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Acquisition of Conditioned Avoidance Response in Rats under the Influence of Addicting Drugs

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Abstract. Four groups of white rats, aged 8—12 weeks, were treated with morphine, D-lysergic acid diethylamide, DL-amphetamine and ethanol, respectively, while being trained in a conditioned avoidance response (CAR) schedule. Morphine caused deterioration in the acquisition of CAR, as manifested by significant increases in the number of training sessions required for 100% correct CAR and in the reaction time (RT), when compared to those of a control group. The RT decreased after withdrawal of morphine and was associated with a revival of the conditioned emotional responses (CER). LSD and ethanol insignificantly retarded the acquisition of CAR, while withdrawal of LSD caused significant increases in the RT, error and CER. Amphetamine facilitated the acquisition rate associated with increased CER; during withdrawal, the CER was negligible whereas the error increased significantly. In another series of rats, tolerance was seen to morphine and, to a less extent, to ethanol and amphetamine after 8—12 days of continued treatment; whereas the withdrawal effects lasted for 3—4 days only. These effects of the addicting drugs on conditioned learning are discussed in the light of their influence on the emotional responses of the animals and the degree of development of drug-dependence.

Key-Words: Conditioned Avoidance Response — Addicting Drugs — Emotional Responses — Tolerance — Withdrawal Effects.

While the behavioural literature abounds in reports on the effect of various drugs on established conditioned behaviour in animals, relatively fewer studies have been undertaken to assess how the process of acquiring conditioned learning might be modified by drugs, particularly by those with addiction liability. The most widely studied drugs are notably the tranquillizers (Ader and Clink, 1957; Kamano and Arp, 1964), the psychostimulants and psychotomimetics (Key, 1964; Domino *et al.*, 1965) and the centrally acting cholinergic agents and their antagonists (Chalmers and Ericson, 1964; Goldberg *et al.*, 1965). Addicting drugs like morphine have been shown to disrupt or depress prelearned conditioned behaviour (Cook and Weidley, 1957; Verhave *et al.*, 1959), while amphetamine-like drugs facilitate it (Weissman, 1959; Carlton and Didamo, 1961), whereas the psychotogenic drugs usually produce a dose-dependent

biphasic effect (Jarrard, 1963; Smythies and Sykes, 1964). There has however been little uniformity in the nature of conditioning or in the parameters of training. A comparative assessment of the effects and after-effects of addicting drugs, given concomitantly, upon the acquisition of conditioned behaviour in a single species and under similar experimental situation has, therefore, been the objective of a pilot study reported in this paper.

Methods and Materials

The animals were trained in a discriminated conditioned avoidance response (CAR), using a simple pole-climbing apparatus (Cook and Weidley, 1957). The conditioned stimulus (CS), an electric bell-ring, was paired 3 sec later with the unconditioned stimulus (UCS) of a mild foot-shock; pole-climbing within the first 3 sec was the correct response and failure to do so was counted as the error. Training sessions of 10 trials each given at 1-min intervals were held on alternate days. The training was assessed in 4 ways: (i) the number of sessions required for a full acquisition of the CAR, (ii) the reaction time (RT) to elicit the CAR, (iii) the frequency of error (ER %) after a 100% correct CAR and, (iv) the degree of the conditioned emotional response (CER). The criterion for a full training was repetitive 100% correct CAR, allowing only one (i.e., $<5\%$) error out of 3 consecutive sessions of 10-trials each. The CER was graded quasi-objectively, using a 5-plus rating scale (Banerjee, 1971) from the gross behaviour of the rats triggered by the CS, e.g., pole-clinging, piloerection, trembling and shaking, jaw-working, squeaking, micturition, defaecation etc. Grades ranging from 1+ to 5+ were given depending on whether these responses occurred singly or in combination of 2, 3 or 4 symptoms at a time.

