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Influence of novel and habituated testing conditions on cocaine sensitization

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Abstract

Female Swiss-Webster mice were treated daily for 10 days with cocaine (15 mg/kg i.p.) followed by 10 days with saline or ethanol (1.6 g/kg i.p.) or the reverse; following each injection in the experimental conditions locomotion was quantified in photocell cages. In animals given cocaine first, cocaine-induced locomotion was initially high and did not increase further with successive injections. In animals given prior saline or ethanol treatments, cocaine-induced locomotion was initially low but increased with successive cocaine treatments. There was no evidence of sensitization to the locomotor-stimulating effects of ethanol or of cross-sensitization between ethanol and cocaine. With respect to subsequent cocaine sensitization, the essential feature of prior saline or ethanol treatment appeared to be the handling and injection experience itself; a control group receiving prior saline injections in the home cage also showed a low level of cocaine-induced locomotion on the first day of cocaine testing but increasing locomotion with repeated cocaine testing. Thus, cocaine sensitization, rather than a progressive augmentation of motor function, may reflect a progressive reversal of the behavioral suppression caused by habituation to aspects of the testing situation or to some form of situational anxiety that precludes normal exploratory responses.

Keyword Cocaine; Sensitization; Reverse tolerance

1. Introduction

While there is tolerance to many of the effects of repeated drug treatments, the locomotor effects of the psychomotor stimulants often become progressively greater with repeated administration. Such progressive enhancement is known as psychomotor stimulant sensitization or 'reverse tolerance' (Babbini and Davis, 1972; Kilbey and Ellinwood, 1977; Segal and Mandell, 1974). Psychomotor stimulant sensitization is reflected in an increase in the locomotor responses and oral stereotypies associated with repeated administrations of such drugs as amphetamine and cocaine. Sensitization is progressive and relatively permanent (Robinson and Becker, 1986); moreover, sensitization to the locomotor-stimulating effects of one drug can be produced by experience with another drug – a phenomenon termed 'cross-sensitization' (DuMars et al., 1988; Stewart and Vezina, 1987; Vezina et al., 1989) – or even by repeated experience with stress (Antelman et al., 1980).

The degree of locomotor sensitization in response to repeated cocaine injections depends on the environmental context in which the injections are given. When cocaine injections are given in the same environment where the locomotor effects of cocaine are to be subsequently tested, maximal evidence of cocaine sensitization is seen; when cocaine injections are given repeatedly in the home cage they cause substantially less sensitization to cocaine subsequently given in distinct test chambers (Jackson and Nutt, 1993; Post et al., 1981; Weiss et al., 1989). Thus, a portion of the augmented locomotor response seen after repeated stimulant injections is due to a conditioned association – presumably involving Pavlovian conditioning (Tilson and Rech, 1973) – between the drug and the environment in which it is given (Stewart and Eikelboom, 1987).

The degree of sensitization seen with repeated cocaine treatment varies considerably from study to study. In some cases the incremental increases in locomotor scores continue to build over a period of weeks (e.g. Post and Rose, 1976), whereas in other cases asymptotic increases are

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seen within a few cocaine administrations (e.g. Borowsky and Kuhn, 1991). Differences in habituation to the testing situation may contribute to differences in apparent sensitization; whereas some experiments test animals in their 'home' cages (e.g. Segal and Kuczenski, 1992), most experimenters test animals in distinctive activity boxes, sometimes with a pre-exposure (habituation) period (e.g. Post and Rose, 1976) and sometimes without such pre-exposure (e.g. Hoffman and Wise, 1993). To the degree that Pavlovian conditioning contributes to psychomotor sensitization, pre-exposure to the test situation could be an important variable; pre-exposure to a neutral stimulus before it is paired with an unconditioned stimulus generally reduces the effectiveness of Pavlovian conditioning (Lubow, 1973; Weiner, 1990).

In the present experiment the degree of sensitization of the locomotor-stimulating effects of cocaine was compared across animals with varying habituation to the test environment. The observations were made as part of an experiment in which we assessed the possibility of cross-sensitization between the locomotor-stimulating effects of cocaine and those of ethanol.

