Enhancement of Amphetamine- and Cocaine-Induced Locomotor Activity after Chronic Ethanol Administration

S. J. MANLEY and H. J. LITTLE
Psychology Department, Durham University, Science Laboratories, South Road, Durham DH1 3LE, U.K. 1Department of Pharmacology, School of Medical Sciences, Bristol, BS8 1TD United Kingdom
Accepted for publication February 4, 1997

ABSTRACT
The effects of amphetamine and cocaine on locomotor activity in mice were studied after 3 weeks of chronic administration of ethanol by liquid diet. When testing was started 24 h after cessation of the ethanol treatment, no differences were seen on the first administration between the effects of the psychostimulants in controls and ethanol-treated animals, but after subsequent daily injections of amphetamine and cocaine, at doses that were insufficient to cause sensitization in controls, sensitization to both of these drugs was seen in ethanol-treated mice. When testing was started on the sixth day after cessation of the ethanol treatment, the effects of amphetamine on the first administration were significantly greater in ethanol-treated animals than in controls. After subsequent repeated daily injections, the locomotor stimulant effects of cocaine were greater in ethanol-treated mice than in controls. Administration of amphetamine for the first time 2 months after cessation of ethanol treatment also had a greater stimulant effect, compared with that in control animals. Two months after cessation of ethanol treatment, the first dose of cocaine caused a locomotor stimulation that was not seen in control animals, but sensitization was not seen after repeated cocaine administration in either group of animals. No differences in the effects of amphetamine or cocaine were seen after only 7 days of ethanol treatment. The results indicate that changes are still present in the CNS long after ethanol withdrawal hyperexcitability has subsided and that these changes result in increases in the effects of amphetamine and cocaine. Analysis of brain concentrations of the two psychostimulants suggested that metabolic changes were not responsible for the differing effects in control and ethanol-treated animals. It is possible that alterations in mesolimbic dopamine transmission are responsible for the effects of the ethanol treatment.

One of the main characteristics of dependence on many different types of drug is the tendency of addicts to relapse into drug taking long after they have recovered from the major withdrawal symptoms. This is characteristic of dependence on ethanol and is one of the main problems in the treatment of alcoholics. Much study has been devoted to alterations in neuronal function that take place during the phase of withdrawal hyperexcitability, but until recently, little attention has been paid to more prolonged changes that might be responsible for the tendency to relapse into excessive drinking. We are currently investigating the existence of functional changes that might persist for a long time after cessation of ethanol intake.

Recently evidence has been accumulating that there are commonalities in the mechanisms involved in dependence on drugs of different types. Compounds such as psychostimulants (amphetamine and cocaine), opiates, nicotine and ethanol exert their initial acute effects through different sites, but they cause similar changes in the mesolimbic dopamine pathway, acutely increasing transmission in the projection to the nucleus accumbens from dopaminergic neurons in the VTA (Di Chiara and Imperato, 1988). Evidence for alterations in transmission in this pathway during the withdrawal phase after repeated administration of these different types of drugs has been obtained from in vivo dialysis measurements of extracellular dopamine concentrations in the nucleus accumbens (Kalivas and Duffy, 1990; Akimoto et al., 1990; Rossetti et al., 1992; Benwell and Balfour, 1992), while neurochemical studies on the VTA and nucleus accumbens have suggested that there are common patterns in the effects of prolonged administration of the different types of drugs (Nestler et al., 1994; Ortiz et al., 1995).

