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Effects of Nortriptyline Treatment on Learned Helplessness in the Rat

J. I. TELNER[1] AND R. L. SINGHAL

Department of Pharmacology, Faculty of Health Sciences, School of Medicine
University of Ottawa, Ottawa, Canada

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TELNER, J. I. AND R. L. SINGHAL. *Effects of nortriptyline treatment on learned helplessness in the rat.* PHARMAC. BIOCHEM. BEHAV. 14(6) 823–826, 1981.—Using an escape delay procedure previously shown to elicit behavioral deficits in mice exposed to uncontrollable shock, rats treated with inescapable but not escapable shock or no shock displayed comparable interference effects when tested in a two-way shuttle box 24 hr later. Treatment with 12.5 mg/kg nortriptyline for 4 or 6 days counteracted the escape deficits produced by inescapable shock while the 0 or 2 day administration regimens were without any appreciable effect. The finding that interference effects produced by inescapable shock were sensitive to sub-acute but not acute drug administration supports the utility of the learned helplessness model in evaluating potential antidepressant agents in experimental animals.

Learned helplessness Nortriptyline Shock Stress Antidepressants

STUDIES have shown that when an organism is exposed to an uncontrollable stressor, it displays avoidance and escape deficits when tested in a later task situation where successful responding results in stress postponement or termination [9]. This phenomenon has been termed "learned helplessness" and has been shown to occur in a variety of species across an array of task situations. Learned helplessness has been proposed as an animal model of depression as it shares many of the behavioral characteristics found in clinical depression, both in terms of etiology and manifestation [11].

Recently, this model has been proposed as a tool to investigate potential antidepressant drugs both in terms of their antidepressant characteristics as well as elucidate their mechanism of action [13]. For example, the antidepressants imipramine and desmethylimipramine, but not the antipsychotic chlorpromazine, have been shown to counteract learned helplessness in rats [7,13]. Furthermore, these same studies demonstrated that the model is sensitive to varying drug doses as well as to sub-acute but not acute administration of these psychoactive agents.

McKinney [10] suggested several criteria that must be met in order for an animal model of human depression to be considered adequate. One of these requirements is that treatment that relieves depression in the human must also reverse the behavioral expression observed in the animal model. Thus, the aim of the present study was to examine the influence of acute and sub-acute nortriptyline on escape learning in animals pre-exposed to inescapable shock. Nortriptyline was the antidepressant chosen as it is a typical tricyclic used in the treatment of depression. Furthermore, this agent has been shown to be a somewhat selective norepinephrine (NE) reuptake inhibitor and may be efficacious in reversing the fall in brain NE which is usually seen in animals exposed to inescapable stress.

EXPERIMENT 1

The interference effect produced by inescapable shock has been reliably produced in a variety of species including the rat. However, with the rat, it has been observed that testing must involve a fairly complex task in order for the behavioral impairment to be expressed. For example, interference effects have been observed in the rat if a FR3 bar press or a FR2 shuttle escape served as the test task, but not if lower schedules were employed [8,12]. Anisman *et al.* [2], using mice as subjects, have devised a procedure whereby an escape delay is imposed on animals pre-exposed to shock or no-shock conditions. The 4 or 6 sec delays, but not the 0 or 2 sec delays, reliably differentiated between subjects exposed to the two pre-shock conditions. The purpose of the first experiment in the present study was to replicate the escape delay procedure in rats using the typical triadic design of escapable shock, inescapable-yoked shock and no shock.

METHOD

Subjects

Eighteen male Holtzman rats weighing 175–200 g served as subjects. They were housed and maintained under standard laboratory conditions with free access to food and water.

Apparatus

Preshock. The apparatus for shock presentation consisted of three identical wooden chambers (30×36×39 cm) with a

[1]J. I. Telner is a Research Fellow of the Ontario Mental Health Foundation.

transparent observation wall. The floor consisted of a removable aluminum pan which served as the ground electrode. At the back of the box was a metal shelf (7 cm above the floor) which served as a jump-up platform for escape responding. The shelf of the inescapable shock chamber was connected to the floor in order that shock would be inescapable. Overhanging the shelf was a piece of wood, hinged to the ceiling, that served to deposit the animal onto the floor prior to the start of each trial as well as to prevent shelf-jumping during inter-trial intervals. Shock was delivered via a safety pin electrode implanted subcutaneously in the upper back [12].