Seventy-three young white Norwegian rats of both sexes, aged 8—12 weeks, were used in the acquisition study. They were born of mixed inbred stock and were raised under the same environmental situation. The rats were placed in 2 series according to their age at the onset of training. The first series (11—12 weeks old and weighing 148 to 240 g) consisted of 3 groups: a control (A), a morphine-treated (B) and an LSD-treated (C) group. Likewise, the second series consisted of 3 other groups (8—9 weeks old and weighing 128—210 g), namely, the control (D), the amphetamine-treated (E) and ethanol-treated (F) rats. The training, including a post-drug period, was usually concluded within 3—4 weeks except for the morphine rats which required nearly 6 weeks.

A third series of 20 newly trained rats (12—15 weeks old and weighing 204—310 g) were treated with morphine, amphetamine and ethanol for 16 days in 3 respective groups. Training, using the same parameters as

in the first 2 series, was given to these rats for investigating the phenomenon of tolerance and withdrawal syndrome.

The drugs used in the experiment were: morphine hydrochloride (T & H Smith), D-lysergic acid diethylamide (LSD; Sandoz), DL-amphetamine sulphate (Sigma Chemicals) and absolute ethanol (E. Merck). Morphine and amphetamine were injected subcutaneously every day; LSD was given on alternate days, 30—45 min before the training sessions; ethanol was chronically administered as a 5% solution in the drinking water, the average consumption being 2—2.5 ml of ethanol per rat per day. The drugs were administered until most of the rats in the group acquired a 100% correct CAR or the 12th training session, whichever was earlier. The training however was continued for five more sessions after the drugs in order to assess the effect of withdrawal of these drugs.

The statistical significance of the results was assessed by the Student *t*-test.

Results

Group A: Control Rats

The untreated rats were fully trained after an average of 6 sessions; they suffered from minimal post-acquisition error and had a moderate degree of CER (Table 1). The other group (D) behaved similarly.

Group B: Morphine-Treated Rats (0.25 mg/kg, SC)

The dosage of morphine was aimed to be devoid of any significant analgesic effect, tested by Haffner's tail-pinch method, in order to eliminate any probable effect of analgesia on CAR acquisition. Since 1 mg/kg dose of morphine was known to have a good analgesic effect (Verhave *et al.*, 1959), both 0.5 mg/kg and 0.25 mg/kg doses were tried and the latter was finally chosen.

All morphine-treated rats showed significant deterioration in the acquisition of CAR. They required a mean of 12.5 training sessions against 6 for their control litter-mates. The mean RT was also significantly higher ($P < 0.01$) than that of the control group; the error increased insignificantly. There was however a sharp decrease in the RT ($P < 0.001$), even lower than that of the control group, after the withdrawal of morphine while the CER increased appreciably as compared to the corresponding values during morphine administration (Table 1). The rats appeared to be much more perturbed, agitated and apprehensive after withdrawal of the drug than during its administration, which possibly contributed to the increase in CER.

Group C: LSD-Treated Rats (0.05 mg/kg, SC)

LSD, in doses of 0.1 mg/kg or higher, often caused frank excitation; hence a lower dose was used. The effects of this dose of LSD on the

Table 1. *Influence of addicting drugs on the C.A.R. training in naive rats*

Rat groups	Drug treatment	Sessions required for acquisition of 90% CAR	Sessions required for acquisition of 100% CAR	Reaction time (RT) (sec)	Frequency or errors (ER%)	Degree of CER
A (21)	None	4.2 ± 0.1[a]	6 ± 0.3[a] ($P < 0.001$)[c]	0.94 ± 0.01[a] ($P < 0.01$)[c]	2 ± 0.6[a]	3+[b]
B (15)	*Morphine*	8.1 ± 0.7	12.5 ± 1.1	1.26 ± 0.08 ($P < 0.001$)	2.9 ± 0.7	2.2+
B	(Post-withdrawal)		—	0.83 ± 0.07	3.4 ± 0.6	3.7+
C (8)	*LSD*	5.7 ± 0.2	7 ± 0.3	0.97 ± 0.04 ($P < 0.001$)	3 ± 0.8 ($P < 0.02$)	2.5+
C	(Post-withdrawal)		—	1.2 ± 0.04	8 ± 1.5	3.8+
D (6)	None	4.8 ± 0.2	7 ± 0.4 ($P < 0.01$)	1.0 ± 0.04	2 ± 1.0	2.2+
E (9)	*Amphetamine*	3.5 ± 0.2	5 ± 0.3	0.92 ± 0.06	2 ± 0.9 ($P < 0.01$)	3.7+
E	(Post-withdrawal)		—	0.98 ± 0.04	9.5 ± 1.3	0.5+
F (14)	*Ethanol*	5.7 ± 0.5	8.8 ± 0.95	1.12 ± 0.08	6 ± 1.8	1.5+
F	(Post-withdrawal)		—	0.95 ± 0.96	3.6 ± 1.1	3.4+

