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Tricyclic Antidepressants: Effects on Extinction and Fear Learning

GAYLORD ELLISON, JUDI HANDEL, RICHARD ROGERS AND JUDY WEISS

Department of Psychology, University of California, Los Angeles 90024

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ELLISON, G., J. HANDEL, R. ROGERS AND J. WEISS. *Tricyclic antidepressants: effects on extinction and fear learning.* PHARMAC. BIOCHEM. BEHAV. 3(1) 7–11, 1975. – Rats trained to run an alley for a food reward were extinguished following injections of different antidepressants. When retested several days later, the animals extinguished following pretreatment with the NE reuptake blocker protriptyline showed faster running speeds than did the other groups. Other rats given electrical shocks following pretreatment with protriptyline avoided the compartment in which they had been shocked less than did animals shocked following pretreatment with other antidepressants. This implies an interference with some aspect of the learning or consolidation process which is correlated with the degree of NE reuptake blockage. It is hypothesized that NE terminals are deactivated following frustrative nonreward or punishment by the conversion and reuptake of the released NE to an altered extinction molecule.

Tricyclic antidepressants Extinction Fear learning Autoproduction of 6-OHDA Reinforcement theory

RAPID developments in psychopharmacology have led to substantial advances in the description and treatment of the alterations in brain chemistry present in emotional or affective disorders, particularly at the biogenic amine level [1, 7, 25], but this body of knowledge has not as yet been successfully integrated with more psychological or behavioral conceptions of the causes of emotional disorders. Central to many psychological theories of depression [33] is the role of learning and the assertion that it is a prolonged period of excessive negative reinforcements (frustrative non-reward or punishment) which is critical in triggering the diffuse biochemical alterations which develop in dysphoric states such as depression and anxiety. An excellent animal model of this is the phenomenon of learned helplessness [26], whereby prolonged inescapable punishment eventually leads to a complete lack of response initiation much like the behavior in novel environments of animals with extensive monoaminergic depletions [8].

These reinforcement models imply some special relationship between negative reinforcers and depression and suggest that those pharmacological agents which have been found effective in treating depression might attenuate the negative reinforcement process. The present experiments were designed to study whether pretreatment with various antidepressants would alter the degree of control over behavior exerted by two different kinds of negative reinforcers – the frustrative nonreward of an appetitive approach response and the suppressive effects of punishment by painful electrical shocks.

EXPERIMENT 1: EXTINCTION OF AN APPETITIVE APPROACH RESPONSE

Method

Forty-eight female albino rats initially weighing 215–280 g were housed in individual cages, tamed with daily handling, and then placed on a 23 hr food deprivation schedule. After they had habituated to this schedule (2 weeks later) they were trained (2 trials per day) to run a 7 ft straight alley for a highly preferred food reward (30 sec access in the goal box to a mixture of chocolate chip cookies and water). Training was conducted in a dim, quiet room with a masking noise present. After 50 training trials the animals were split into 4 equal groups matched for terminal running speed and, on extinction day, given 3 massed extinction trials (with confinement in the empty goal box for 60 sec). This combination of training parameters (well-trained animals, with no previous exposure to partial reinforcement, given massed extinction trials) was chosen because it maximizes the frustration effect in runway studies and produces rapid extinction [2].

One experimental group was given these extinction trials following an i.p. injection of the tricyclic antidepressant protriptyline HCl (7 mg/kg). Protriptyline is one of the most potent blockers of norepinephrine (NE) reuptake known [14] and at this dose is a potent but selective inhibitor of NE reuptake [4]. A second group was extinguished after pretreatment with chlorimipramine (7 mg/kg),

