The Use of Conditioned Suppression to Evaluate the Nature of Neuroleptic-Induced Avoidance Deficits[1]

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Accepted for publication March 17, 1980

ABSTRACT

Beninger, Richard J., Stephen T. Mason, Anthony G. Phillips and Hans C. Fibiger: The use of conditioned suppression to evaluate the nature of neuroleptic-induced avoidance deficits. J. Pharmacol. Exp. Ther. 213: 623–627, 1980.

Three groups ($N = 8$) of rats received five 10-trial sessions of one-way avoidance training in which each trial was initiated by a 10-sec tone stimulus and terminated either by a shuttle response during the tone (avoidance) or by a response during the electric shock (escape). Rats in groups treated with pimozide (0.5 or 1.0 mg/kg i.p.) failed to acquire the avoidance response although they escaped readily when shock was presented, whereas control rats consistently avoided the shock.

The same rats then received several sessions of food-reinforced lever-pressing in a different apparatus; no drugs were given during these sessions. When responding had stabilized, the tone that had signalled shock in the avoidance sessions was presented for a 1-min period. A significant decrease in responding during the tone was observed in all groups when compared to unshocked controls, demonstrating that the pimozide-treated rats, although failing to acquire the avoidance response in the shuttle box, had learned the association between the tone and shock. The results suggested that the neuroleptic-treated animals failed to avoid because of a deficit in the ability to initiate responses rather than a deficit in associative learning.

Numerous studies have shown that depletion of brain catecholamines produced a deficit in conditioned avoidance responding (Moore and Rech, 1967; Rech et al., 1966; Seiden and Carlsson, 1963; Seiden and Hanson, 1964). Several additional studies have shown that the selective depletion or blockade of dopamine (DA) systems produced a similar deficit (Cooper et al., 1973; Fibiger et al., 1975; Neimegeers et al., 1969; Smith et al., 1973), whereas interference with noradrenalin neurons did not (Fibiger and Mason, 1978; Mason and Fibiger, 1979), suggesting that DA was the relevant CA in this effect. Furthermore, the results of a number of experiments suggested that the nigrostriatal DA bundle (NSB) was the particular DA system involved (Delacour et al., 1977; Fibiger et al., 1974; Mitchum and Thomas, 1972; Zis et al., 1974).

From these data it is not possible to determine whether the observed effects on avoidance responding are caused by 1) a deficit in conditioning to the preshock stimuli or 2) a deficit in initiating the avoidance response. Fibiger et al. (1975), Lenard and Beer (1975) and Seiden and Hanson (1964) reported that during the preshock stimulus DA-disrupted animals urinated and defecated and showed other behavioral signs which suggested that they had associated the stimulus with shock; how-

ever, they failed to avoid the shock. On the other hand, these animals could move and they readily escaped from the electrified grid. Posluns (1962) and Fibiger et al. (1975) suggested that neuroleptics selectively blocked the initiation of avoidance and that these drugs did not block escape responding because shock-induced reflexive responses (flinch, jump, etc.) began the motor sequences that were not initiated by the conditioned stimulus alone. Patients with Parkinson's disease who are known to suffer from degeneration of the NSB (Ehringer and Hornykiewicz, 1960) cannot initiate movement but can overcome this deficit when confronted with strong environmental stimuli (Denny-Brown, 1966). Further, chlorpromazine-treated rats avoided more frequently when the duration of the preshock stimulus was extended (Posluns, 1962). On the other hand, Ranje and Ungerstedt (1977a,b) concluded that central DA neurons are involved in learning as well as motor functions.

The present experiments used the conditioned suppression procedure (Estes and Skinner, 1941) to determine whether conditioning had occurred to a preshock stimulus. Neuroleptic-treated animals received avoidance training with an auditory preshock stimulus and then were trained to lever-press for food while undrugged. The auditory preshock stimulus was subsequently presented during the lever-pressing session. Suppression of responding during the auditory stimulus would indicate that conditioning to the preshock stimulus had occurred during the avoidance sessions and would confirm that avoidance deficits observed in animals treated with neuroleptic drugs are related to an impairment in initiating motor responses.