Repeated administration of the psychostimulants amphetamine and cocaine causes sensitization to their locomotor stimulant actions (Segal and Mandell, 1974) and to the liability of rodents to self-administer these drugs (Horger et al., 1990; Wolverton et al., 1984). A similar prolonged sensitization has been seen with opiates (Babbini and Davis, 1972) and nicotine (Ksir et al., 1985). This sensitization lasts long after cessation of the drug treatment, which suggests that it may be involved in dependence on these drugs (Robinson and Berridge, 1993).
Cross-sensitization between different drugs has been demonstrated. Pretreatment with amphetamine increased the locomotor stimulant actions of cocaine (Kalivas and Weber, 1988), and a prolonged effect of amphetamine in increasing the acquisition of cocaine self-administration has been reported (Valadez and Shenk, 1994). More complex interactions have also been seen. Nicotine, for example, was not found to increase the locomotor stimulant actions of cocaine but did increase the predisposition of rats to self-administer this substance (Schenk et al., 1991; Horger et al., 1992). Amphetamine administered directly into the VTA caused sensitization to morphine given systemically; this effect was demonstrated not to involve conditioning effects (Vezina and Stewart, 1990). Although ethanol can cause sensitization, this effect is strain-dependent (Phillips et al., 1994). Concurrent administration of ethanol and cocaine increased the anxiogenic effects of cocaine after cessation of chronic treatment (Prather et al., 1991). There has been little investigation of the effects of prior administration of ethanol on sensitization to other drugs.

In the present study, we have investigated the effects of chronic ethanol treatment on the subsequent responsiveness to single and repeated administration of the psychostimulant drugs amphetamine and cocaine. The primary aim of this project was to determine whether, at comparatively long time intervals after cessation of the ethanol treatment, there was any evidence for changes in the effects of the psychostimulants that might indicate persistent neurochemical changes that could be involved in relapse. In addition, after the effects of the 3-week administration of ethanol had been discovered, we studied the effects of a shorter, 1-week treatment to see whether the same effects were produced.

Materials and Methods

Animals

Male TO mice (Bantin and Kingman, Hull, UK) were used in all studies. The weights ranged from 25 to 35 g, with no more than a 5-g range in any single experiment. Between tests the mice were housed seven per cage, at 21°C ± 1°C and 55% ± 10% relative humidity, with a 12-h light/dark cycle, the light phase being between 09:00 h and 21:00 h. They had free access to tap water and laboratory rodent chow (SDS, Edinburgh, Scotland). Fresh tap water was available throughout the liquid diet treatment.

Each treatment group contained seven mice. All drugs were given by the i.p. route, at a volume of 10 ml/kg.

Production of Physical Dependence on Ethanol

Three-week ethanol administration. Ethanol was administered in a liquid diet schedule (Dyets, Bethlehem, PA; Green et al., 1990). In the liquid diet administration schedule, all mice received a control liquid diet for an initial 3-day period to acclimatize them to the diet. Ethanol-treated mice then received a diet containing 3.5% ethanol for 2 days, followed by a diet containing 5% ethanol for 9 days and then a diet containing 8% ethanol for a further 9 days. Control groups were pair-fed a control diet balanced isocalorically to match the ethanol-containing diet (Green et al., 1990). There were no significant differences between the weights of the ethanol-treated and control mice at the end of the treatment periods.

The amount of ethanol drunk by the groups of mice was measured every 3 or 4 days during the treatment. The following are the means and S.E.M. values calculated for the amounts drunk by each cage of animals, expressed in g/kg/24 h (day 1 = first day of administration of liquid diet).

<table>
<thead>
<tr>
<th>Day</th>
<th>Amount drunk (g/kg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>24.6 ± 2.2</td>
</tr>
<tr>
<td>10</td>
<td>22.9 ± 1.8</td>
</tr>
<tr>
<td>13</td>
<td>23.2 ± 1.5</td>
</tr>
<tr>
<td>16</td>
<td>25.0 ± 1.0</td>
</tr>
<tr>
<td>20</td>
<td>31.0 ± 0.7</td>
</tr>
<tr>
<td>23</td>
<td>28 ± 0.3</td>
</tr>
</tbody>
</table>

The days after the end of the ethanol treatment have been numbered, with the first day (i.e., the day of withdrawal) as day 1. The effects of amphetamine and cocaine were measured, after the ethanol treatment, in separate groups of mice, beginning after either 24 h (on day 2) or 6 days (on day 7) from cessation of the ethanol treatment. The effects of amphetamine were also examined, in another separate group of animals, 2 months (on withdrawal day 61) after cessation of the ethanol treatment. The treatment schedule is illustrated in table 1.

