J. Pharm. Pharmacol. 1990, 42: 257–260
Received December 18, 1989

A Comparison of the Effects of Nicotine and (+)-Amphetamine on Rat Behaviour in an Unsignalled Sidman Avoidance Schedule

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Abstract—In agreement with the results of previous studies, the withdrawal of nicotine from rats trained on an unsignalled Sidman avoidance schedule under the influence of the drug (0·4 mg kg^{-1} given subcutaneously 3 min before each training session) was associated with a reduction in lever-pressing responses ($P < 0·05$) and an increase in the number of shocks received ($P < 0·01$). The number of shocks received by the withdrawn rats was also greater ($P < 0·05$) than the number of shocks received by rats trained and tested with saline, whereas the number of lever-pressing responses recorded for saline-treated rats was not influenced significantly by the drug used during training. The subcutaneous administration of (+)-amphetamine (0·5 mg kg^{-1} 30 min before the test session) stimulated lever-pressing in rats trained with saline or nicotine and abolished the increase in the number of shocks received by the nicotine-withdrawn rats, but had no significant effect on the number of shocks received by rats trained with saline. The number of shocks received by the rats trained on the schedule with (+)-amphetamine but tested after an injection of saline was also greater ($P < 0·05$) than the number of shocks received by rats trained and tested with saline. It is concluded that the disruption in shock avoidance performance observed for the nicotine- and (+)-amphetamine-withdrawn rats may reflect the development of dependence upon the stimulant properties of these drugs.

It is now widely accepted that nicotine plays an important part in the tobacco smoking habit and that many smokers become dependent upon the nicotine present in the smoke (Balfour 1984, 1990; Clarke 1987; Gilbert 1979). There is also evidence that infra-human species can be trained to self-administer the drug although, when compared with many other drugs of dependence, nicotine is a relatively weak substrate in this paradigm (Balfour 1984, 1990; Clarke 1987). In addition few groups have been able to demonstrate any effects of nicotine-withdrawal on animal behaviour. One important exception to this is the study reported by Morrison (1974a,b) in which she showed that the withdrawal of nicotine from nicotine-treated rats trained on unsignalled Sidman avoidance task evoked a significant disruption of the avoidance behaviour. When the stress of the procedure was diminished by the addition of warning or feedback signals to the experimental protocol, the effects of nicotine-withdrawal were less marked. Morrison argued, therefore, that nicotine dependence appeared to develop more readily in a stressful environment. Subsequent studies which have shown that nicotine self-administration is also enhanced if the animals are exposed to stressful environmental stimuli (Hutchinson & Emley 1985) provide some support for this conclusion. There is also evidence that, in man at least, nicotine can exert a "tranquillising" effect (Gilbert 1979). Balfour (1984) suggested that stress influenced the development of nicotine dependence because a primary rewarding property of the drug lay in its ability to ameliorate the effects of stress. However, studies with experimental animals have failed to provide any convincing evidence that the drug has any of the properties of a conventional anxiolytic agent such as diazepam (Morrison & Stephenson 1972; Balfour et al 1986).

Studies in a number of laboratories have shown that the chronic administration of nicotine to experimental rats causes stimulation of locomotor activity and there is a growing body of evidence to suggest that this effect of nicotine is associated with increased dopamine secretion in the mesolimbic system of the brain (Clarke 1987). There is clear evidence that the psychostimulant response to (+)-amphetamine is also mediated by the mesolimbic dopamine system (Kelly et al 1975; Vale et al 1988) and that many of the behavioural properties of nicotine are similar to those of (+)-amphetamine (Balfour 1984). The purpose of this study was to test the hypothesis that the withdrawal of (+)-amphetamine from rats trained on unsignalled shock avoidance under the influence of the drug, would evoke a disruption in avoidance performance similar to that seen for nicotine-withdrawn rats.

Materials and Methods

Animals

Male Wistar rats, supplied by Charles River (UK) Ltd, were used. They weighed ca 250 g at the beginning of the experiment and were housed in pairs in a room which was illuminated between 0700 and 1900 h daily.