Methods

Subjects. Thirty-two male albino rats of the Wistar strain with *ad libitum* weights of from 258 to 355 g were housed individually in a climatically controlled colony room on a 12-hr light-dark cycle. All rats were deprived to 80% of their free-feeding weights and were maintained at those weights throughout the experiment by daily feeding with measured rations.

Apparatus. Two different apparatuses were used, one for lever-press training and the other for one-way avoidance training. For the former, three similar experimental environments each consisted of a cubicle (23.4 × 20.4 × 19.5 cm) with Plexiglas sides and top, aluminum plate ends and a grid floor. In the middle of one of the ends at a height of 5.5 cm was a lever (Scientific Prototype) that was 5.0 cm wide and had a force requirement of about 0.11 N. To the left of the lever at a height of 1.5 cm was a feeder cup. Each cubicle was located in a ventilated sound-attenuating box illuminated by an overhead light and fitted with a 2900 Hz tone generator (Sonalert). Environmental control and data collection were performed by a Data General Nova 3 computer.

The one-way avoidance apparatus consisted of a shuttlebox (25 × 78 × 33 cm deep) divided in half by a partition. One-half was painted flat black and the other was metallic gray. The partition could be removed by raising a 13 cm wide guillotine door. The grid floor on the black side could be electrified by a scrambled 2.0 mA d.c. current (BRS/LVE). A 2900 Hz tone generator (Sonalert) was mounted below the grid floor in the black side of the box. Electromechanical relays and timers were used for environmental control and data collection.

Procedure. Twenty-four rats were randomly assigned to three groups, the control ($N = 8$), pimozide 0.5 ($N = 8$) and pimozide 1.0 ($N = 8$) groups. At approximately the same time each day for 8 days, each rat received a 40-min session in the operant chamber. During the first three sessions, a continuous reinforcement schedule was in effect; each lever-press was followed by the presentation of one 45-mg food pellet (P. J. Noyes Co., Lancaster, NH). For the next five sessions, a random interval (RI) 64-sec schedule was in effect; lever-press-contingent food pellets became available only after RIs with an average duration of 64 sec. These training sessions were given to establish lever-press responding which was to be used after avoidance training to test for conditioned suppression.

One-way avoidance training followed. Each rat received five 10-trial sessions in which the intertrial interval was 30 sec. Ninety minutes before each session i.p. injections were given. The shocked control group received vehicle (tartaric acid), the pimozide 0.5 group received 0.5 mg/kg of pimozide dissolved in boiling tartaric acid (1:6) and cooled to about 40°C before injection and the pimozide 1.0 group received 1.0 mg/kg of pimozide. At the start of each session, the rat was placed into the gray side of the shuttle box. After 30 sec, the rat was placed into the dark side facing the end opposite the guillotine door. The trial then began with the onset of the tone and the opening of the door. If the rat moved into the gray side during the 10-sec tone period, the tone was turned off, the door was lowered and an *avoidance* response was recorded. If the rat failed to avoid during the 10-sec period the offset of the tone was contiguous with the electrifying of the grid floor on the black side. The subsequent movement to the gray side was followed by replacement of the door and an *escape* response was recorded. Entry into the gray side always began the next intertrial interval of 30 sec after which the animal was replaced in the black side by hand. Dependent variables were latency and number of avoidance and escape responses.

On the third day after the end of avoidance training, the first of three additional 44-min sessions of responding on the RI 64-sec schedule occurred. During the second and third of these sessions, the same tone that had signalled shock in the avoidance trials was presented for one 60-sec period. Tone presentation occurred after 8 to 18 min of responding. The purpose of these sessions was to measure conditioned suppression to the tone. The number of responses during the 1 min preceding the tone and during the tone period was recorded for this purpose. After these RI sessions, the original three groups each received

three additional one-way avoidance sessions in the shuttle box. No drugs were given before these sessions.