The 3-week ethanol schedule produced clear withdrawal signs of hyperexcitability for several hours after cessation of ethanol administration, as evidenced by increased convulsive responses to handling, but these signs had all ceased by 24 h after the cessation of treatment.

Shorter ethanol administration. The effects of a shorter ethanol treatment were also studied. During this treatment the mice drank a total of 196 g/kg over 7 days. After the initial days of control diet, these animals were given a diet containing 3.5% ethanol for 2 days, followed by a diet containing 7% ethanol for 5 days. We then examined the effects of amphetamine and cocaine, using the times at which the maximal changes were seen after the longer ethanol treatment, because these results were available when this section of the study was started. The amphetamine administration was therefore begun after 6 days (withdrawal day 7), and cocaine treatment after 24 h (withdrawal day 2), from the cessation of the ethanol treatment phase.

Repeated Administration of the Psychostimulants

The following treatment schedules were used to measure the effects of amphetamine and cocaine on locomotor activity and to produce sensitization to these effects.

TABLE 1

<table>
<thead>
<tr>
<th>Schedule of drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of ethanol treatment</td>
</tr>
<tr>
<td>Interval after end of ethanol treatment</td>
</tr>
<tr>
<td>First psychostimulant administration and locomotor activity measurements</td>
</tr>
<tr>
<td>First sensitization regime (daily injections of psychostimulant)</td>
</tr>
<tr>
<td>Second locomotor activity measurements</td>
</tr>
<tr>
<td>Second sensitization regime (daily injections of psychostimulant)</td>
</tr>
<tr>
<td>Third locomotor activity measurements</td>
</tr>
</tbody>
</table>

Day numbers refer to days after withdrawal from the chronic ethanol treatment, day 1 being the day the ethanol diet was removed. For the experiments involving 3 weeks of ethanol administration, the times refer to studies on both amphetamine and cocaine.
1) First administration of drugs, measurements of effect on locomotor activity, on either day 2, day 7 or day 61 after the end of the ethanol treatment.

2) Injections of amphetamine, cocaine or saline, once daily for 8 days, beginning 24 h after the first locomotor measurements, from withdrawal day 3 to withdrawal day 10 (when tests started on withdrawal day 2), from withdrawal day 7 to withdrawal day 15 (when tests started on withdrawal day 7) or from withdrawal day 62 to withdrawal day 69 (when tests started on withdrawal day 61).

3) Test of effects of drugs on locomotor activity, 24 h after last injections (first sensitization test), on withdrawal day 10 (when tests started on withdrawal day 2), on withdrawal day 15 (when tests started on withdrawal day 7) or on withdrawal day 69 (when tests started on withdrawal day 61).

4) Injections of amphetamine, cocaine or saline, once daily for 6 days, beginning 24 h after the second locomotor measurements, from withdrawal day 11 to withdrawal day 16 (when tests started on withdrawal day 2), from withdrawal day 16 to withdrawal day 21 (when tests started on withdrawal day 7) or from withdrawal day 70 to withdrawal day 75 (when tests started on withdrawal day 61).

5) Test of effects of drugs on locomotor activity, 24 h after last injections (second sensitization test), on withdrawal day 18 (when tests started on withdrawal day 2), on withdrawal day 21 (when tests started on withdrawal day 7) or on withdrawal day 76 (when tests started on withdrawal day 61).

A dose of 3 mg/kg amphetamine was used for both the tests and the repeated injections. A dose of 20 mg/kg cocaine was used for all the repeated injections, and 10 mg/kg was used for the tests. (We had originally intended to use a higher dose of amphetamine, 5 mg/kg, for the repeated injections, but preliminary experiments showed this to be too toxic).