Avoidance training

The rats were trained on an unsignalled shock avoidance task using a protocol similar to that described by Morrison (1974a). During the training period the rats were given daily subcutaneous injections of nicotine (0·4 mg kg^{-1}), (+)-amphetamine (0·5 mg kg^{-1}) or 0·9% NaCl (saline). Following each injection the rats were placed in a two-lever rat chamber (Campden Instruments Ltd) which was housed in a sound-attenuating box and trained to avoid shocks delivered through the grid floor of the cage. During training the shocks were delivered every 5 s unless the rat pressed one of the

levers, in which case each lever-press delayed the presentation of the next shock for 25 s. Initially, a shock level of 0·1 mA was used. This was increased steadily during training to a level of 0·4 mA. The rats given nicotine were placed in the chamber 3 min after the injection; those trained with (+)-amphetamine were placed in the chamber 30 min after the injection. Each training session lasted 60 min. Separate control groups, tested in the chamber 3 and 30 min after an injection of saline, were used for the nicotine and (+)-amphetamine experiments, respectively. Training continued until the rats, when transferred to the final schedule, in which shocks were delivered every 30 s and each lever-press delayed presentation of the next shock for 30 s, successfully avoided 75% of the maximum number of shocks they could have received on three consecutive days.

Drug testing schedule

The rats were tested in the chamber for 40 min per day on five consecutive days per week for three weeks. The behaviour (shocks received and number of lever-pressing responses) of the rats was recorded on day 4 of each of the five day blocks using electromechanical accumulators to record the number of lever-pressing responses and a cumulative recorder to record the shocks received. On that day the animals were treated with saline, nicotine or (+)-amphetamine using a counter-balanced design. On the remaining four days of each block the rats were tested in the chambers after an injection of saline (saline-trained rats), nicotine (nicotine-trained rats) or (+)-amphetamine ((+)-amphetamine-trained rats).

Measurements of locomotor activity

The locomotor responses to the drugs under test were studied using a 40 cm square activity box with 25 cm high sides mounted above an Animex activity meter (LKB Instruments). Activity measurements were made using rats treated acutely or chronically (12 daily injections) with nicotine (0·4 mg kg^{-1}), (+)-amphetamine (0·5 mg kg^{-1}) or saline. The animals were tested in the apparatus for 60 min starting 3 (nicotine experiment) or 30 min ((+)-amphetamine experiment) after the injection.

Statistical analysis

The effects of the drugs on locomotor activity were analysed using an ANOVA for repeated measures with drug treatment and the duration of treatment as the factors analysed. The effects of training on the avoidance behaviour of the rats (numbers of lever-presses and shocks avoided) were analysed using a one-way ANOVA with the drug used in training as the factor analysed. Analysis within training groups was performed using an ANOVA for repeated measures. Subsequent analyses were performed using Duncan's multiple range test.

Drugs

The nicotine solutions were prepared by dissolving nicotine hydrogen tartrate (British Drug Houses Ltd) in saline and correcting the pH to 7·4 by the addition of a small quantity of NaOH. The (+)-amphetamine solutions were prepared by dissolving (+)-amphetamine sulphate (Sigma Chemical Company) in saline. The drug doses are expressed as free base.

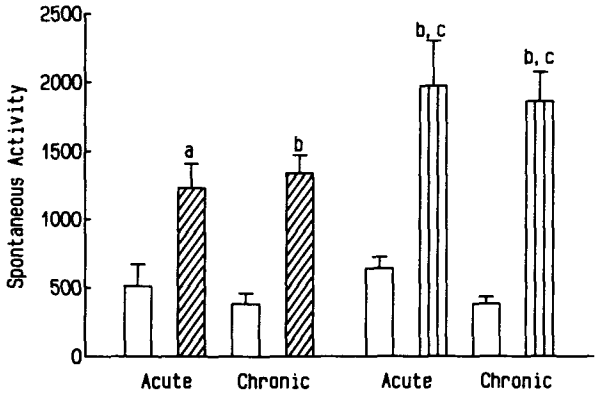

FIG. 1. The effects of nicotine and (+)-amphetamine on the spontaneous locomotor activity of rats. The spontaneous activity was measured in rats treated acutely or chronically (12 daily injections) with subcutaneous injections of saline (open columns), 0·4 mg kg^{-1} nicotine (hatched columns) or 0·5 mg kg^{-1} (+)-amphetamine (striped columns). Each experimental session lasted 60 min. The bars represent the means ± s.e.m. of 6 results. Significantly different from appropriate saline-treated control; a = $P < 0.05$; b = $P < 0.01$. Significantly different from rats treated with nicotine; c = $P < 0.05$.

Results

Preliminary studies with the activity box confirmed that the doses of the drugs selected for the study evoked significant increases in locomotor activity ($F(3,19) = 19.2$: $P < 0.001$) (Fig. 1). Further analysis showed that the effects were significant following treatment with both acute and chronic nicotine ($P < 0.05$ for acute nicotine; $P < 0.01$ for chronic nicotine) and (+)-amphetamine ($P < 0.01$ for both acute and chronic (+)-amphetamine). The increase in activity evoked by the dose of (+)-amphetamine used was also greater ($P < 0.05$ for both acute and chronic drug) than that observed for nicotine.

