-treated mice, and that the brain level of DA, but not of NE, was correlated with the return of avoidance responding. Similar results have been reported to follow α-methyltyrosine (α-MT)- instead of reserpine-induced depletion [11,15].

Avoidance decrements have been observed in cases in which DA has been selectively depleted. These results have come from experiments in which rats, after a pretreatment that protected NE neurons from destruction, were injected with 6-hydroxydopamine (6-HD), which depletes brain CA [10, 18, 28, 29]. Selective depletion of DA after the direct injection of 6-HD into rat's substantia nigra [14] has also resulted in impaired avoidance. Selective depletion of NE with 6-HD did not cause decreases in avoidance responding [10,28], but in these experiments, NE levels were not as low as those in experiments in which avoidance decrements have been shown [10, 19, 21].

For the most part, the behavioral effects observed when NE transmission has been selectively altered by means other than 6-HD have been more subtle and of a smaller magnitude than those seen after manipulation of DA transmission. Much of the evidence relating NE to avoidance has come from studies showing the occurrence of

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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.

decrements in avoidance after selective depletion of NE resulting from inhibition of dopamine-β-hydroxylase (DBH), the enzyme that converts DA to NE. Decreases in avoidance responding have followed treatment with the inhibitors of DBH, diethyldithiocarbamate [16] and FLA-63 [2]. Although disulfiram, another inhibitor of DBH, did not produce avoidance decrements when given alone, avoidance decrements produced by the combination of reserpine and disulfiram were less easily reversed by the subsequent administration of L-DOPA than were decrements produced by reserpine alone [27]. This finding suggested that NE synthesized in the brain after treatment with L-DOPA might play a role in the reversal of reserpine-induced decrements. A similar result was reported when FLA-63 was used instead of disulfiram [1]. Ahlenius and Engel [2] reported recently that a small (25 percent) decrease in avoidance responding in mice that occurred after the oral administration of FLA-63 alone was reversed by the subsequent administration of DL-threo-3,4-dihydroxyphenylserine (DOPS), a compound that is converted directly to NE by DOPA-decarboxylase, thus bypassing the DBH step in the synthesis (12). Finally, Lenard *et al.* [21] demonstrated that avoidance decrements induced in rats by the intraventricular administration of 6-HD could be completely reversed by the subsequent intraventricular administration of L-NE.

The experiments reported in the present paper were designed to evaluate further the relative roles of NE and DA in avoidance behavior. The means chosen to examine this issue was the stimulation, in the brains of rats, of noradrenergic and dopaminergic receptor sites depleted of both NE and DA by the administration of 6-HD. Rats with low avoidance baselines after treatment with 6-HD were given a series of noradrenergic and dopaminergic agonists in an attempt to improve avoidance performance. Those rats that did improve after the injection of an agonist were then given that agonist again, along with spiroperidol, an agent that blocks dopaminergic receptor sites, in an attempt to block that improvement.

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. Food and water were available ad lib.

Procedure

Surgery. Each rat was implanted with a permanent cannula (Plastic Products, Co.) in either the left or right lateral ventricle. The details of the procedure have been described elsewhere [19]. Briefly, the rats were anesthetized by intraperitoneal injections 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 ear drum. 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.* [3]. Training did not begin until at least 7 days after surgery.

Avoidance procedure. Avoidance behavior was measured in shelf-jump avoidance chambers based on design by Tenen [30]. Shock (2.0 mA, provided by a BRS/Foringer SG-901 shock generator) was delivered to the grid at appropriate times via a shock-scrambler circuit.

Each test session consisted of 50 trials. A trial was initiated by the presentation of the conditioned stimulus (CS) (withdrawal of a 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. The rat was then gently pushed by the moving wall off the shelf and back onto the grid, 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 the start of the CS, and series of shock pulses (each 0.5 sec in duration, with a 2 sec shock-shock interval) was initiated. If the rat then jumped onto the shelf, it escaped from the shock, ending the trial and initiating an ITI as described above. A maximum of 10 shocks was presented if no escape response occurred.