Eight additional rats were used to demonstrate the neutrality of the tone in the absence of tone-shock pairings (unshocked control). Each rat received six 40-min sessions of training on the RI 64-sec schedule. During the last of these sessions, the tone was presented for two 60-sec periods at various times in the session. Number of responses in the 1-min period preceding and during the tone was recorded.

Five sets of data were treated independently. The number of avoidance responses in each session for each group during the five training sessions was analyzed with a two-way analysis of variance with repeated measures on the sessions variable. The avoidance data for the three drug-free sessions were analyzed similarly. Response latencies for each group for each trial of the five training sessions were subjected to a three-way analysis of variance with repeated measures on the sessions and trials variables. Latencies for the three drug-free sessions were analysed in a similar fashion. Finally, the suppression ratios (see below) during the first two tone presentations for the four groups in the lever-pressing sessions were compared using a two-way analysis of variance with repeated measures on the probe variable. Whenever significant main effects were observed, post hoc tests of simple main effects and individual comparisons were carried out.

Results

Avoidance. An avoidance response was recorded if the rat left the dark side of the shuttle box during the 10-sec tone period before shock onset. If no avoidance response occurred shock was presented and the shuttle response was recorded as an escape. The mean number of avoidance responses for each group for each of the five training sessions is shown in figure 1. The control group improved in performance from session 1 to session 2 ($P < .05$), whereas the two pimozide groups showed no significant change in mean number of avoidance responses from session to session. The control group made more avoidance responses than both pimozide groups in every session ($P < .05$) but the pimozide 0.5 group made more avoidance responses than the pimozide 1.0 group only on sessions 4 and 5.

The avoidance data from the three drug-free avoidance sessions which followed conditioned suppression testing also are shown in figure 1. The control group made more avoidance responses than both pimozide groups ($P < .05$) but the pimozide groups did not differ significantly from each other.

Comparison of the avoidance performance of the pimozide 1.0 group in the first drug-free session (Session 6) and the control group in the very first test session in the apparatus (Session 1) revealed that the pimozide 1.0 group made more
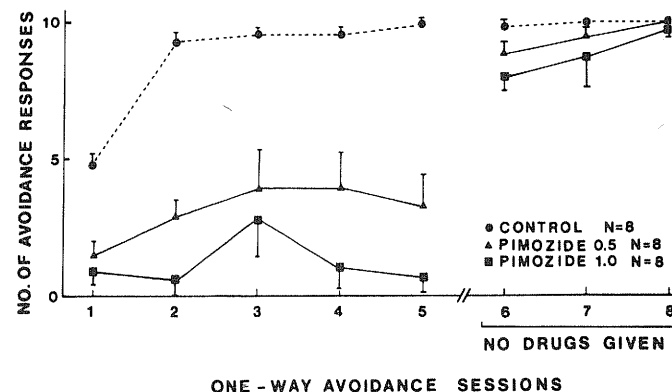

Fig. 1. Mean number (±S.E.M.) of avoidance responses for each group for each of the first five 10-trial avoidance sessions during which drugs or vehicle were given and during the last three avoidance sessions during which no drugs were given.