Statistical analysis of the data for the avoidance experiments showed that rats trained and tested with nicotine made the same number of lever-pressing responses and avoided the same number of shocks as those trained and tested with saline (Fig. 2). However, when nicotine was withdrawn from the rats trained with nicotine and replaced by an injection of saline, the reduction in lever-pressing responses ($F(2,16) = 18.7$; $P < 0.001$; Duncan's test; $P < 0.05$) and the increase in shocks received ($F(2,16) = 9.3$; $P < 0.01$; Duncan's test; $P < 0.05$) were significant. The number of shocks received by the nicotine-withdrawn rats was also significantly greater ($F(1,14) = 9.0$; $P < 0.01$: Duncan's test; $P < 0.05$) than that received by rats trained and tested with saline, whereas the number of lever-pressing responses made by rats tested with saline was not influenced significantly by the drug used during training. The administration of nicotine to rats trained with saline had no significant effect on avoidance performance.

The administration of (+)-amphetamine to rats trained with nicotine caused an increase ($F(2,16) = 18.7$; $P < 0.001$) in the number of lever-pressing responses when compared with nicotine-trained rats tested with saline or nicotine (Duncan's test; $P < 0.01$) (Fig. 2). Injections of (+)-amphetamine also abolished the increase in the number of shocks

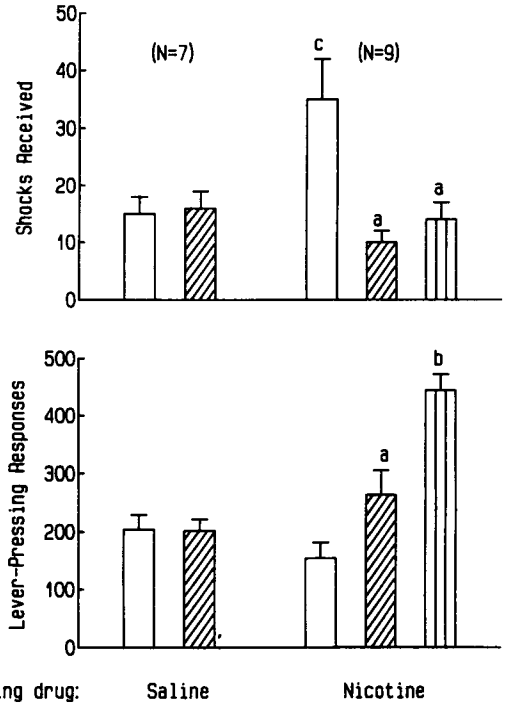

FIG. 2. The avoidance performance of rats trained with nicotine or saline. The rats were trained with saline or 0·4 mg kg^{-1} nicotine and then tested after injections of saline (open columns), 0·4 mg kg^{-1} nicotine (hatched columns) or 0·5 mg kg^{-1} (+)-amphetamine (striped columns). The bars represent the means ± s.e.m. of the numbers of observations given in parentheses. Significantly different from rats tested after saline; a = $P<0·05$; b = $P<0·01$. Significantly different from rats trained and tested with saline; c = $P<0·05$.