Escape learning. Escape training was carried out in an automated two-way shuttle box (60×21×30 cm) fashioned out of clear Plexiglas with walls lined with thin aluminum sheets. The floor consisted of two aluminum pans (28×21 cm) which served as the ground electrode. A vertically movable gate divided the box into two compartments and served as a hurdle (5 cm from the floor) in the down position. The shuttle box was enclosed in a sound-attenuated chamber and white noise was delivered throughout the experiment via two speakers located at the top of the test box. Shock (1 mA) was delivered from a 1200 V shock source via a safety pin electrode. Gate opening and shock delivery as well as recording of escape latencies and failures were controlled by a Commodore PET microcomputer.

Procedure

Rats were randomly assigned to one of three treatment groups and run as triplets in the preshock boxes. Subjects in the escapable shock group received 1 mA, 12 sec shocks with a 48 sec intertrial interval for one hour, where jumping on the shelf terminated shock for each trial. Subjects in the inescapable shock group were yoked to their escapable shock partners and received an identical pattern of shocks with no response-shock contingency. Control animals were pinned, but spent an uneventful time in the chamber.

Escape training was carried out 24 hr later in the two-way shuttle box. Animals were placed individually in the box (side chosen at random) and allowed to habituate to the test environment for 10 min with the gate in open position. Following this period, subjects received 15 shock trials with the gate in the closed position at the start of each trial. A 3 sec escape delay was employed as extensive pilot work had demonstrated this time interval to be the most effective in producing the interference effect in rats pre-exposed to inescapable shocks. Escape consisted of jumping across the hurdle to the other compartment within 30 sec after gate opening, which resulted in shock offset and gate closing. A variable 60±20 sec intertrial interval was employed. Number of escapes as well as escape latencies were recorded for each animal.

RESULTS

Analysis of variance of the number of successful escapes revealed a significant effect of pre-shock treatment, $F(2,15)=6.43$, $p<0.01$. Subsequent Duncan multiple comparisons ($\alpha=0.05$) of the means revealed that the group exposed to inescapable shock escaped less frequently than either the escapable or no-shock groups. Analysis of variance of the latency data revealed a significant influence of prior shock treatment on escape time, $F(2,15)=5.81$, $p<0.05$. Duncan multiple comparisons revealed that subjects pre-exposed to inescapable shock exhibited longer latencies in the escape task than either animals in the escapable or no-shock condition. These findings are depicted in Table 1.

TABLE 1

EFFECT OF PRIOR SHOCK CONDITION ON ESCAPE LEARNING

	No Shock	Escapable Shock	Inescapable Shock
Latency (sec)	3.7±1.3	5.8±2.0	17.7±4.6*
Successes	14.0±0.8	14.2±0.4	7.7±2.6†

Rats were exposed to 15 trials of no shock or 1 mA of escapable or inescapable-yoked shock and tested 24 hr later in a shuttle box using an escape-delay procedure. Data are expressed as mean latencies to escape for each group (n=6)±S.E.M.

*Significantly different from other groups ($p<0.05$).

†Significantly different from other groups ($p<0.01$).

TABLE 2

TIME-COURSE EFFECTS OF NORTRIPTYLINE ON ESCAPE LEARNING IN RATS PRE-EXPOSED TO SHOCK OR NO SHOCK

Treatment	Day 0	Day 2	Day 4	Day 6
Shock-drug	5.3±3.1	7.5±3.1	17.5±1.0*	16.0±1.4*
Shock-saline	7.0±2.4	8.8±2.9	9.2±2.2	6.2±1.9
No shock-drug	14.3±2.8	18.2±0.8	18.8±0.5	17.8±1.0
No shock-saline	14.5±2.4	16.5±1.9	18.0±1.2	18.8±0.5

Rats were exposed to 12 sec shocks for 1 hr and administered 12.5 mg/kg nortriptyline or saline for 0, 2, 4 or 6 days and tested in a shuttle box using an escape delay procedure. Data represent mean number of escapes for each group (n=6)±S.E.M.