Locomotor Activity

Spontaneous locomotor activity was measured using Opta-Varimex-Mini activity meters operated by the interruption of 15 infrared beams. A clear perspex cage (50 × 32 × 15 cm) containing a small amount of sawdust was placed between a metal frame containing the infrared emitters and sensors placed 1 inch apart. Prior to the test period, the animals were kept in their home cages, each of which contained seven animals. The locomotor activity measurements were all made between 9 A.M. and 2 P.M., and the studies on the different contained seven animals. The locomotor activity measurements were grouped together were obtained from experiments carried out concurrently, and spaces left between the columns indicate results that were obtained on different occasions.

Results

Tests started 24 h after cessation of ethanol treatment (day 2). When the effects of amphetamine and cocaine were tested with the first administration on withdrawal day 2, 24 h after cessation of the ethanol administration, there were no significant differences between the effects of amphetamine, cocaine and saline in animals that had been given the control diet compared with the animals that received the ethanol treatment (fig. 1A). Significant increases in the ambulant locomotor activity (P < .001) were seen at this time, when the effects of amphetamine and cocaine were compared with the effects of saline administration, except when cocaine was given after the ethanol treatment; when although the

Treatment of Animals for Measurements of Brain Concentrations of Amphetamine and Cocaine

Mice were administered ethanol by the long-term (3-week) liquid diet schedule, and controls were pair-fed control liquid diet. Beginning either on withdrawal day 6 (for amphetamine measurements) or on withdrawal day 2 (for cocaine measurements) after cessation of ethanol treatment, animals received 16 daily injections of 3 mg/kg amphetamine or 10 mg/kg cocaine. They were then left for 26 days, until withdrawal day 43 (for cocaine) or withdrawal day 48 (for amphetamine), with standard laboratory chow and water and no drug treatment. After this time interval, animals were administered 3 mg/kg amphetamine or 20 mg/kg cocaine, and the locomotor activity was measured between 20 and 30 min after the injections. The whole brains were then removed 30 min after drug administration.

Measurements of Amphetamine and Cocaine Concentrations

The brains were weighed and homogenized in 3 ml 0.1 M sulphuric acid, and 0.5 ml of the homogenate or standard solution was added to 50 μl aqueous (1 mg/ml) internal standard solution (mephentermine for the amphetamine assay and amylocaine and iprindole for the cocaine assay). Then 100 μl of 4 M ammonium hydroxide and 50 μl of n-butyl acetate were added, and the samples were mixed and centrifuged at 9500 × g for 3 min. Next, 2 μl of the upper organic layer was injected onto the chromatography column. Gas chromatography was performed on a Hewlett-Packard 5890 gas chromatograph in splitless injection mode with nitrogen phosphate detection. Calibration graphs were prepared for amphetamine, cocaine and methylecgonine. Norcaine was quantified against the cocaine standards.

Drugs

Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) and cocaine hydrochloride (BDH) were dissolved in saline (Baxter Healthcare, Norfolk, OK). The doses refer to the salt weights of the drugs.

Statistical Analysis

Statistical comparisons were made by two-way analysis of variance, followed by the Scheffe’s F test for comparison between samples. P = .05 was taken as the level of significance. The withdrawal hypere excitability scores were analyzed by a nonparametric two-way analysis of variance designed for repeated measures on the same animals (Meddis, 1984). Throughout the figures, the sets of results grouped together were obtained from experiments carried out concurrently, and spaces left between the columns indicate results that were obtained on different occasions.
mean value was increased, the difference was not significant (P < .1).

