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Amphetamine and conditioned 'anxiety'

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Summary

1. Rats pressed a bar for milk reward at a steady rate, but this baseline responding was suppressed in the presence of an auditory stimulus associated with electric shock (conditioned suppression). The effects of (+)-amphetamine sulphate on this conditioned suppression were studied in two experiments.

2. (+)-Amphetamine sulphate (0·5, 1·0 or 2·0 mg/kg) reduced the baseline rate of responding and also reduced the conditioned suppression, i.e. responding in the presence of the auditory stimulus was partially restored. Both these effects were dose related.

3. In a further experiment the effects of 1·0 mg/kg on two levels of conditioned suppression were studied. Regardless of its degree, (+)-amphetamine attenuated suppression.

4. The results were compared to previous research which found that amphetamine increased baseline responding and exaggerated conditioned suppression. It was concluded that the conditioned suppression procedure should be used with caution as an animal model of anxiety in psychopharmacological investigations.

Introduction

A continuing effort of behavioural pharmacology has been to develop animal models of clinically important human states and processes. One such model is the 'conditioned emotional response' (CER), a special case of which is the conditioned suppression of food or water rewarded bar-pressing behaviour. Although variations in procedure are possible, this conditioned suppression typically takes the following form. Hungry or thirsty rats are trained to press a bar for a food or water reward and conditions are arranged so that this behaviour is maintained at a steady rate. When this adequate baseline responding has been attained, the animals are given an auditory or visual signal which terminates in an unavoidable electric shock. After sufficient training, this auditory or visual signal comes to act as an aversive conditioning stimulus (CS) and the rate of responding is reduced or suppressed altogether in its presence. The rate of responding in the absence of the CS and shock remains at the original steady baseline rate. It has been suggested that conditioned suppression may be an index of conditioned 'fear' or 'anxiety' (Brady, 1956), but many investigators (see Davis, 1968, for a review) have rejected these concepts as being scientifically unproductive. Nevertheless, the conditioned suppression method has assumed an important place among the techniques of behavioural pharmacology (Kelleher & Morse, 1968).

Since investigators are more interested in developing and testing drugs which reduce anxiety rather than increase it, research on drugs and conditioned suppression has concentrated on 'anti-anxiety' compounds almost exclusively (Davis, 1968; Kelleher & Morse, 1968). A notable exception to this rule is the small

amount of work on amphetamine (Brady, 1956; Lauener, 1963). In his widely cited investigation, Brady (1956) reported that amphetamine intensified conditioned suppression in both rats and monkeys while increasing response rate in the absence of the CS which preceded shock. In a much less frequently cited study, Lauener (1963) found that amphetamine had no effect on conditioned suppression in rats; moreover, it is unlikely that this negative result reflected a procedural inadequacy, since substantial effects were observed using the same procedure and different drugs.

Conditioned suppression is not the only model of fear or anxiety in animals which has been used to assess the effects of amphetamine on 'emotional' behaviour. Active avoidance in which the subject takes some positive action following the conditioned stimulus to avoid receiving shock is considered to be maintained by fear (Rescorla & Solomon, 1967) and may be improved by amphetamine (Hearst & Whalen, 1963; Kriekhaus, Miller & Zimmerman, 1965); however, it is possible to interpret this improvement in terms of the drug's effect on activity rather than fear. Rushton & Steinberg (1964) observed that amphetamine increased the exploration of rats in a novel environment, as did amylobarbitone. It was suggested that these results reflected decreased fear in the case of amylobarbitone but increased activity in the case of amphetamine; however, the methods employed by Rushton & Steinberg did not permit a separation of drug effects on fear and activity. Clearly, increased exploration under amphetamine could be ascribed to a fear-reducing effect of the drug in the absence of appropriate control conditions. When such conditions were provided in an extension of the Rushton & Steinberg work by Kumar (1968), the latter was led to conclude that amphetamine had the dual effect of increasing fear and activity simultaneously and independently. In summary, there is some evidence from the avoidance and exploration literature which is consistent with the hypothesis that amphetamine increases fear.

Thus, while there has been some progress with other behavioural models, the effects of amphetamine on conditioned suppression, which is perhaps the most widely employed animal model of fear or anxiety, have not been investigated adequately. Not only is there a paucity of empirical results, but the existing data contain inconsistences and no dose-response information is available. In the research described below, two types of experiment provided additional information. The first type which was designed primarily to obtain dose-response data, yielded a 'paradoxical' drug effect. Since the rats in this experiment had previous experience with mescaline, the second type of experiment was designed as a partial replication with naïve rats. In addition it included two levels of conditioned suppression; although the work of Appel (1963) indicates that this is an important consideration, with few exceptions, experimenters have failed to manipulate this variable in psychopharmacological investigations.