Implanted rats were trained to a criterion of at least 90 percent avoidance during 2 consecutive sessions. When the criterion had been met, they were injected intraperitoneally with pargyline, 50 mg/kg, 30 min prior to an intraventricular injection of 6-HD, 250 μg. A second intraventricular injection of 6-HD, 250 μg, was given 24 hr later, not preceded by pargyline. The intraventricular injection procedure has been described in detail elsewhere [19]. Avoidance testing resumed on a daily basis 5 days after the second injection of 6-HD.

Those rats treated with 6-HD that showed consistent avoidance decrements after 4 daily sessions were used, and each received various agonists prior to testing. The agonists given were DA, L-DOPA (along with R04-4602, a drug that inhibits extracerebral DOPA-decarboxylase, apomorphine, L-NE, and clonidine. If a dose of an agonist improved avoidance behavior substantially, the same dose of that agonist was given again prior to the following day's session, along with spiroperidol, 0.25 mg/kg. Saline was also given to each rat by both the intraventricular and intraperitoneal routes on separate occasions. At least 2 daily sessions of baseline responding were allowed before treatment with a different agonist.

Pharmacological agents., The following compounds were prepared and injected as indicated: 6-HD HBr (Regis) was dissolved in 0.9 percent saline with 0.05 percent ascorbic acid and was kept on ice until just prior to intraventricular administration. Pargyline (Eutonyl® – Abbott) was dissolved in 0.9 percent saline and injected intraperitoneally 30 min prior to the administration of 6-HD. L-NE HCl (Aldrich) and DA HCl (Regis) were each dissolved in 0.9 percent saline and injected intraventricularly 5 min prior to testing. L-DOPA (Sigma) was dissolved in 0.9 percent saline with 0.1 percent ascorbic acid and injected intraperitoneally 30 min prior to testing. RO4-4602 [DL-serine-N^2 (2,3,4-trihydroxybenzyl) hydrazide HCl (Hoffman-La Roche)] was dissolved in 0.9 percent saline and injected 30 min prior to the injection of L-DOPA. Apomorphine HCl (Merck) was dissolved in 0.9 percent saline and injected intraperitoneally 15 min prior to testing. Spiroperidol (Janssen) was dissolved in 0.01 percent tartaric acid solution and injected 90 min prior to testing. Clonidine (Catapres® – Boehringer-Ingelheim) was dissolved in 0.9 percent saline and injected intraperitoneally 30 min prior to testing. Doses of all compounds are always expressed as the

weight of the salt. The volume for each injection of 6-HD was 20 μl, that for injections of L-NE or DA was 10 μl. Injections were always given to awake animals that were moving freely.

Biochemical assay. For the purposes of the biochemical assay, 4 rats of the same age and weight as those receiving 6-HD received no drug treatment and were designated as controls. Two of these rats, each with an implanted cannula, were tested in the shelf-jump procedure in the standard manner. The other 2 rats were housed in individual cages for the duration of the experiment, but were never tested. Since no significant differences in CA levels were found among these control rats, the data from the assays of their brains were pooled.

About 3 weeks after the conclusion of the experiment, all the rats were decapitated and their brains were excised and immediately frozen on dry ice. NE and DA were measured fluorometrically by the trihydroxyindole procedure. Whole brains were homogenized in 4 volumes of fresh 0.4N perchloric acid at 0–4°C, as described by Brodie *et al.* [7]. The homogenized samples were centrifuged at 0–4°C for 20 min at 9000 rpm. The CA were adsorbed onto alumina at pH 8.5, then eluted into 0.1N acetic acid and oxidized according to the method described by Chang [8]. After oxidation, the samples were read for NE on a spectrophotofluorometer (Aminco-Bowman), excitation wavelength 385 mμ/emission wavelength 485 mμ. The samples were stored overnight under a fluorescent light and were read the next day for DA at 320/380 mμ.

RESULTS

Ten rats that had been treated with 6-HD and pargyline showed substantial avoidance decrements after treatment and were, therefore, selected for further testing. Of these, 3 died in the course of testing, the avoidance responding of two others eventually returned spontaneously to levels that were too high to permit testing for agonist-induced recovery, and one rat lost its cannula. Thus, not all rats received every agonist. NE and DA levels for the animals that survived are shown in Table 1.