2. Materials and methods

2.1. Design

This study was originally designed as a test of the hypothesis that prior experience with the locomotor-stimulating effects of ethanol would sensitize animals to the locomotor-stimulating effects of cocaine. Consequently, the primary experimental groups received a series of repeated ethanol or saline injections followed by a series of repeated cocaine injections; the intended 'control' groups received a series of repeated cocaine injections followed by a series of repeated ethanol or saline injections. Because of the unexpected findings, the initial experiment was fully replicated, with the addition of control groups that received habituation to the injection regimen or the locomotor-testing environment prior to repeated injections of cocaine.

2.2. Subjects

Seventy-eight female Swiss-Webster mice, weighing 21–28 g at the start of the experiment, were used. They were randomly assigned to 13 treatment groups. The six animals comprising each treatment group were housed together with free access to food and water. The animals were maintained according to the regulations of the Canadian Council on Animal Care.

2.3. Apparatus

Six 22-cm plastic cylinders served as locomotor test chambers. The cylinders were divided by two photocell beams, perpendicular to one another and 2 cm above the floor. Beam interruptions were recorded by a microprocessor.

2.4. Procedure

Testing was done in two phases: a sensitization phase and a cross-sensitization phase (a summary of the treatment conditions is given in Fig. 1). Each phase consisted of 10 consecutive days of locomotor testing following ethanol (1.6 g/kg i.p.), cocaine (15 mg/kg), saline, or no injection; with the exception of one control group all animals were given 1 week without treatment between the two phases of the experiment. The five primary treatment conditions were examined in two independent squads (replications) of five groups of six animals each.

Three additional control groups ($n = 6$ each) were tested. The first of these groups was habituated to saline injections in the home cage during the sensitization phase, given a 1-week break without treatment, and then tested with cocaine in phase II. The second group was habituated to saline injections in the test chamber during phase I, given a 1-week break without treatment, and tested with cocaine in phase II. The third group was habituated to saline injections in the test chamber during phase I and given cocaine in phase II without the normally intervening 1-week break.

In each of the activity tests the animals were placed in the text chamber immediately after injection and activity counts were taken at 30-s intervals for 10 min. The animals were then returned to their home cages and carried back to the animal colony where they were left until the subsequent day.

2.5. Drugs

95% ethanol was injected at 20% (v/v) concentration in saline. Cocaine hydrochloride was obtained from the National Institute on Drug Abuse and dissolved in saline at a concentration of 1.5 mg/ml. Cocaine dose was calculated as the salt.

3. Results

Ethanol produced reliable elevations in locomotion which were evident in both the initial experiment (Fig. 1, upper left: binomial sign test, $P < 0.001$) and in replication (Fig. 1, middle left: $P < 0.001$). Cocaine caused a much more dramatic elevation in each case. In the initial experiment the effects of cocaine were constant across days, but locomotion progressively decreased in both the ethanol and saline groups. This was reflected by analysis of variance in a treatments × days interaction ($F(36,216) = 2.54$, $P < 0.0001$). In the replication experiment there was again a treatments × days interaction ($F(36,225) =$