*Significantly different from day 0 and day 2 shock treated drug groups and all shock treated saline groups ($p<0.05$).

EXPERIMENT 2

Experiment 2 involved an examination of the effects of a widely used tricyclic antidepressant, nortriptyline, on behavioral deficits induced by inescapable shock. Specifically, it was suggested earlier that a valid animal model should be sensitive to treatments known to effectively alleviate clinical depression. Thus, acute versus sub-acute administration of the drug on reversal of interference effects produced by inescapable shock was examined.

METHOD

Subjects and Apparatus

Ninety-six male Holtzman rats served as subjects. All subject and apparatus specifications were identical to those described in Experiment 1.

Procedure

Preshock. All animals were pinned for shock and individually placed into the pre-shock chambers. Half the subjects received 1 mA, 12 sec shocks with a 48 sec intershock interval for 1 hr, while the remaining animals spent an equivalent

shock-free period in the chambers. Rats were then kept in individual holding cages until the time of testing.

Drug administration. Experiment 2 consisted of a 2 (shock/no shock)×2 (drug/vehicle)×4 (days of injection) factorial design with 6 subjects per group. One day following shock exposure, subjects received 0, 2, 4 or 6 days of 12.5 mg/kg nortriptyline HCl (Aventyl, Lilly) IP or an equivalent amount of saline, with the last injection administered 30 min before test.

Escape training. Escape training was carried out in an identical manner as described in Experiment 1, except that 20 shock trials were presented. For each subject, mean number of successful escapes as well as mean overall latency were recorded.

RESULTS

The mean number of successful escapes for each treatment group is shown in Table 2. A three way analysis of variance yielded a significant shock×drug×days interactions, $F(3,80)=2.79$, $p<0.05$. Analysis of variance of the preshock condition data revealed that animals exposed to shock displayed significantly fewer escapes than those exposed to the no-shock condition, $F(3,80)=53.14$, $p<0.001$. Although the preshock×drug interaction did not reveal any statistical significance, $F(1,80)=2.87$, $p>0.05$, Duncan multiple comparisons ($\alpha=0.05$) were conducted to evaluate the drug effect separately for shock and no-shock rats. Among no shock subjects, nortriptyline had no effect on performance when compared to saline subjects. In the shock group, drug treatments for 4 and 6 days, but not for 0 and 2 days, did prevent the interference effects.

The mean escape latencies as a function of preshock treatment and drug administration over days are shown in Fig. 1. A three way analysis of variance yielded a significant shock×drug×days interaction, $F(3,80)=2.75$, $p<0.05$. Analysis of variance of the preshock condition (shock×no shock) showed that animals exposed to preshock displayed significantly longer escape latencies than those exposed to the no shock situation. Analysis of variance revealed a significant preshock×drug interaction, $F(1,80)=3.96$, $p<0.05$. Duncan multiple comparisons conducted at each injection day for drug and placebo groups ($\alpha=0.05$) revealed that animals pre-exposed to shock and administered the drug for 4 or 6 days had shorter escape latencies than either the 0 and 2 day treatments as well as all preshock placebo groups. There were no significant differences in escape latencies for drug- and saline-treated animals in the no-shock group.