Saline, whether administered by the intraventricular or the intraperitoneal route, did not affect the behavior of any rat treated with 6-HD. The administration of DA, L-DOPA, apomorphine, or L-NE resulted in an increase in general activity and in stereotyped behavior, such as sniffing, gnawing on food pellets and circling. Each of these agonists was effective also in restoring avoidance responding. Figure 1 shows the results for the 9 rats that received intraventricular injections of DA, 10 μg. Improvement in avoidance was evident in 8 of the 9 rats. Rat 416, for example, did not avoid at all during the immediate nonagonist sessions, but avoided on 88 percent of the trials after the administration of DA; Rats 434, 485, and 489 avoided on 100 percent of the trials after the administration of DA, even though their nonagonist baselines were very poor; Ray 428 avoided 95 percent of the time after the administration of DA, but started to convulse shortly after the session and died within 12 hr.

L-DOPA (+R04-4602, 50 mg/kg, I P) was particularly

TABLE 1

LEVELS OF NE AND DA IN THE BRAINS OF RATS TREATED WITH 6-HD

	NE		DA	
Rat	ng/g	Percent of Control	ng/g	Percent of Control
416*	–	–	–	–
418	69.7	16	n.d.†	0
425	3.4	1	n.d.	0
426	139.4	32	1328.5	90
427	69.7	16	637.7	43
428*	–	–	–	–
433	91.8	21	607.3	41
434	69.7	16	235.3	16
485	13.6	33	89.2	6
489	23.8	55	75.9	5
Control (n = 4)	435.6 ± 21.4	–	1469.1 ± 55.7	–

*Died before brain could be removed for assay
†Not detectable; below the level of sensitivity of the assay

effective in restoring avoidance to normal levels (Fig. 1). After an intraperitoneal injection of L-DOPA, 5 or 10 mg/kg, Rats 418, 425, 428, 433, and 434 avoided on 90–100 percent of the trials. L-DOPA produced no change in the avoidance behavior of Rats 416 and 427 after an injection of 5 mg/kg, and produced only a small increase in Rat 416 after a 10 mg/kg dose. Regardless of whether avoidance responses occurred, however, stereotyped behavior and increased activity were always apparent in all rats after the injection of L-DOPA. R04-4602, by itself, produced no change in the behavior of any animal.

Apomorphine was also quite effective in reversing 6-HD-induced avoidance decrements (Fig. 1). After receiving a dose of 0.1 mg/kg, Rat 425 recovered to the 96 percent avoidance level from a baseline of almost no avoidance behavior; after 0.2 mg/kg, Rats 418, 433, and 434 avoided at levels comparable to those before treatment with 6-HD. In a result that parallels that seen after the administration of L-DOPA, Rats 416 and 427 showed almost no avoidance, either before or after the injection of apomorphine, 0.1–0.2 mg/kg. After 0.4 mg/kg, however, Rat 416 recovered to 64 percent avoidance, but Rat 427 failed to avoid even when the dose was increased to 0.8 mg/kg. Stereotyped behavior was evident in all animals, including Rats 416 and 427, after all doses of apomorphine. This was also seen after treatment with L-DOPA.

In a replication of earlier results [21], L-NE was found to be effective in reversing the avoidance decrements produced by 6-HD (Fig. 2). After an intraventricular injection of L-NE, 5μg, avoidance responding increased in 5 of 6 rats. Rat 416, for example, avoided on 96 percent of the trials after L-NE, although this rat did not avoid at all during the nonagonist sessions that immediately preceded or followed this treatment; Rat 434 showed no change in behavior after the 5 μg dose, but did improve when the dose was increased to 10 μg; all 4 rats given the 10 μg dose avoided more frequently at that dose than they had after the smaller dose. Rat 427 avoided on 39 percent of the trials after 5 μg and on 60 percent after 10 μg, despite the fact that it had shown virtually no change in avoidance behavior after treatment with DA, L-DOPA, or apomorphine. It should be emphasized here that, in all rats treated with 6-HD, intraventricular administration of L-NE, produced stereotyped behavior that was indistinguishable from that produced by DA, L-DOPA, or apomorphine.