Fig. 2. Mean (±S.E.M.) latency (sec) of the shuttle response (avoidance or escape) for each group for each of the first five 10-trial avoidance sessions during which drugs or vehicle were given and during the last three avoidance sessions (Sessions 6 to 8) during which no drugs were given.
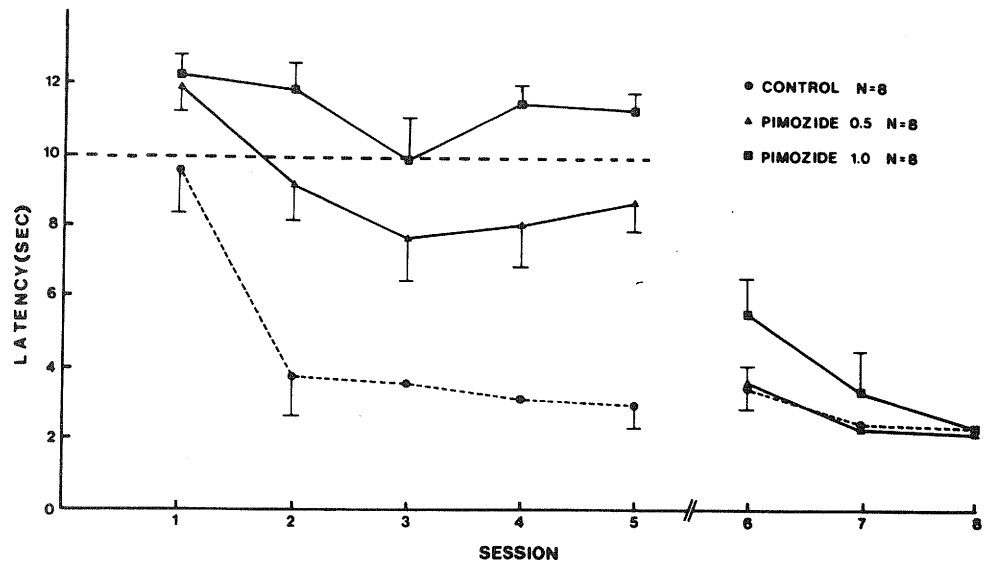
avoidance responses than the control group (P < .05). Although the animals treated with pimozide were severely impaired in avoidance performance, when retested in a drug-free state they made more avoidance responses than naive animals in the very first training session.

Latency. Latency was defined as the time from tone onset until the animal entered the gray side of the shuttle box. The group mean latencies for each session (collapsed over trials) are shown in figure 2. The results were similar to the avoidance results described above. The data indicate that over sessions the mean latency to shuttle to the safe side was significantly longer for the pimozide-treated rats than for controls.

Figure 3 indicates that the relationship of the three groups from trial to trial in Session 1 differed from that relationship in Session 5. Thus, there was no significant group effect in Session 1 (P > .05) but the groups differed in Session 5 (P < .01). Also, in Session 1 there was a groups by trials interaction (P < .01), whereas in Session 5 this interaction was insignificant (P > .05).

The latency data from the first five sessions indicate that the control group had the shortest latencies and the pimozide 1.0 group was slower than the pimozide 0.5 group (see fig. 2). In the first test session, the three groups had similarly long latencies which decreased at about the same rate for about the first six trials. During the last four trials, the control group began to avoid effectively, whereas the two pimozide groups did not. This initial training effect had disappeared by Session 5 when all three groups were performing consistently but at different levels (see fig. 3).

The latencies for the three drug-free test sessions also are shown in figure 2. The groups did not differ significantly (P > .05).

The drug groups did avoid on some trials. Thus, the pimozide 0.5 and pimozide 1.0 groups made 124 and 49 avoidance responses with a mean (±S.E.M.) latency of 3.11 (±0.22) and 4.95 (±0.39) sec, respectively. The mean latency for the 342 avoidances made by the control group in the first five sessions was 2.78 (±0.11) sec. The avoidance latencies of the pimozide 1.0 group were longer than those of both the control (P < .001) and the pimozide 0.5 group (P < .001), whereas the latter two groups did not differ significantly (P > .05). These data suggest that a high dose of pimozide interferes with the ability of the animals to perform the avoidance response on the rare occasion when they make one.
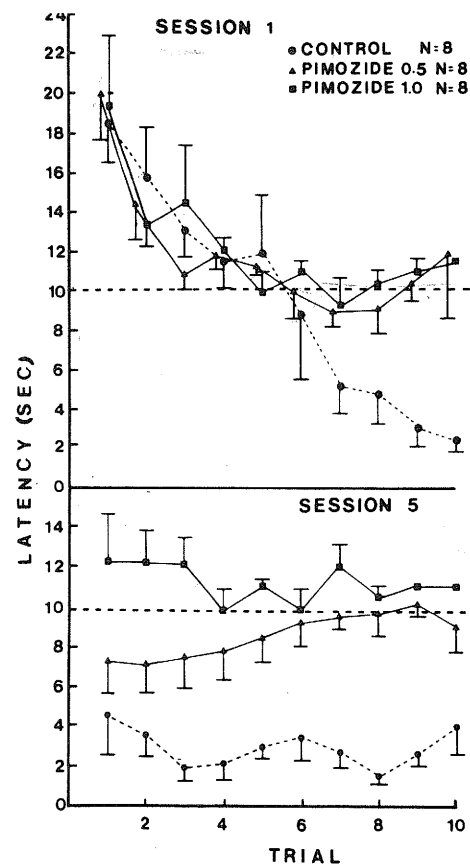