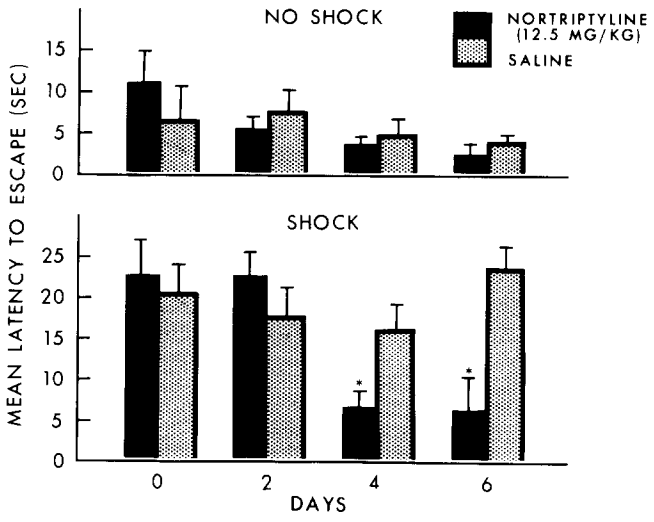

FIG. 1. Time-course effects of nortriptyline on escape learning in rats pre-exposed to shock or no shock conditions. Drug animals were administered 12.5 mg/kg nortriptyline for 0, 2, 4 or 6 days prior to testing. Each bar represents the mean escape latency (N=6)±S.E.M. *Significantly shorter latencies than all other shock groups ($p<0.05$).

DISCUSSION

The results of Experiment 1 confirm previous findings [5, 8, 12] that pre-exposure to inescapable, but not escapable stress interferes with later adaptive behaviour. Furthermore, these findings support the hypothesis forwarded by learned helplessness theorists that it is not the stress *per se* that produces interference effects, but rather the amount of control that the organism has over the stressor. As well, this experiment reproduced in rats the escape delay procedure used by Anisman *et al.* [2] in mice. The learned helplessness hypothesis advocates a cognitive interpretation of the mechanisms underlying the behavioural impairment produced by inescapable stress [9,11]. This hypothesis has been challenged by others [2, 15, 16] who suggest that whereas neurochemical and motoric factors are the salient mechanisms in determining the response deficits, cognitive or motivational factors are probably not involved. Although, arguments have been proposed on both sides, the issue still remains unresolved.

The results of Experiment 2 show that the interference effect produced by prior inescapable shock is reversed by nortriptyline. Furthermore, only the 4 and 6 day drug treatments were effective in reversing this effect, whereas the shorter treatment schedules (0 and 2 days) produced no significant effect. These findings correlate with those of Sherman *et al.* [13] who counteracted shock-induced interference effects by administration of imipramine for 4 days.

Numerous studies have demonstrated the profound neurochemical changes produced by stressors such as shock, cold and restraint [1,14]. Furthermore, it has been shown that these changes are more often elicited by stress that is uncontrollable than by insult that is controllable. The most dramatic changes are seen in norepinephrine (NE), although changes are seen in other transmitter systems as well. Weiss and his associates [15,16] observed substantial decreases in endogenous levels of NE in whole brain as well as in the hypothalamus and brainstem of animals exposed to unavoidable/inescapable shock. Furthermore, pharmacological treatments have been shown to both produce and eliminate interference effects. For example, several studies [3,6] have shown that agents that deplete NE and/or dopamine (DA) produced behavioral deficits similar to those elicited by inescapable shock. Recently, Anisman *et al.* [4] eliminated escape deficits in animals pre-exposed to shock by administering DA and NE receptor agonists, apomorphine and clonidine, respectively. Taken together, these studies point to a catecholaminergic system mediating the interference effects produced by inescapable stress. The results of the present study would appear to support this interpretation as

nortriptyline, a somewhat selective NE reuptake inhibitor, was shown to reverse the behavioral impairment engendered by inescapable shock.

As mentioned earlier, McKinney [10] has suggested that one requirement that must be met in order to propose a workable animal model of depression is that treatments that alleviate clinical depression also act effectively on the behavioral parameters which define the model. It is our contention that the results of the present study strengthen the validity of the learned helplessness model as a prototype of depression, since it has been shown that the behavioral impairment inherent in the model is reversed by an agent known to produce antidepressant effects in man. More importantly, a delayed action of the drug on the interference effects which, although shorter, parallels the development of psychological change in the human depressive. The present study thus suggests the utility of the learned helplessness model for future studies relating to potential antidepressant drugs.

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