In contrast to treatment with the other agonists, treatment with clonidine resulted in decreased muscle tone, ataxia, no increase in locomotor activity, and no apparent stereotyped behavior. Nevertheless, clonidine, 0.4 mg/kg, was effective in increasing avoidance responding in all 4 rats that received this treatment (Fig. 2), including Rat 427, which had failed to improve after all other agonists except L-NE. The extent of the recovery after clonidine was

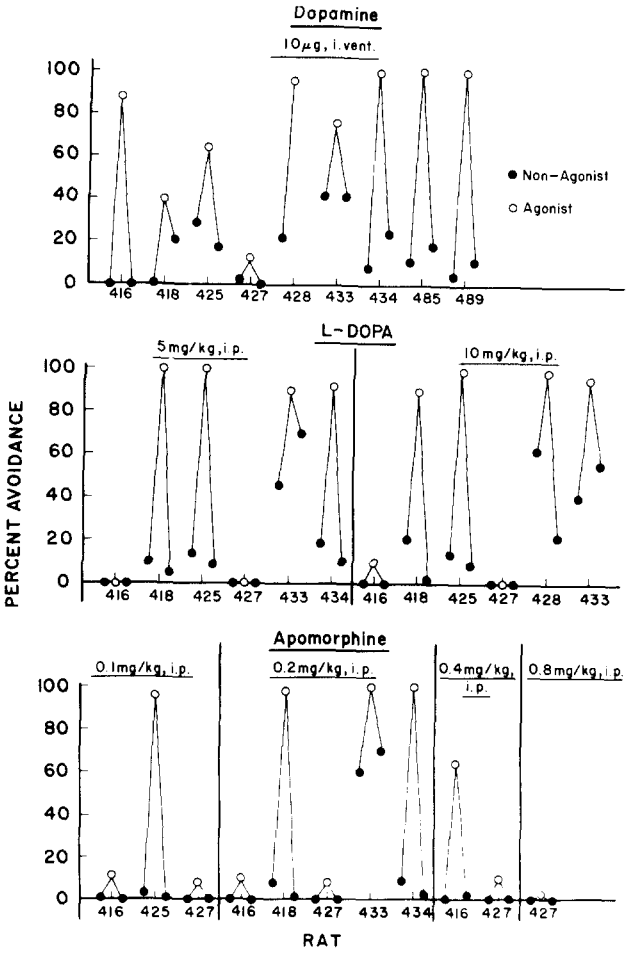

FIG. 1. Effects of DA, L-DOPA, and apomorphine on avoidance behavior in rats treated with 6-HD. Non-agonist refers to the session immediately before or immediately after the agonist session.

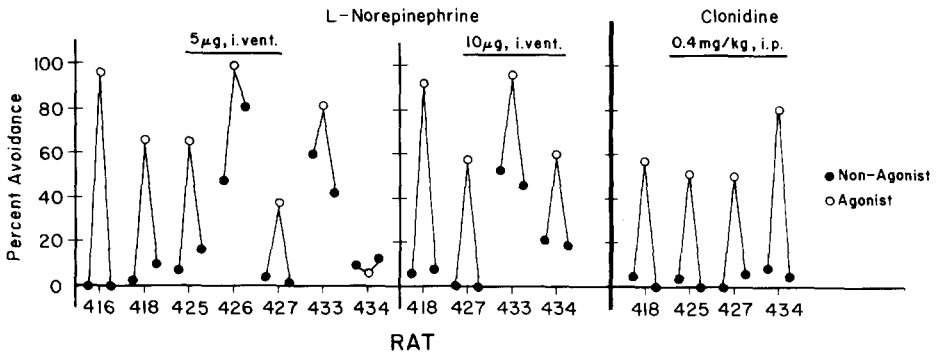

FIG. 2. Effects of L-NE or clonidine on avoidance behavior in rats treated with 6-HD. Non-agonist refers to the session immediately before or immediately after the agonist session.

generally less than that produced by the other agonists. This observation, plus the failure of clonidine to produce stereotyped behavior, suggested that the dose of clonidine selected may have been too small to restore avoidance to the high levels observed after treatment with the other agonists. To test this possibility, another group of rats (n = 11), trained for avoidance and treated with 6-HD and pargyline as described above, was given clonidine, 0.04, 0.1, 0.4, and 1.0 mg/kg, but in random order. Avoidance improved in a dose-dependent manner after clonidine, and 0.4 mg/kg was found to be the optimal dose for improving avoidance behavior.