also a tricyclic antidepressant but at this same dose an equally potent and selective blocker of serotonergic (5HT) reuptake with poor NE reuptake blocking properties [5]. A third experimental group was extinguished following an i.p. injection of the monoamine oxidase inhibitor nialamide (100 mg/kg). Nialamide has been found to have moderate MAO inhibition with poor uptake blocking properties [11], and this dosage of nialamide has been found to be comparable to that used for the tricyclics in studies comparing tricyclic antidepressants and MAO inhibitors in their ability to alter thermoregulation and monoamine turnover [6], induce changes in histofluorescence [19], and reverse reserpine sedation [23]. This group therefore served as a control for increased levels of monoamines during extinction without primary monoaminergic reuptake blockage. A control group was extinguished after saline injections. All of these injections were given 15 min prior to the first extinction trial, and all testing was done blind. Three days after these massed extinction trials all animals were retested in the alley nondrugged (2 trials separated by 30 min).

Results

Mean running speeds (reciprocal of time to sec to break photocell beams mounted 15 cm from the start and goal boxes) on the 3 extinction trials were comparably decreased in all drug treated groups (mean running speed ± standard deviation for Saline animals was 0.402 ± 0.15, for the Protriptyline group 0.285 ± 0.12, for Chlorimipramine 0.288 ± 0.3, Nialamide 0.277 ± 0.11; $F(3,45) = 3.60$, $p<0.05$). A different pattern between groups was found when the animals were given two nondrugged retest trials 3 days following the day of extinction (Fig. 1; $F(3,45) = 3.27$, $p<0.05$). The animals which had been extinguished following injections of protriptyline ran significantly faster on these postextinction retests than did the saline controls ($t = 2.45$, $p<0.05$); none of the other drug treated groups were significantly different from saline controls. Whether this faster retest running speed in the Protriptyline group was due to a drug state dependent extinction was tested with a second retest 3 more days later, when the Saline and Protriptyline groups were given 2 further retest trials 15 min after a 7 mg/kg injection of protriptyline. Again the animals extinguished following the protriptyline injections showed faster running speeds (mean running speed across these 2 drug retest trials ± standard deviation was 0.25 ± 0.21 for the Protriptyline group and 0.14 ± 0.09 for the Saline group; $t = 1.93$, $p<0.05$). Thus, animals in which a food approach behavior was extinguished following pretreatment with the norepinephrine reuptake blocker protriptyline 3 days later behaved as though a part of the extinction process had been blocked; this effect was present whether the animals were tested while drugged or nondrugged; two other antidepressants did not share this effect.

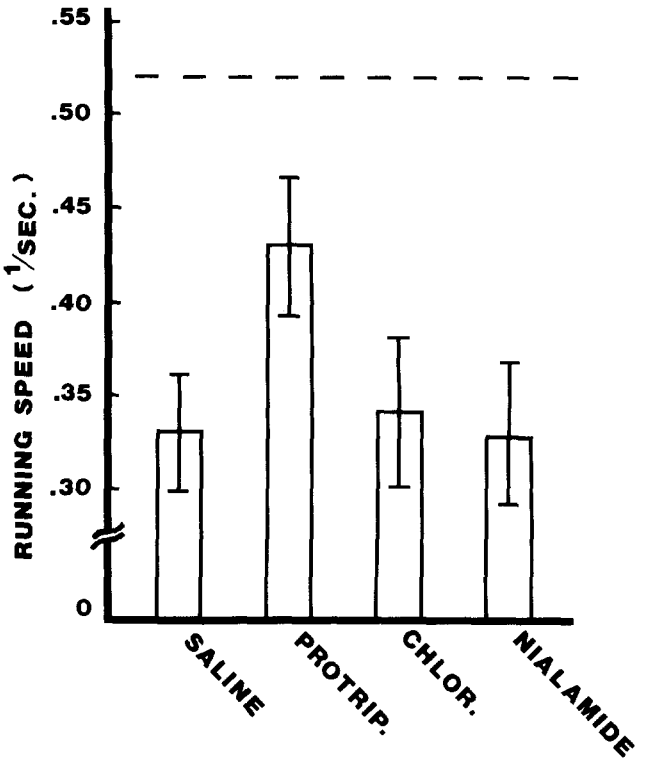

FIG. 1. Mean running speeds (± standard error) across two postextinction test trials in a straight alley. Groups of rats had been extinguished following pretreatment under the drug conditions listed. The broken line indicates mean running speed (all groups) on the last 4 trials prior to extinction.