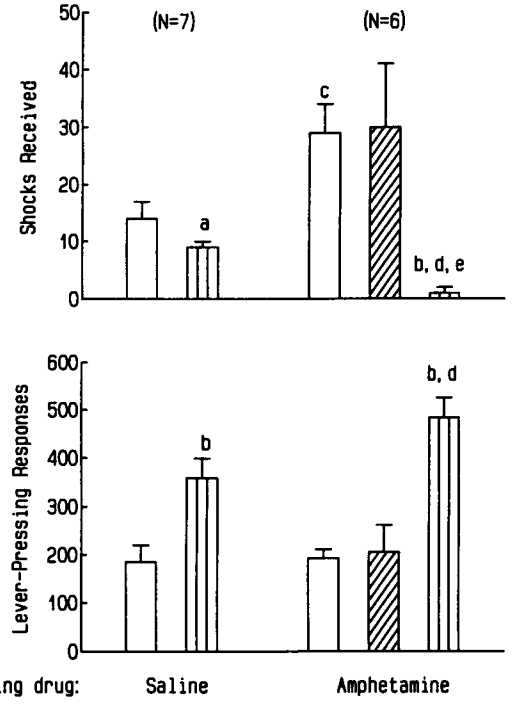

FIG. 3. The avoidance behaviour of rats trained with (+)-amphetamine or saline. The rats were trained with saline or (+)-amphetamine and then tested after injections of saline (open columns), 0·4 mg kg^{-1} nicotine (hatched columns) or 0·5 mg kg^{-1} (+)-amphetamine (striped columns). The bars represent the mean ± s.e.m. of the numbers of observations given in parentheses. Significantly different from rats tested after saline; a = $P<0·05$; b = $P<0·01$. Significantly different from rats trained and tested after saline; c = $P<0·05$; d = $P<0·01$. Significantly different from rats trained with saline but tested after (+)-amphetamine; e = $P<0·01$.

received following nicotine-withdrawal ($F(2,16)=9·3$; $P<0·01$; Duncan's test; $P<0·05$), the number of shocks received by these rats being not significantly different from those received by rats trained and tested with nicotine.

Statistical analysis of the results of the experiment with (+)-amphetamine showed that the increase in lever-pressing responses evoked by administration of the drug was significant ($F(1,11)=86·6$; $P<0·001$) and that the decrease in the number of the shocks received was also significant ($F(1,11)=42·5$; $P<0·001$) (Fig. 3). These responses to (+)-amphetamine were influenced by the drug used during training (drug × training drug $F(1,11)=5·5$; $P<0·05$ for lever-presses: drug × training drug $F(1,11)=20·0$; $P<0·001$ for shocks). Further analysis showed that the rats trained and tested with (+)-amphetamine made more lever-pressing responses ($P<0·01$) and received fewer shocks ($P<0·01$) than the rats trained and tested with saline. The acute administration of (+)-amphetamine also increased the number of lever-pressing responses ($P<0·01$) and decreased the number of shocks ($P<0·05$) received by the rats trained with saline. However, rats trained and tested with (+)-amphetamine received significantly fewer shocks ($F(2,11)=21·5$; $P<0·001$) than the rats trained with saline but tested after an injection of (+)-amphetamine. The difference between the number of lever-presses made by rats trained and tested with (+)-amphetamine and those made by saline-trained rats treated acutely with drug also approached significance ($P=0·057$).

The administration of saline to rats trained with (+)-amphetamine reduced the number of lever-pressing responses ($F(2,11)=23·3$; $P<0·001$): Duncan's test; $P<0·01$) to a level which was not significantly different from that measured for rats trained and tested with saline (Fig. 3). The withdrawal of (+)-amphetamine also increased the number of shocks received by the rats ($F(2,11)=7·2$; $P<0·05$; Duncan's test; $P<0·05$) to a level which was significantly higher ($F(1,11)=8·5$; $P<0·05$) than that recorded for rats trained and tested with saline. The behavioural performance of (+)-amphetamine-trained rats treated acutely with nicotine was not significantly different from that measured for (+)-amphetamine-trained rats tested with saline.

Discussion

The results of the study have confirmed the previous report by Morrison (1974a) which showed that the withdrawal of nicotine from rats trained to perform an unsignalled shock avoidance task under the influence of the drug is associated with disruption of avoidance performance. The study has extended these observations in that it has shown that the withdrawal of another stimulant drug, (+)-amphetamine, from rats trained on the same schedule also causes significant disruption of avoidance performance and that the administration of (+)-amphetamine to nicotine-withdrawn rats attenuates the effects of withdrawal. These data, therefore,

appear consistent with the hypothesis that, in this schedule, rats can become dependent upon the stimulant properties of drugs administered during training. The acute administration of nicotine, however, did not attenuate the effects of (+)-amphetamine withdrawal. The reason for this remains to be established although it may be significant that Morrison (1974a) reported that the effects of nicotine in this behavioural paradigm are most pronounced during the early stages of the trial whereas the stimulant response to acute, but not chronic, nicotine could be expected to peak towards the end of the trial (Clarke & Kumar 1983).