The administration of spiroperidol blocked the recovery of avoidance behavior induced by each of the agonists. Despite their failure to avoid under these conditions, all rats continued to escape shock. In each case of the combined administration of DA and spiroperidol, recovery of avoidance to the levels that preceded treatment with 6-HD was blocked, so that the rats avoided at essentially the same level at which they had avoided during the nonagonist sessions (Fig. 3). Similar results were seen after the combined administration of L-DOPA and spiroperidol (Fig. 3), except that the recovery induced by L-DOPA in Rat 425 was only partially blocked by spiroperidol. The complete recovery of avoidance induced by apomorphine was totally blocked by spiroperidol (Fig. 3) in every instance. Figure 4 shows the effects of spiroperidol on L-NE-induced recovery. Each animal avoided much less during the combined treatment than it did after treatment with L-NE alone. As noted above, the administration of clonidine generally resulted in less recovery of avoidance behavior than did the administration of any other agonist, but spiroperidol seemed to be less effective in blocking that recovery than it had been in blocking recovery induced by the other 4 agonists (Fig. 4) (mean percent avoidance for clonidine − (clonidine + spiroperidol) = 26 percent compared with differences of 86, 95, 92, and 70 percent for DA, apomorphine, L-DOPA, and L-NE, respectively).

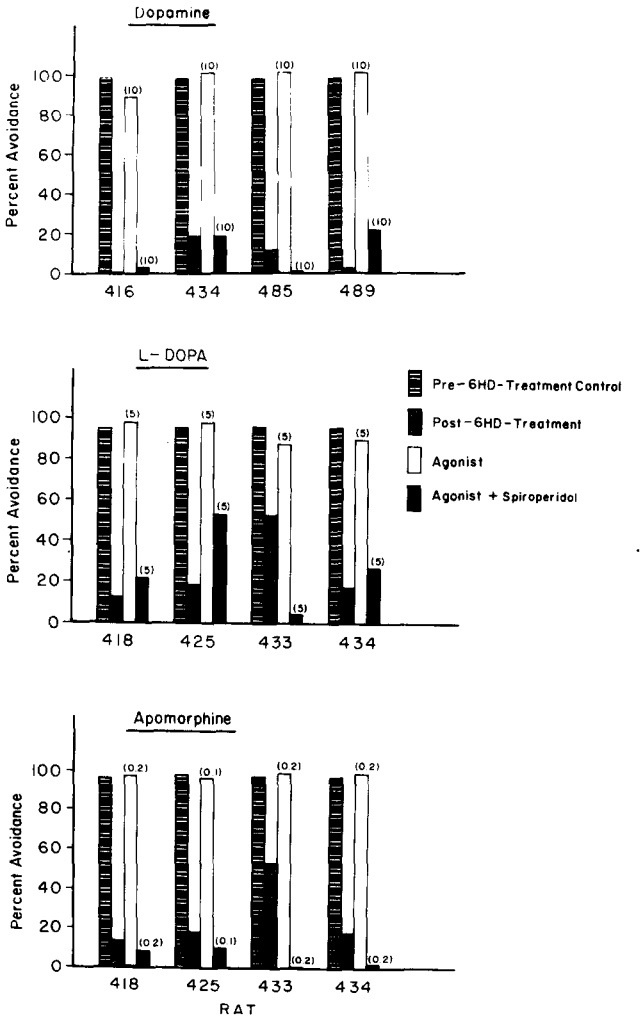

FIG. 3. Effects of DA, L-DOPA, or apomorphine on avoidance behavior of rats treated with 6-HD and then given spiroperidol, 0.25 mg/kg, I P. Pre-6-HD-Treatment refers to the mean of the last two sessions before 6-HD treatment; Post-6-HD-Treatment refers to the mean of all non-agonist sessions after 6-HD treatment; numbers in parentheses refer to the dose of the agonist; dose of DA is expressed in μg, i. vent.; doses of L-DOPA and apomorphine are expressed in mg/kg, I P.