EXPERIMENT 2: ACQUISITION OF A SPATIAL AVOIDANCE BASED ON FEAR

Method

The experimental apparatus [20] consisted of a tilt box divided into a white illuminated compartment (30 × 28 × 30 cm) connected via a round access hole (7 cm dia) to a smaller black and poorly illuminated compartment (7 × 7 × 30 cm). This apparatus was housed in a soundproofed chamber with a quiet masking noise present. Noiseless lever switches sensed the position of the rat in the apparatus (black vs. white compartment). Eight-four experimentally naive female hooded rats were habituated to the apparatus (allowed 3 min periods of ad lib exploration on each of 3 successive days). They were then divided into 7 groups of 12 each equated on the basis of black side preference during habituation. On the fourth experimental day all animals were placed into the black compartment for 2 min with access hole blocked and a cover placed over the tilt box. One experimental group (No shock controls) was merely confined; the other groups were given four foot shocks (1.6 mA, 1 sec duration, 30 sec intershock interval). These inescapable shocks were administered to the different groups 15 min after i.p. injections of either of two monoamine oxidase inhibitors (nialamide 100 mg/kg or tranylcypromine 10 mg/kg) or following injections of tricyclic antidepressants (protriptyline 7 mg/kg, or chlorimipramine 7 mg/kg, or a mixture of these two, each at 3.5 mg/kg, or following saline injections. The mixture of chlorimipramine and protriptyline at a halved dose was used to simulate the mixed pattern of monoaminergic uptake blockade found in many other tricyclic antidepressants [4,15].

On the third day following this drug–shock treatment, the animals were tested for the degree to which they had acquired a spatial avoidance of the black side. Each animal was placed into the white side of the tilt box with the access hole open for a 15 min test during which the latency to first enter the black compartment, the total number of

crossings, and the total time spent in the black compartment were recorded.

Results

There were significant differences between groups in the amount of time spent in the black compartment (Fig. 2; $F(6,77) = 4.50$, $p<0.001$). The animals shocked after saline injections avoided (spent less time in) the black compartment compared to nonshock controls, but both experimental groups preinjected with protriptyline prior to receiving shocks avoided the black compartment significantly less than did the animals shocked following saline injections ($p<0.05$, t tests). While the animals shocked following injections of nialamide or tranylcypromine were not significantly different from the saline controls, the chlorimipramine treated animals approached statistical significance ($t = 1.85$, one tailed $p<0.05$). Rats given a mixture of monoaminergic reuptake blockers showed the maximal memory disruption on this test. A similar pattern between groups was observed when the amount of spontaneous activity in the tilt box (mean number of crossings) was analyzed (Table 1). The animals shocked following saline injections were inactive compared to the nonshocked controls, but all three groups shocked following injections of tricyclic antidepressants made significantly more crossings than did the animals shocked following saline injections ($p<0.05$, t tests).

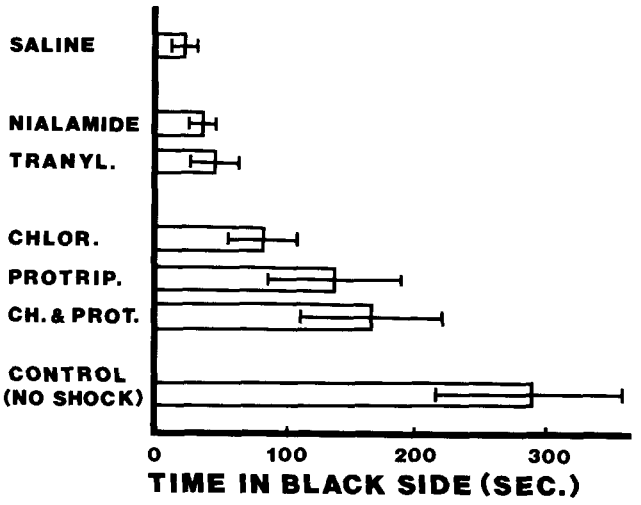

FIG. 2. Mean time (± standard error) spent in the black side of a tilt box during a 15 min test. Animals had been shocked in the black side 3 days previously under the listed drug conditions.