Methods

Establishment of a dose-response relationship

Subjects

The subjects were 12 individually housed male Wistar rats. They were kept on a schedule of *ad lib* access to water and Purina Rat Chow sufficient to maintain weight at approximately 250 g.

Procedure and apparatus

Each rat had received extensive exposure to variations of a conditioned suppression procedure in earlier work (Cappell, Webster & Ginsberg, unpublished) with mescaline. The prior experience consisted of nine 1-hour sessions of training on a variable interval (VI) 60 s schedule of reinforcement, on which a bar-press is rewarded (reinforced) if it occurs after a given interval from the previous reinforcement: the apparatus was so programmed in this case that these intervals varied randomly around a mean of 60 seconds. Such a reinforcement schedule results in a steady rate of responding throughout a 1 h session. Each reinforcement consisted of 0·1 ml of sweetened evaporated milk (30 g of table sugar per 15 oz of milk). This training was followed by 33 h of conditioned suppression training in 1-hour daily sessions and by an experiment in which each rat received four injections of mescaline hydrochloride ranging from 12·5 to 25·0 mg/kg.

Prior to testing with amphetamine, the rats were maintained drug-free for 14 days. During this period, and for the remainder of the experiment, conditioned suppression training continued as follows: while responding for milk on the VI 60 s schedule, the rats were exposed to a tone CS of 65 dB, 3,000 Hz which invariably terminated in a scrambled grid shock of 1·5 mA. The tone was presented twice during each 1-hour session, beginning at minutes 18 and 39. Tone duration was 3 min; shock was delivered during the last 0·5 s of the tone, and shock and tone terminated simultaneously. On the next 6 days, rats received an intraperitoneal injection 5 min before each session. Control and drug days alternated as follows: control; drug (1·0 mg/kg); control; drug (0·5 mg/kg); control; drug (2·0 mg/kg). The drug solution was the appropriate concentration of (+)-amphetamine sulphate dissolved in isotonic saline; the control solution was isotonic saline. Injection volume was always 4 ng/kg. The apparatus consisted of two liquid dispensing Grason-Stadler rat chambers enclosed in sound-attenuating chests; with the exception of a home-made shock generator, the programming and recording of experimental events were done with the appropriate Grason-Stadler programming and data collection relay equipment.

Manipulation of shock intensity

Rationale

While the results of the foregoing experiment were clear cut, there were two important limitations in the procedure which required further investigation. First, in spite of the fact that two drug-free weeks elapsed prior to testing with amphetamine, it is possible that the previous experience with mescaline influenced the rats' behaviour. Secondly, the shock level selected for the dose-response investigation had the effect of producing complete response suppression during the tone on control days. Thus the only *possible* change in responding during the tone was an increase. Therefore, a second investigation was designed to meet these limitations.

Subjects

The subjects were 16 male individually housed Wistar rats. They were kept on a schedule of *ad lib* water and Purina Rat Chow sufficient to maintain weight at approximately 300 g throughout the experiment. The subjects were drug-naïve prior to the experiment.