DISCUSSION

Avoidance decrements after the depletion of NE and DA by the administration of 6-HD were reversed by both noradrenergic and dopaminergic agonists. In many cases, animals treated with 6-HD that almost never avoided during nonagonist sessions showed normal avoidance behavior after the administration of such an agonist.

Compounds that produced the behavioral signs characteristic of activity in dopaminergic neurons in the brain seemed to be particularly effective in restoring avoidance behavior. This finding suggests a major involvement of brain dopamine in avoidance behavior. Apomorphine, which stimulates DA receptors selectively [13], L-DOPA, which is converted almost exclusively to DA in rats treated with 6-HD [23], and DA itself all restored avoidance behavior that had been impaired by 6-HD. These agents also produced stereotyped behavior typical of the stimulation of dopaminergic neurons in the brain. It seems likely, moreover, that L-NE, which consistently reversed the avoidance decreases as well, was also active at dopaminergic receptor sites, because intraventricular injection of L-NE also produced stereotyped behavior.

Tha administration of clonidine also brought about recovery of avoidance responding. Because clonidine has been shown to stimulate α-adrenergic receptors in the central nervous system selectively [5], and, in the present experiment produced none of the behavioral signs characteristic of dopaminergic activity, these data suggest a noradrenergic involvement in avoidance behavior.

The possibility has not been ruled out that all the agonists, including clonidine, brought about recovery through their activity at dopaminergic receptors, but this hypothesis implies that clonidine was active at dopaminergic receptors, despite the lack of stereotyped behavior after this agent had been administered. Zis *et al.* [32] have recently shown that the appearance of stereotyped behavior is not a prerequisite for L-DOPA-induced improvement of avoidance acquisition. Certain other results also bring into question the relationship between stereotyped behavior and the recovery of avoidance. For example, Rats 416 and 427 showed less improvement of avoidance after L-DOPA or apomorphine than did other rats; but both rats also showed

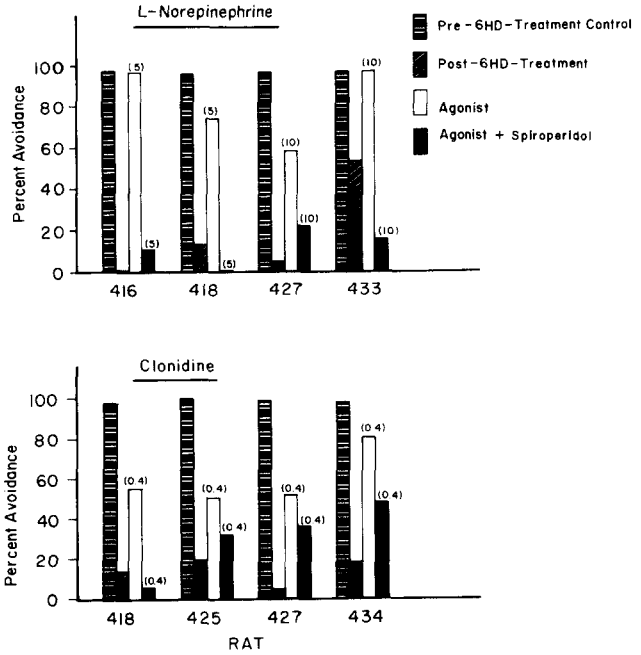

FIG. 4. Effects of L-NE or clonidine on avoidance behavior of rats treated with 6-HD and then given spiroperidol, 0.25 mg/kg, IP. Pre-6-HD-Treatment refers to the mean of the last 2 sessions before 6-HD-treatment; Post-6-HD-Treatment refers to the mean of all nonagonist sessions after 6-HD treatment; numbers in parentheses refer to the dose of the agonist; dose of L-NE is expressed in μg, i. vent.; dose of clonidine is expressed in mg/kg, I P.

evidence of dopaminergic stimulation resulting from these treatments. In their cases, at least, clearcut dopaminergic activity was not correlated with recovery of avoidance responding. Noradrenergic agonists, on the other hand, were effective in improving avoidance for both these rats.