Fig. 3. Mean (±S.E.M.) latency (sec) of the shuttle response (avoidance or escape) for each group for each trial of the first and fifth sessions during which drugs or vehicle were given.

Conditioned suppression. The mean (±S.E.M.) responses per minute on the RI schedule for the unshocked control, control, pimozide 0.5 and pimozide 1.0 groups in the one minute preceding the first tone presentation were 24.6 (±3.3), 26.0 (±2.9), 22.8 (±2.7) and 25.0 (±3.1), respectively and did not differ significantly. Suppression ratios were calculated by dividing the number of responses made during the 1-min tone period by the sum of the responses made in the minute preceding the tone and during the tone. Thus, if responding ceased during the
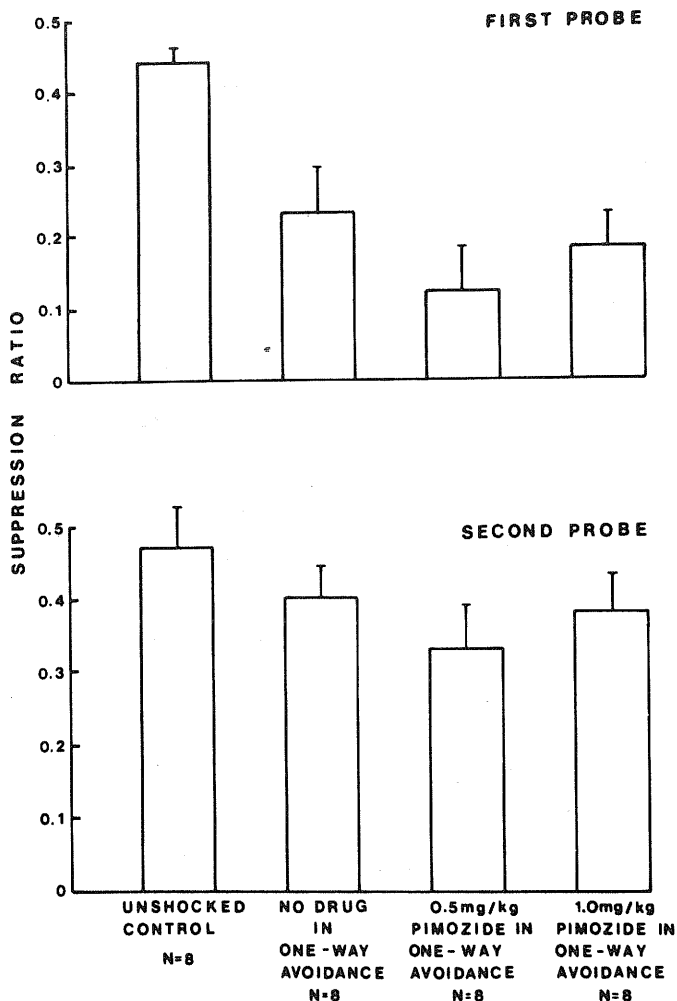
Fig. 4. Mean (±S.E.M.) suppression ratio measured on an operant base line of each of the three groups which had received avoidance training and of control animals which had never received tone-shock pairings. Probes one and two indicate the first and second 1-min presentation of the tone which had signalled shock during avoidance trials.

tone this ratio would be 0, whereas no effect of the tone on responding would yield a ratio of 0.5. Ratio values between 0 and 0.5 would indicate partial suppression of responding during the tone.