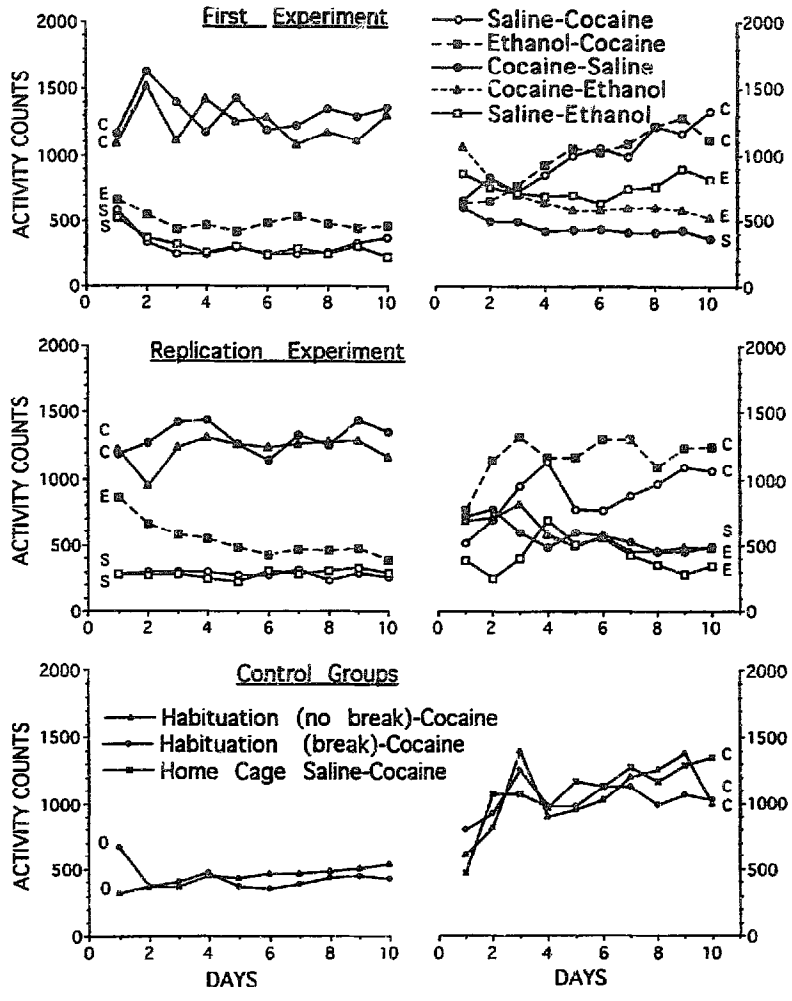

Fig. 1. Effects of daily drug and saline treatments on locomotor activity. Graphs on the left show data from the sensitization phase; graphs on the right show data from the same animals for the cross-sensitization phase. Treatments are indicated two ways: by the symbol and line combinations (the same in top and middle panels) and by the letters that identify cocaine (C), ethanol (E), saline (S), or no injection (O).

2.10, $P < 0.0005$); in this case the only progressive change was a decrease in locomotion in the ethanol condition.

On the first day of testing in the cross-sensitization phase, there were no significant differences between groups (Fig. 1, top and middle right). There were, however, significant treatment × days interactions, due to the fact that locomotion increased in the cocaine-treated groups but not the ethanol- or saline-treated groups. Locomotion was reliably higher in the two ethanol-treated groups ($P < 0.001$ in each case) than in the saline-treated group in the first experiment but this trend was not statistically significant in the replication.

In the three control groups – one habituated to saline injections in the home cage, one habituated to saline injections in the test chamber and tested with cocaine 1 week later, and one habituated to saline injections in the text box but tested with cocaine beginning the next day – low levels of locomotion were seen in response to the first cocaine injection, but progressively higher levels were seen in response to subsequent injections (Fig. 1, bottom right).

4. Discussion

Under the conditions of the present experiment, there was no evidence of psychomotor sensitization in response to repeated ethanol treatment (but see Crabbe et al., 1982; Cunningham et al., 1992; Cunningham and Noble, 1992; Masur and Dos Santos, 1988; Masur et al., 1986; Phillips et al., 1991). Indeed, if anything, there was a tendency towards tolerance in the replication experiment. The failure of sensitization might be a function of dose, number of ethanol exposures, or any of a number of other differences between this and other ethanol studies. Because there was no sensitization under our testing conditions, we cannot interpret the lack of cross-sensitization between ethanol and cocaine observed in the present experiment.

Despite the negative findings with respect to ethanol, two important observations about cocaine sensitization arise from the present experiments. First, cocaine sensitization was seen only in animals that were previously habituated to the handling and injection procedure that accompanied subsequent cocaine treatment. Second, in the cases where

progressive sensitization to the effects of cocaine was seen that progressive sensitization served to bring animals to the level of cocaine-induced locomotion that was seen on the first day with non-habituated animals; it did not elevate cocaine-induced locomotion to an abnormally high level. Thus, cocaine sensitization did not require the kind of cellular changes that are typically invoked (see e.g. Kalivas and Stewart, 1991; Robinson and Becker, 1986; White and Wolf, 1991) to explain psychomotor sensitization. These findings suggest that much of the unexplained variability in the strength of cocaine – and presumably other psychomotor stimulants – sensitization derives from the variety of habituation conditions associated with different experiments. The fact that low levels of activity were seen on the first day of cocaine treatment both in animals having 2 weeks of prior exposure to the test chamber and in animals having 2 weeks of saline injections in the home cage suggests that it is habituation to the presumed stress of handling and injection more than habituation to the environment itself, that is critical.