In her paper, Morrison (1974a) reported that the acute administration of nicotine to rats trained on the schedule with saline did not cause disruption of the avoidance behaviour and argued that this excluded the possibility that the effects of nicotine-withdrawal reflected the development of state-dependent learning on nicotine. The results of the present study have confirmed those reported by Morrison in this respect and shown that acute injections of (+)-amphetamine to the saline-trained rats actually evoked a modest improvement of shock avoidance, again indicating that the disruption evoked by the withdrawal of (+)-amphetamine did not reflect state-dependent learning. Following both nicotine- and (+)-amphetamine-withdrawal, the increase in the number of shocks received was associated with a significant reduction in lever-pressing responses. In addition, the administration of (+)-amphetamine to rats trained with saline increased lever-pressing and decreased the number of shocks received. These data suggest that there could be a simple relationship between lever-pressing activity and the number of shocks the rats avoided. Some of the other results, however, suggest that the relationship between lever-pressing and shock avoidance is not a simple one. For instance, the numbers of shocks received following the withdrawal of both nicotine and (+)-amphetamine were higher than those recorded for rats trained and tested with saline, whereas the numbers of lever-pressing responses made by rats tested after an injection of saline were independent of the drug used during training. In contrast, the number of shocks received by rats tested after an injection of (+)-amphetamine were influenced by the drug used during training, whereas the number of lever-pressing responses was not. These results suggest that nicotine and (+)-amphetamine may have an effect on the "efficiency" of avoidance performance. Further studies are required to clarify this issue.

The neural mechanisms which mediate the effects of nicotine and (+)-amphetamine in this schedule must remain a matter of speculation. Nevertheless, reports from a number of laboratories suggest that the locomotor stimulant properties of both drugs are related to their ability to stimulate preferentially the mesolimbic DA system of the brain (Kelly et al 1975; Di Chiara & Imperato 1988; Clarke et al 1988). Studies in other laboratories have also shown that neuroleptics disrupt avoidance performance and that the free avoidance schedule used in this study is particularly sensitive to this effect of the drugs (Lehr 1980). Thus, the data appear consistent with the hypothesis that the rats may have become dependent upon the enhanced levels of DA secretion evoked by administration of the stimulant drugs during training and that the locomotor stimulant properties of these agents by themselves may not be responsible for their effects on shock avoidance behaviour in this paradigm. Clearly, further studies are needed to confirm the hypothesis.

Acknowledgement

The author is grateful to the Wellcome Trust for financial support for this project.

References

Balfour, D. J. K. (1984) The pharmacology of nicotine dependence: a working hypothesis. In: Balfour, D. J. K. (ed.) Nicotine and the Tobacco Smoking habit (International Encyclopedia of Pharmacology and Therapeutics—Section 114) Pergamon Press, New York pp 101–112

Balfour, D. J. K. (1990) Nicotine as the basis of the tobacco smoking habit. Pharmacol. Ther. In Press

Balfour, D. J. K., Graham, C. A., Vale, A. L. (1986) Studies on the possible role of brain 5-HT systems and adrenocortical activity in behavioural responses to nicotine in the elevated X-maze. Psychopharmacology 90: 528–532

Clarke, P. B. S. (1987) Nicotine and smoking: a perspective from animal studies. Ibid. 92: 135–143

Clarke, P. B. S., Kumar, R. (1983) Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br J. Pharmacol. 80: 587–594

Clarke, P. B. S., Fu, D. S., Jakubovic, A., Fibiger, H. C. (1988) Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. Pharmacol. Exp. Ther. 246: 701–708

Di Chiara, G., Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. 85: 5274–5278

Gilbert, D. G. (1979) Paradoxical tranquilizing and emotion-reducing effects of nicotine. Psychol. Bull. 86: 643–661

Hutchinson, R.R., Emley, G.S. (1985) Aversive stimulation produces nicotine ingestion in squirrel monkeys. Psychol. Rec. 35: 491–502

Kelly, P. H., Seviour, P. W., Iversen, S. D. (1975) Amphetamine and apomorphine response in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 96: 507–522

Lehr, E. (1980) Testing antipsychotic drug effects with operant behavioral techniques. In: Hoffmeister, F., Stille, G. (eds) Handbook of Experimental Pharmacology Vol 55 (part 1) Springer-Verlag Berlin. pp 90–95

Morrison, C. F. (1974a) Effects of nicotine and its withdrawal on the performance of rats on signalled and unsignalled avoidance schedules. Psychopharmacologia (Berl.) 38: 25–35

Morrison, C. F. (1974b) Effects of nicotine on the observed behaviour of rats during signalled and unsignalled avoidance experiments. Ibid. 38: 37–46

Morrison, C. F., Stephenson, J. A. (1972) Drug effects on unconditioned light-avoidance in the rat. Ibid. 24: 456–461

Vale, A.L., Graham, C. A., Balfour, D. J. K. (1988) Behavioural responses to nicotine in rats with selective lesions of the mesolimbic dopamine system. Psychopharmacology 96: 558