() Number of animals.
[a] Mean ± S.E.
[b] Average grade.
[c] Significance levels are shown interposed between the comparing pairs of data.

acquisition of CAR were far from remarkable; there was no significant retardation in the acquisition process comparative to the control rats (Group A). In general, the rats appeared to be less affected by the aversive situation of CAR-training and were initially more or less withdrawn from the environment. However, about half of the treated rats showed signs of some excitement and increased alertness after receiving 6—7 doses of LSD, as manifested by hyperkinesia, much quicker response and even jumps and myoclonic jerks. These symptoms promptly disappeared upon withdrawal of the drug. The RT and ER% increased significantly ($P < 0.02$) and the CER appreciably during withdrawal as compared to the corresponding figures during LSD-administration, or to those of the control rats (Table 1).

Group E: Amphetamine-Treated Rats (1.5 mg/kg, SC)

Like morphine, the dose of amphetamine was selected to be relatively free from causing marked excitation of general behaviour that could

have interfered with the conditioning procedure. Even with this dose, however, all the treated rats appeared to be comparatively more alert and apprehensive than the others and exhibited appreciably higher degree of the emotional responses. Quivering, trembling, pole-clinging, squeaking as well as an increased frequency of urination and defaecation were always encountered during the training. All these features contributed to a high degree of CER. The rats required a significantly lower ($P < 0.01$) number of training sessions for a 100% correct CAR than their litter-mate controls (Group D) but the RT and ER% were about the same. But when the drug was withdrawn, the ER% increased significantly ($P < 0.01$) while the CER was almost totally abolished (Table 1).

Group F: Ethanol-Treated Rats (5% v/v, Orally)

Ethanol was administered as a 5% solution in drinking water to ensure a chronic intake; a good portion of the average daily intake (2—2.5 ml per rat) was consumed during the morning hours, 1—2 h before the training sessions. There was hardly any deterioration in the acquisition and performance of CAR at this dosage; the number of sessions, RT and ER% were somewhat higher in these rats as compared to the controls (Group D), but none of these changes were statistically significant. The CER was slightly decreased during ethanol administration but soon went up to and beyond the normal level upon withdrawal of the drug. The post-withdrawal RT and ER% were also nearer to those of the control group (Table 1).

Additional features of the effects of these addicting drugs upon the acquisition of CAR were brought out in a closer analysis of the day-to-day progress of the various groups of rats. This has been illustrated in Figs. 1 and 2. Fig. 1 shows the mean per cent acquisition of CAR plotted against the number of consecutive training sessions. The steady progress of the control group (only one is represented for clarity) stands out clearly and, while the amphetamine- and LSD-treated groups closely followed the control pattern, they suffered from immediate regressions (i.e., increasing errors) upon withdrawal of the drugs. Whereas the morphine- and ethanol-treated groups exhibited trends of regression, more than once, during the course of the drugs but showed quick improvements upon withdrawal.