It is also possible that recovery could have been due, at least in part, to stimulation of noradrenergic receptors by all the agonists. Although there are no independent behavioral measures of noradrenergic activity, such as exist for dopaminergic activity, the implication that dopaminergic agonists could have been active at noradrenergic receptor sites seems reasonable in light of two pieces of evidence: first, Langer *et al.* [17] reported that the cat nictitating membrane, which is noradrenergically innervated, becomes supersensitive to DA and other agonists, in addition to NE, after denervation; second, the reverse effect, i.e., noradrenergic stimulation of dopaminergic receptors, was demonstrated in the present experiment when L-NE produced evidence of dopaminergic activity.

The blockade of avoidance recovery by spiroperidol seems to eliminate the possibility that only noradrenergic neurons were responsible for recovery. Spiroperidol, in small doses, specifically blocks dopaminergic receptors [4]. The dose of spiroperidol used here (0.25 mg/kg, I P) was 1/20 that reported by Andén *et al.* [4] to have threshold effects on the blockade of noradrenergic function, but was above the threshold for dopaminergic blockade. The spiroperidol-induced blockade of agonist-induced recovery of avoidance implies that this behavioral effect was largely a function of the stimulation of DA receptors by at least four of the agonists. On the other hand, clonidine again had a behavioral effect in rats treated with 6-HD that was different from the effects produced by the other 4 agonists. Clonidine reversed avoidance decrements without producing stereotyped behavior. Further, the improved avoidance brought about by clonidine did not seem to be blocked as completely by spiroperidol as was the improvement that followed treatment with agonists that did produce signs of dopaminergic activity. If clonidine were causing recovery through its actions on noradrenergic neurons, precisely such results might be expected.

A third possibility is that both NE and DA neurons were involved in the recovery of avoidance. This may imply separate functions for noradrenergic and dopaminergic neurons. If such separate functioning were the case, it would probably mean that apomorphine and clonidine was each reversing avoidance decrements by stimulating a separate system. There is some evidence that separate systems are involved. For example, selective depletion of DA results in severe avoidance decrements in untrained rats, but in only small decrements in pre-trained animals [9,14]. The concurrent depletion of both NE and DA results in severe decrements regardless of whether the animals have been pre-trained [9]. Also, Lenard and Beer [20] have shown that both NE and DA levels are correlated with avoidance performance in animals depleted of both CAs, but that the magnitude of each correlation depends on when the behavior is measured.

The doses of apomorphine and L-DOPA that were effective in restoring avoidance in the present study (0.1–0.2 and 5-10 mg/kg, respectively) were exceptionally small; Schoenfeld and Uretsky [22], for example, saw the most consistent increases in stereotyped behavior in normal rats only after doses of apomorphine as large as 1–3 mg/kg. Their effective doses in rats treated with 6-HD were more in line with the present results. In another experiment, Schoenfeld and Uretsky [23] saw no increase in activity levels in normal rats given L-DOPA (+R04-4602) until the dose had reached 300 mg/kg, I P; in rats treated with 6-HD, they saw no increases in activity until the dose had reached 30 mg/kg. Zis *et al.* [32] have reported that the acquisition of an avoidance response in rats treated with 6-HD could be improved by the intraperitoneal injection of L-DOPA, 1.5 mg/kg, (+R04-4602). All these findings, in addition to the present results, support the contention of several authors [6, 22, 23, 31] that some form of denervation supersensitivity does occur in the brain after the depletion of brain CA by 6-HD.

The present results are consistent with data showing that avoidance behavior is readily affected by altering dopaminergic transmission in the brain. They support the argument that the integrity of DA-containing neurons is important for avoidance behavior in rats [9, 18, 28, 29]. Nevertheless, the effects of clonidine, the high correlation of NE levels with avoidance noted by Lenard and Beer [20], and the evidence from studies with inhibitors of DBH suggest strongly that NE-containing neurons also play a major role in avoidance behavior. Thus, both DA and NE neurons in the brain may have major roles in the maintenance of avoidance behavior, but the exact nature of those roles is still to be determined.

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