The group means of these suppression ratios for the first and second probe presentation are shown in figure 4. The group differences during probe 1 were significant (P < .01) but not during probe 2 (P > .05). During probe 1 the unshocked control group differed from the pimozide 0.5 group, the pimozide 1.0 group and the shocked control group. However, the three groups which had received one-way avoidance training did not differ significantly from one another during probe 1.

Discussion

That the DA-receptor blocker pimozide (Pinder *et al.*, 1976) impairs avoidance acquisition and performance is consistent with previously reported effects of neuroleptic drugs (Fibiger *et al.*, 1975; Niemegeers *et al.*, 1969). To the extent that the effects of chlorpromazine on avoidance responding are related to that drug's DA-receptor blocking properties, the present results also are consistent with an extensive early literature (*e.g.*, Cook and

Kelleher, 1963; Dews and Morse, 1961; Herz, 1960; Posluns, 1962).

The results of this experiment indicate that neuroleptic-treated animals, although impaired in initiating responses, are not impaired in associative learning. This result is consistent with the finding of Hunt (1956) that chlorpromazine did not disrupt classical conditioning and with the observation of Posluns (1962) that the avoidance behavior of chlorpromazine-treated rats was affected by the location of the warning signal. That previously drugged animals avoided more in the first drug-free session than undrugged naive animals is in agreement with the results of Davidson and Weidley (1976) and Fibiger *et al.* (1975) and again suggests that there were no associative learning deficits. Finally, the similar trial by trial improvement in escape latencies for the pimozide-injected and control rats during session 1 also suggests that learning occurred in the drugged animals. That the two pimozide groups never avoided consistently suggests that the drug impaired the initiation of responses in the absence of shock.

The effects of the neuroleptic pimozide seem to be specific to DA neurons (Pinder *et al.*, 1976) and numerous studies have shown that neuroleptics and other techniques for decreasing DA function have similar effects on avoidance behavior (see above). Thus, it seems that DA neurons are involved in response initiation mechanisms but not in associative learning mechanisms. The precise DA system cannot be deduced from the present study, although other data implicate the projection to caudate-putamen (Delacour *et al.*, 1977; Mitchum and Thomas, 1972).

Some authors have studied discrimination learning in NSB-lesioned animals with procedures designed to overcome deficits in the initiation of responses. Price and Fibiger (1975) used an electrified Y-maze to demonstrate that animals with 6-hydroxydopamine lesions of the SN could learn a brightness discrimination as well as unoperated controls indicating that DA-disruption fails to affect associative learning processes. Ranje and Ungerstedt (1977a,b) required DA-disrupted animals to perform a brightness or spatial discrimination by swimming underwater to safety in the correct arm of a Y-maze. In one study (Ranje and Ungerstedt, 1977a), animals injected with spiroperidol learned a spatial discrimination but not a brightness discrimination. However, the spiroperidol-treated rats did improve, albeit more slowly than controls, from an error rate of about 70 to 80% in the first session to 40% on Session 4. Unfortunately, the experiment ended in the fourth session. Possibly, the attenuated learning rate was due to motor impairments in performing the task when DA systems were disrupted.

In a related study, Ranje and Ungerstedt (1977b) reported that animals with bilateral destruction of the NSB learned neither a spatial nor a brightness discrimination in the underwater maze. The discrepancy between these data and those of Price and Fibiger (1975) may be related to more complete lesions of the NSB by Ranje and Ungerstedt (1977b). Thus, animals with almost complete loss of striatal DA might have a severe response initiation deficit even when submerged in water. That half of the lesioned rats from one of their spatial discrimination experiments (Experiment IV) failed to complete more than one trial in the first session lends support to this hypothesis.

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