Fig. 2, on the other hand, shows the changes in the reaction time plotted against the training sessions. The control group, as above, shows a steady drop in RT, almost exponentially, as the sessions progressed; this pattern was even more prominent in the amphetamine-treated group, but the RT tended to go up on withdrawal. All the other drug-treated groups show rather irregular patterns in the reductions of RT against progressive training sessions, registering sharp fluctuations or frequent

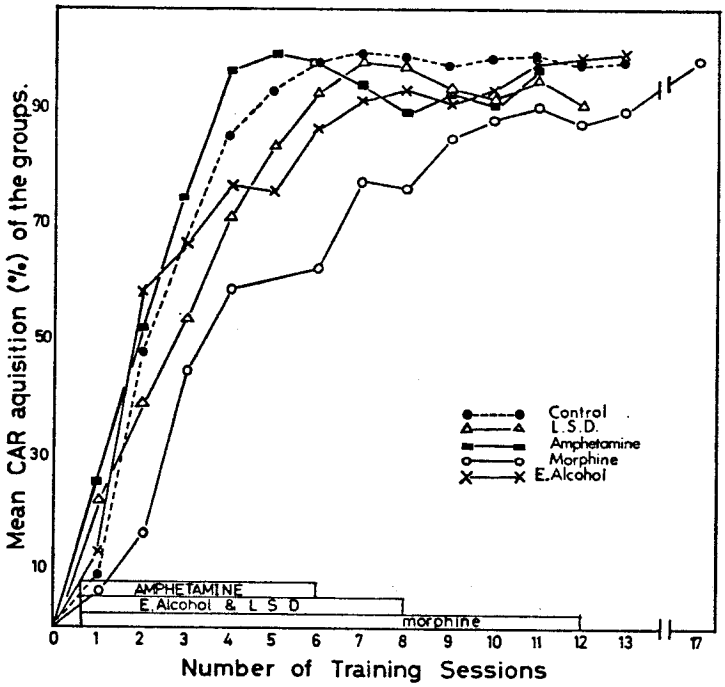

Fig. 1. Mean acquisition of CAR in groups of rat, four drug-treated and a control, plotted against training sessions. The durations of drug treatment are indicated by the horizontal bars at the bottom. Withdrawal caused errors in the amphetamine- and LSD-treated rats while it improved performance in the morphine- and ethanol-treated groups

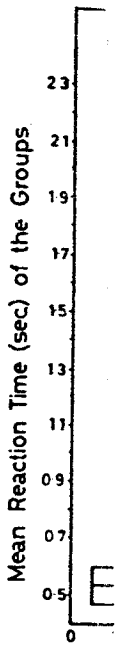

Fig. 2. Mean rea
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regressions. The RT however decreased progressively upon withdrawal of morphine and ethanol, while it increased steeply after LSD-withdrawal.

The newly trained rats of the third series were used to further investigate tolerance and withdrawal effects of these drugs. The grouped mean performances computed from the individual mean performances under the influence of the drugs (like the data presented in Table 1), were not significantly different from those during the initial training, except for the increase in RT with morphine. However, when the data were analysed along the individual training sessions (in the manner of the data presented in Figs. 1 and 2), relevant features in the CAR performance were noticeable (Table 2).

The RT in the morphine-treated rats went up until the 5th session, then decreased during the 6th—8th sessions while the drug was still

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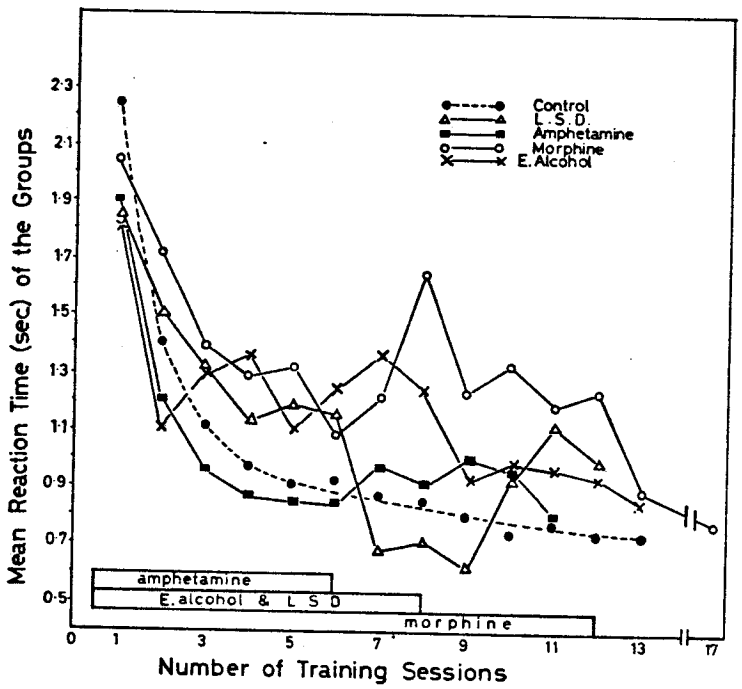