The most important suggestion from the present findings may be that psychomotor sensitization, rather than producing an abnormally elevated motoric sensitivity to cocaine and related compounds, merely reverses the inhibitory effects of environmental habituation or situational anxiety or both. That is to say, repeated treatment with psychomotor stimulants may not produce abnormal sensitivity to the drug. Rather, the drug may block or overcome processes that normally desensitize the animal to novel or otherwise arousing environmental stimuli, restoring sensitivity that would be typical of an inexperienced animal. This suggests, in effect, a new hypothesis of psychomotor stimulant action.

The psychomotor stimulants form a class of drugs that is difficult to define (Wise and Bozarth, 1987). They are traditionally distinguished from the 'central nervous system' stimulants, such as strychnine, picrotoxin, pentylenetetrazol, and the methylxanthines, but the nature of the distinction has not been prominently articulated. The term 'psychomotor' was coined a century ago to characterize movements elicited by electrical stimulation of the central nervous system, and it appears to have first been appended to 'stimulant' (Meier et al., 1954) with the marketing of methylphenidate, a drug used in attentional deficit disorder (Ayd, 1957). In humans the psychomotor stimulants are associated with superior performance on vigilance tasks (Hindmarch, 1980) while in animals the psychomotor stimulants are those that increase locomotion (Van Rossum et al., 1962) without causing convulsions.

The psychomotor stimulant actions of drugs involve increased locomotion at low doses and various forms of stereotyped behavior at high doses. The behaviors – both the locomotion and the more focal stereotypies – are repetitive, and give the appearance of being 'driven' motor automatisms. In the rat the dominant movements of psychomotor stereotypy are sniffing, licking, and chewing and associated repetitive head movements (Creese and Iversen, 1975; Randrup and Munkvad, 1967). In the cat head and eye movements predominate (Creese and Iversen, 1975; Stevens et al., 1977). Dogs treated with high doses of amphetamines locomote, perseverating in patterns of locomotion – such as the following of another specific animal – that were in progress at the time of onset of drug action (Ellinwood and Kilbey, 1975; Randrup and Munkvad, 1967). On the basis of observations involving several species, including humans (Randrup and Munkvad, 1967), it has been suggested that psychomotor activation represents 'an exaggeration and perseveration of fragments of species specific exploratory behaviors' (Stevens et al., 1977, p. 809). As a test of the hypothesis that amphetamine stereotypy represents drug-induced responsiveness to sensory stimuli rather than forced muscle movements, Stevens et al. (1977) have shown that blindfolding amphetamine-intoxicated cats eliminates the repetitive head and eye movements that are present when visual stimuli are available to the animals.

The traditional theory of psychomotor stimulant action is reflected in the term 'motor'. In perhaps the most explicit considered statement in this tradition, Lyon and Robbins (1975) have emphasized 'an increasing motor-stimulatory effect of the drug.' The alternative position, suggested by Stevens et al. (1977) is that amphetamine stereotypy is 'not due to activation of a pure motor automatism but represents release or facilitation of a centrally patterned exploratory program which is maintained by sensory feedback' (p. 809). It is this latter perspective (see also Wise and Bozarth, 1987) that fits best with the present data, since repeated stimulant treatment did not elevate locomotion to higher than normal levels but rather merely restored it to the normal levels that were seen in non-habituated animals. Thus, the present data encourage the view that psychomotor stimulants increase investigatory behavior (Pavlov, 1927; Sokolov, 1963) and that they do so by counteracting the consequences of habituation – and thus increasing the sensitivity of the animal to the response-eliciting stimuli in the environment – and also perhaps by counteracting situational anxiety, rather than by increasing the sensitivity of the animal to response-driving actions of the drug itself.

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