TABLE 1

MEAN INITIAL LATENCY, NUMBER OF CROSSINGS, AND TOTAL TIME SPENT IN THE BLACK SIDE DURING THE 15 MIN RETEST

Experimental group	Latency (sec) to initially enter black side	Mean number of crossings in 15 min	Mean time (sec) spent in black side
Saline-shock	691	1.8	19
Nialamide-shock	553	2.4	25
Tranylcypromine-shock	609	2.6	54
Chlorimipramine-shock	537	4.2	88
Protriptyline-shock	431	3.9	135
Chlor. & protrip.-shock	469	4.5	164
No shock	89	6.7	290
Statistical significance: $F_{(6,77)}$	4.39	5.3	4.50
	$p<0.05$	$p<0.05$	$p<0.05$

To test if these differences between groups could be explained by a drug state dependent learning of fear, the results with the mixture of protriptyline and chlorimipramine at 3.5 mg/kg each were replicated using the procedures just described. Four new experimental groups of 8 naive rats each were tamed, habituated to the apparatus, and then shocked following either drug or saline injections. Again all animals were tested 3 days later in the apparatus 15 min after either drug or saline injections. The results were analyzed using a 2 × 2 experimental design. The (shocked following) Saline–(tested following) Saline group spent an average of 0 sec in the black compartment during the 15 min retest; the (shocked following) Saline–(tested following) Tricyclic group averaged 2.5 sec in the black compartment; the (shocked following) Tricyclic–(tested following) Saline group spent 33.4 sec in the black compartment, and the Tricyclic–Tricyclic group averaged 52.8 sec in the black compartment. Thus, the results again were not due solely to state dependency, with the rats shocked after pretreatment with tricyclics spending more time in the black compartment whether tested under saline or drugged conditions, $F(1,28) = 10.3$, $p<0.01$, and the interaction between drug state when shocked and when tested, a measure of the degree of state dependency, being insignificant, $F(1,28) = 0.22$, n.s.

DISCUSSION

Rats pretreated with some kinds of antidepressants before being administered two different kinds of negative reinforcers later behaved as though the degree of behavioral control by these negative reinforcers had been attenuated: they showed an increased tendency to enter environments in which they had been administered either frustrative nonreward or painful electric shocks. This effect, which could be due either to an attenuation of the negative reinforcement process or to the more general disruption of memory consolidation, was maximal in rats pretreated with a tricyclic antidepressant which is a relatively selective inhibitor of NE reuptake, protripyline. However, in the fear–avoidance test rats pretreated with a tricyclic which is a selective inhibitor of 5HT reuptake, chlorimipramine, also showed decreased avoidance. In this test the most effective pharmacological treatment for producing decreased fear of the shocked compartment was a mixture of these two selective reuptake blockers, a mixed pattern of NE and 5HT reuptake blockade like that of the most common tricyclic antidepressants [4, 5, 15]. The results of the present study are very similar to those of Latz *et al.* [18], who studied the effects on maze learning of pretreatment with a wide range of doses of tricyclic antidepressants, monoamine oxidase inhibitors, and d-amphetamine. In addition to finding enhanced water maze learning in rats pretreated with d-amphetamine, these investigators found decreased learning in animals pretreated with tricyclics and no effects on learning produced by pretreatment with monoamine oxidase inhibitors.