Fig. 2. Mean reaction time in four rat groups under drug treatment and a control, plotted against CAR-training sessions. Bottom horizontal bars indicate durations of respective drug treatments. Withdrawal of drugs caused different effects on the reaction time depending on the stimulant or depressant nature of the drugs

being given; the ER% increased till the 4th session, fell to zero by the 6th and remained low until the end of drug treatment. Both RT and ER% rose sharply during the 1st session of withdrawal but came down to near normal levels in the subsequent post-withdrawal sessions. Similar results were obtained in the ethanol-treated group, showing unsteady increases in ER% and RT till the 5th and 7th session, respectively, under drug treatment and decreasing thereafter. The ER% was quickly reduced to zero after an initial increase following withdrawal, while the RT generally decreased to less than the pre-drug control. The CER gradually decreased during the administration of either drug and again increased after withdrawal, particularly in the morphine-treated rats.

Amphetamine-treatment, on the other hand, caused a small but progressive decrement in RT that occurs as well in control rats under training (see Fig. 2). However, the RT was increasing during the last

Table 2. *Mean CAR-performance of newly trained rats under the influence of addicting drugs*

Drugs and performances measured	Before drug*	Sessions under drug-treatment								Mean *	Sessions after drug-withdrawal					Mean *
		1	2	3	4	5	6	7	8		1	2	3	4	5	
Morphine (8)																
a) RT (sec)	0.8 ± 0.07	1.0	1.1	1.03	1.1	1.4	1.34	0.9	1.03	1.12 ± 0.05	1.28	0.9	0.96	1.04	1.0	1.04 ± 0.06
b) ER (%)	3 ± 1.3	2.5	4	6.2	12.5	2.5	0	1.2	2.5	3.9 ± 0.32	6.2	1.25	1.25	1.25	0	2 ± 0.7
Ethanol (6)																
a) RT (sec)	1.0 ± 0.08	1.03	0.97	1.1	0.9	1.03	1.13	1.3	1.25	1.09 ± 0.07	0.98	0.9	0.97	0.85	1.0	0.94 ± 0.05
b) ER (%)	7 ± 2.7	8	3	7	8	17	15	7	3	8.5 ± 0.91	5	2	0	0	0	1.4 ± 0.7
Amphetamine (6)																
a) RT (sec)	1.09 ± 0.04	1.07	1.01	0.98	0.97	0.88	0.82	0.92	0.94	0.95 ± 0.07	1.03	1.1	1.05	0.98	0.95	1.02 ± 0.05
b) ER (%)	3.3 ± 1.1	6	2	5	3	5	3	0	0	3.0 ± 0.9	3	2	0	0	0	1.0 ± 0.6

() Number of animals.
* Grand mean of the group ± S.E.
All other figures are group-means for individual sessions.

two drug sessions and continued to increase after withdrawal till it reached near normal values. The changes in the ER % and CER, either during or after drug treatment, were negligible (Table 2).

Discussion

It is not unusual to expect that concomitant use of addicting drugs would affect any learning situation and, presumably, in a deleterious manner. Since in an avoidance conditioning situation anxiety or fear of pain has strong motivational properties (Mowrer, 1950), morphine would closely resemble the tranquilizers in reducing the alarm reaction and thus disrupting avoidance behaviour. This has been reported by some workers (Cook and Weidley, 1957; Verhave *et al.*, 1959) who, however, used supra-analgesic doses of morphine, while others disclaim such a selective depressant effect of morphine on the CAR (Domino *et al.*, 1958). According to the former view, an improvement in CAR-performance may be expected upon withdrawal of chronically administered morphine which would also be associated with a revival of the attenuated emotional responses. This has been observed in this study, even with a sub-analgesic dose. The results further suggest that the suppression of the alarm reaction by morphine is, at least partly, independent of its analgesic effects.