Procedure and apparatus

Before exposure to conditioned suppression training, each rat received approximately 70 h of training on a VI 60 s schedule of reinforcement in 1-hour sessions. Eight rats bar-pressed for food reinforcement (45 mg Noyes pellets) and 8 for sweetened evaporated milk. Comparable behavioural baselines were generated with both types of reinforcement. The initial intent of the procedure was to train each rat with a distinctive CS for high and low shock in order that comparisons might be made on a within subject basis. Thus 8 rats were given conditioned suppression training in which a tone was paired with high shock intensity and a light was paired with low shock intensity, and 8 rats were trained with the signal-shock pairings reversed. Each rat received 30 hours of such training in 1-hour daily sessions. When it became evident that reliable differential suppression would be difficult to establish in all subjects, a between groups design was adopted. The tone-shock pairings were deleted, and for 10 additional 1-hour sessions, 8 rats continued with only light-low shock pairings while the remainder continued with only light-high shock pairings. For both groups the light stimulus (CS) was a compound of the red and green panel lights situated on the front wall of the operant chamber. The duration of the CS was 3 min ; there were two CS-shock presentations per session beginning at minutes 21 and 51 of the session. Scrambled grid shock was delivered during the last 0·5 s of CS presentation, and CS and shock terminated simultaneously. Low shock intensity was 0·5 mA and high shock was 2·0 mA as determined by the dial setting on Grason Stadler (Model E6070B) shock generators. Differential conditioned suppression was reliably established by this procedure. Thirty minutes prior to the next two sessions, rats were injected intraperitoneally with isotonic saline in order that they might become adapted to any stress associated with the injection procedure. Drug and saline control sessions followed. Each rat received two drug and two control injections whose sequence was counterbalanced according to a Latin-square design. The drug dose was 1 mg/kg of (+)-amphetamine sulphate. Injections were given intraperitoneally 30 min prior to a session. Injections were spaced at three-day intervals although training continued on the intervening days. The injection volume was 4 ml/kg. The apparatus consisted of two liquid and two food dispensing operant chambers with associated relay programming and data collection devices. The operant chambers were housed in sound attenuating chests.

Results

Dose-response determination

The data analysis was based on two behavioural indices: (a) responding during the 3-min intervals during which the preshock stimulus or CS was presented and

TABLE 1. *Effects of (+)-amphetamine on responding during baseline and CS periods in the dose-response study*

Dose (mg/kg)	Response index	
	Baseline responding	Responding during CS
0	136·8±29·7	1·6±0·3
0·5	66·6±13·4	5·1±1·3
1·0	51·4± 9·0	15·9±3·7
2·0	23·7± 6·1	11·7±3·2

Table entries are mean numbers of responses emitted during baseline and CS periods ± standard error.

(b) baseline responding, which was based on responding during the 3 min intervals immediately preceding CS presentation. The total number of responses made during the two CS and baseline periods of each control or drug session was the unit of analysis. Since preliminary analysis revealed no systematic variation in responding over the three saline control days, the mean of these determinations was used as the control dose point. The results are shown in Table 1. Drug effects on both baseline responding and on conditioned suppression were clearly obtained. Compared to control levels, amphetamine depressed baseline responding in a systematic dose-related manner. The overall drug effect was highly significant ($F=8{\cdot}35$, $df=3/33$, $P<0{\cdot}01$); a subsequent trend analysis indicated that only the linear component of the dose-response function was significant ($F=22{\cdot}62$, $df=1/33$, $P<0{\cdot}01$). While baseline responding was clearly depressed by the drug, a totally different pattern emerged when behaviour suppressed by the tone was analysed—in this case responding was *increased* under the drug. The overall drug effect was highly significant ($F=6{\cdot}05$, $P<0{\cdot}01$) but somewhat complex. There was a significant linear component in the dose-response curve ($F=11{\cdot}08$, $P<0{\cdot}01$), but the quadratic component was also marginally significant ($F=2{\cdot}99$, $P<0{\cdot}10$).

Effects of degree of conditioned suppression

The basic data are contained in Table 2, which shows responding during baseline and CS periods calculated as described earlier; the data represent the means of the two saline and two drug determinations. Responding during baseline and CS periods was analysed separately. During the baseline period, responding was unaffected by shock intensity ($F<1{\cdot}00$); baseline responding was lower on drug than on saline days, but the effect was statistically marginal ($F=3{\cdot}17$, $df=1/14$, $P<0{\cdot}10$). During the CS period, responding was strongly affected both by shock intensity and by drug. Differential suppression was clearly evident in that responding during the CS was greater in the low shock condition ($F=10{\cdot}55$, $df=1/14$, $P<0{\cdot}01$). Additionally, responding during CS presentation was greater after drug than saline treatment ($F=10{\cdot}22$, $df=1/14$, $P<0{\cdot}01$). The statistical analysis yielded no other significant effects.