The major finding of the present study, that the greatest alterations in learning or memory consolidation were produced by a pharmacological agent which produced the most selective alterations in NE circuitry, is consistent with other evidence from animal and human studies demonstrating alterations in learning or performance associated with alterations in NE metabolism, but in the majority of studies it is decreased levels of NE which have been associated with decreased learning abilities [3], and increased NE levels which have been found to produce increased learning rate [12]. Protriptyline, by blocking NE reuptake, elevates synaptic NE levels yet it leads to decreased learning. Moreover, were the effect found in the present studies due principally to elevated synaptic levels of NE it would also be expected to be present in the animals treated with monoamine oxidase inhibitors, for they also elevate synaptic levels of the monoamines [29]. This is especially true for tranylcypromine, a monoamine oxidase inhibitor with similarities to amphetamines in structure [11] and NE releasing ability [24]. While tranylcypromine also blocks the NE reuptake process [13], like d-amphetamine this occurs at concentrations 10 to 50 times greater than those for tricyclic antidepressants [14]. These results, in showing that the greatest innoculation against negative reinforcers was produced by a pharmacological agent with the most potent and selective inhibition of the NE reuptake process, are consistent with other evidence on the importance of the reuptake process whereby up to 80% of the released NE is later taken back up into NE neurons and returned to vesicular stores [14,34].

One speculative interpretation of why blocking the NE reuptake process might produce effects in the learning tests employed in this study is based on the application of reinforcement theory to NE circuitry. Other observations on NE circuitry have demonstrated that NE innervations show a remarkable ability to regenerate and sprout following their experimental destruction [16,22], and behavioral tests of animals in which NE lesions are made with 6-OHDA and a recovery period allowed imply that these new innervations become functional [28]. This has introduced the possibility [21] that such sprouting also occurs under more natural conditions, such as during learning – i.e., that in response to environmental influences central NE neurons rearrange or switch their output connections through the physical growth of connections when reinforced and the destruction of terminals when extinguished. Classical reinforcement theories [27,31] emphasized that a fundamental characteristic of goal directed behavior is that the reinforcement or extinction message must work backward in time as the consequences of a response lead to the strengthening or weakening of that response tendency. Applied to NE circuitry, this suggests that the NE molecules released in energizing a behavioral coping response might be modified by experience and when later reabsorbed could modify the circuitry which had released them.

Applied to the negative reinforcement paradigm employed in the present studies, this would imply a simple model whereby the breaking of the appropriate NE terminal connections could occur in response to frustrative nonreward or punishment. This would involve an extinction message sent through the neural circuitry representing an act in which an animal had just engaged [17], converting those NE molecules out in the synaptic cleft to a temporarily suppressive or autotoxic form. The subsequent reuptake of this modified catecholamine would then produce the temporary inactivation or, in greater quantities, the more permanent destruction of only those NE terminals which had energized the recently emitted (and to be extinguished) behavior, for they would be the only synapses with NE in the cleft at the time of the extinction message. 6-Hydroxydopamine serves as a biochemical model of this toxin [30]. The present results are compatible with the Stein-Wise model, for protriptyline prevents the lesioning

action of 6-OHDA [9] while monoamine oxidase inhibitors do not [10]. But blocking the reuptake process would presumably also interfere with the reinforcement or reward message as well as the extinction message, as is implied by the Latz *et al.* [18] finding that maze learning is also disrupted by pretreatment with tricyclic antidepressants.

This hypothesis has the advantage of integrating behavioral and biochemical conceptions of emotional disorders, showing how the prolonged extinction of basic coping mechanisms could set off the diffuse shutdowns of NE metabolism which have been proposed to occur in clinical depression and the chronic or burnt-out schizophrenic. But the decreased learning in animals pretreated with tricyclic antidepressants could equally well be due to a variety of mechanisms, including alterations of sensory mechanisms or general disruptions of memory consolidation. Furthermore, the neurochemical aspects of tricyclics which produce this effect could be anticholinergic effects as well as reuptake blockage of monoamines. More research is needed on this effect; compared to drugs such as d-amphetamine there have been relatively few studies on the effects of tricyclic antidepressants on learning.

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