Ethanol, however, caused only insignificant deterioration in the acquisition of CAR and no overt behavioural abnormalities either. In fact, there was a transient improvement in the rate of learning during the first 1 or 2 sessions under ethanol (Figs. 1 and 2) which decayed subsequently. As in the case of morphine, the deterioration and improvement in CAR-performance under ethanol and afterwards was associated with an attenuation and then revival of the CER, respectively. The lack of a significantly detrimental effect of ethanol upon CAR-learning could be due to a relatively low dose actually consumed by the rats and, possibly, an inadequate duration of treatment. For the rats were drinking the ethanol-mixed water less and less towards the end of the drug treatment, whereas the contrary would be expected to have occurred with the development of an effective drug-dependence.

Treatment with both amphetamine and LSD produced interesting features in the respective response patterns. Amphetamine is known to have both facilitatory and disruptive influences on learning, depending on various factors like dosage, spacing of trials and other training parameters (Quarton, 1967). The present study confirms the facilitatory effects which were presumably exerted through augmented emotional response and alertness. This assumption is also borne out by the fact that the emotional responses were almost totally abolished during withdrawal and which was associated with concomitant increases in error and, to a smaller extent, in RT. Similar increases in ER % and RT also occur-

* Grand mean of the group ± S.E.
All other figures are group-means for individual sessions.

red upon LSD-withdrawal but, paradoxically, the emotional reactions were usually augmented rather than reduced.

LSD, which is not an addicting drug in the true pharmacological sense, is known to affect the sensory input to the brain, even to the extent of sensitizing the central nervous structures (Taeschler *et al.*, 1960). It did not, however, produce facilitation of learning but rather caused a deterioration in the rate of learning which has been reported by others (Domino *et al.*, 1965). Nonetheless, the RT was getting increasingly shorter towards the end of the experiment and increased again after withdrawal of the drug. The initial deterioration in performance could possibly be due to distracting ambient stimuli, other than the CS, that are known to influence the effect of LSD adversely on the CAR performance (Key, 1964).

Proceeding from the premise that morphine and ethanol tended to retard while amphetamine facilitated CAR-learning, it may be logical to consider any trend in performance contrary to the expected profile of the drug action as tolerance to the respective drug. Such deviations or regressions, opposite to the general trend of the respective drug effects were frequently observed in the morphine- and ethanol-treated rats (Figs. 1 and 2). This phenomenon was particularly investigated in the third series of rats, the earlier training data of which served as a frame of reference for their subsequent performances. In these rats evidence of tolerance to morphine was seen in the abrupt falls in both RT and ER % which had hitherto been increasing steadily until the 4th—5th drug sessions; whereas in the ethanol-treated rats a sharp fall in the ER % in the last two drug-sessions was the only possible sign of tolerance, although there was a concomitant reduction in the consumption of the drug. Similarly, a small and late increase in RT occurring abruptly after a progressive drop during the first 6 sessions of treatment could be interpreted as tolerance to amphetamine (Table 2).

The respective effects of the drugs on the CER of the rats also appeared to be attenuated towards the end of the treatment, except in the case of LSD. However, the changes in the CER, noted under the individual drugs, were more prominent and abrupt after discontinuation of the drugs thus reflecting the behavioural effects of withdrawal. Withdrawal effects usually lasted for the first 1 or 2 post-drug sessions and either diminished or disappeared thereafter, suggesting that only a mild degree of drug-dependence was achieved with morphine treatment and, possibly, with amphetamine and ethanol treatment also to a smaller extent. No satisfactory evidence of either tolerance or drug-dependence was obtained in the LSD-treated rats.

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