Discussion

While the results of our dose-response investigation are clearly inconsistent with the findings of Brady (1956) in that amphetamine elevated suppressed responding, there was an important limitation on the conclusions which could be drawn. The shock intensity in our experiment was sufficiently intense to produce complete

TABLE 2. *Effects of* 1·0 *mg/kg of* (+)*-amphetamine on responding suppressed by high or low shock in the differential suppression study*

	Treatment			
	Control		Drug	
Shock intensity	Baseline responding	Responding during CS	Baseline responding	Responding during CS
Low	160·0±74·4	20·6±4·2	115·7±20·7	39·6±7·1
High	130·4±21·9	5·6±3·5	127·6±25·5	11·2±6·3

Table entries are mean numbers of responses emitted during baseline and CS periods ± standard error. Each entry is the mean of two drug and two control determinations.

suppression. In contrast, the findings reported by Brady appeared to be based on animals whose responding during the CS was only partially suppressed. Since a further decrement could not possibly be demonstrated in our investigation, it could be argued that the failure to corroborate Brady's findings was simply due to the differences in the degree of suppression present when the drug was introduced. However, it should be noted that our finding of a dose-related decrease in baseline responding, which is also discrepant with Brady's result, is not subject to a similar methodological limitation. When shock intensity was manipulated in order to generate high and low levels of suppression, the methodological problem was solved and the experimental conclusions remained unchanged. Once more, baseline response rate tended to be reduced by amphetamine while responding during the preshock stimulus was elevated by the drug. Moreover, these observations did not depend upon drug history or, in the case of responding suppressed by conditioned 'anxiety', upon near-zero response rates; responding during the CS was increased on drug days whether conditioned suppression was moderate or severe.

In spite of the internal consistency of the results in the present investigation, it remains the case that Brady observed that amphetamine increased baseline responding and decreased responding during a preshock stimulus. However, the failure to corroborate the results of pharmacological investigations is certainly not new in the conditioned suppression literature; as Kelleher & Morse (1968) observed, Brady's (1956) observation that reserpine attenuated conditioned suppression has also failed to receive corroboration in other experiments. Kelleher & Morse suggested that such inconsistencies 'undoubtedly resulted from the use of different schedules, shock intensities, and other parameter values'. Since it is difficult to make comparisons between experiments conducted in different laboratories in order to specify the behavioural parameters which might account for such discrepancies, it is necessary to attempt to *manipulate* potentially relevant experimental variables in order to determine their contribution to observed effects. When this strategy was adopted by manipulating the degree of conditioned suppression in the present experiment, amphetamine's effect on conditioned suppression was not altered; whether this would be true of other pharmacological agents remains to be seen.

Finally, our results re-emphasize the admonition (Davis, 1968; Kelleher & Morse, 1968) that conditioned suppression may be a poor model of anxiety or fear for psychopharmacological investigations. Were such a model to be accepted, it would be necessary to accept the contention that amphetamine reduced anxiety in our experiments. While we consider that to postulate internal states like 'anxiety' or 'fear' is unproductive and we prefer to avoid doing so, it is appropriate to suggest several mechanisms worthy of consideration in interpreting the reduction of conditioned suppression by amphetamine. One such mechanism involves the effect of amphetamine on activity. In a conditioned avoidance situation, Kumar (1968) demonstrated that 1·0 mg/kg of (+)-amphetamine increased rats' avoidance of a compartment which had been associated with shock (i.e., amphetamine increased 'fear'); at the same time, the drug increased activity in an adjacent environment which was 'safe' for the rats. Additionally, Hearst & Whalen (1963) attributed an improvement in avoidance behaviour to the breaking up of the 'freezing' postures which rats characteristically adopt in response to a conditioned stimulus which signals inescapable shock. While the improvement of avoidance seems consistent with the hypothesis that amphetamine increases fear,

such an interpretation seems at odds with the hypothesis that the mechanism is a reduction in 'freezing', since the latter would seem to be indicative of reduced fear. Thus a difficulty with the otherwise attractive hypothesis that reduced conditioned suppression reflected a reduction in 'freezing' by amphetamine is that the effects of the drug on 'fear' and activity are inevitably confounded in the conditioned suppression situation. If, as Kumar (1968) maintained, amphetamine may have independent fear-increasing and activity-increasing effects in a 'fearful' situation, any drug-induced change in a conditioned suppression experiment could be accounted for by the *post hoc* assumption of the prepotency of one of these mechanisms over the other. Yet another possibility (Teitelbaum & Derks, 1958) is that amphetamine interferes with the control over behaviour exerted by conditioned stimuli in situations involving the stress of electric shock. Such a mechanism could account for a reduction in conditioned suppression. It is clearly impossible to determine which, if any, of these mechanisms might account for our 'paradoxical' findings. One thing, however, emerges quite clearly. In view of the multiplicity of variables which seem to be involved, the simple notion that conditioned suppression is a direct index of fear or anxiety does little to enhance our understanding of drug effects.

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