However, when the effects of amphetamine and cocaine were compared on withdrawal day 10, after the first set of repeated injections, significant sensitization was seen to both amphetamine and cocaine in the animals that had previously been given ethanol, but not in those that received the control diet (fig. 1B). When we compared the locomotor activity measurements after either amphetamine or cocaine at the first administration (withdrawal day 2) and after the first set of repeated injections (withdrawal day 10), the values were significantly different (P < .01) for ethanol-treated animals, but not for controls (P > .1). The difference between the effects of cocaine on withdrawal day 10 in those animals given control diet and those given ethanol was significant for cocaine (P < .01), but the corresponding difference for amphetamine was not significant (P > .1). The locomotor activity after saline injections on day 10 was not significantly different in controls and ethanol-treated mice (P > .05).

On withdrawal day 18, after the second set of repeated injections (after a total of 16 daily injections, including those on withdrawal days 2 and 11 after the liquid diet treatment), there was no difference between the effects of amphetamine in mice that were given ethanol and those that were given control diet (fig. 1C). The effects of cocaine, however, were again significantly greater in ethanol-treated animals than in controls (P < .01). In control animals, the effects of cocaine were in fact significantly lower on withdrawal day 18 than the effects on the first administration (P < .01). The effects of saline injections did not differ significantly between controls and ethanol-treated animals on withdrawal day 18 (P > .1).

Tests started on day 7 after cessation of ethanol treatment. A different pattern was seen when the effects of amphetamine and cocaine were tested for the first time 6 days (withdrawal day 7) after the cessation of liquid diet treatment (fig. 2), in separate groups of mice from those in the study described above. In this case, the first time that amphetamine was administered after the liquid diet treatment, its effects were considerably greater in mice that had received ethanol than in controls (P < .01). No differences were seen in the effects of cocaine on day 7. A significant increase in locomotor activity was seen on day 7 in all groups receiving either amphetamine or cocaine, when comparison was made with the effects of saline (P < .01 for the groups given ethanol treatment; P < .05 for controls).

On withdrawal day 15 (fig. 2B), after the first set of repeated injections (10 daily injections, including those on withdrawal day 7), the effects of amphetamine were again significantly greater in ethanol-treated animals than in controls (P < .001), whereas the effect of cocaine or saline did not differ between these mouse group.

When amphetamine and cocaine were tested on withdrawal day 21, after the second phase of repeated administration (i.e., after a total of 16 daily repeated injections of the psychostimulants, including those on withdrawal days 7 and

\[ \text{A} \quad \text{B} \quad \text{C} \]

Fig. 1. The effects of amphetamine or cocaine on ambulant locomotor activity when these drugs were given starting on withdrawal day 2, 24 h after withdrawal from the ethanol treatment. Figure 1A shows the effects of the first administration of the psychostimulants. There were no significant differences between the effects of drugs administered to ethanol-treated animals and the effects of drugs administered to controls that were not given the ethanol treatment (P > .1). Figure 1B shows the effects of amphetamine and cocaine given to the same sets of animals on withdrawal day 10, after 10 days of repeated injections (including those on withdrawal day 2). A significant increase was seen in the effects of amphetamine in ethanol-treated animals compared with that on the first administration on withdrawal day 2. Figure 1C shows the effects of withdrawal day 18, after the second phase of repeated injections (a total of 16 daily repeated injections of the psychostimulants, including those on withdrawal days 2 and 10), again in the same groups of mice. The effects of cocaine on withdrawal day 17 were significantly greater in animals that had been given ethanol than in controls. Key: Columns without patterning indicate results after saline injections: white columns = control diet, and black columns = ethanol diet. Stippled columns indicate results after amphetamine injections, and striped columns indicate results after cocaine injections. In both cases, the lighter columns illustrate the results after control diet, and the darker columns illustrate the results obtained after ethanol treatment. A = significantly different from the effects of saline administration on the same day and after the same liquid diet treatment. B = significantly different from the effects of the drugs on the first administration, 24 h after withdrawal. C = locomotor activity significantly different in ethanol-treated animals than in controls.
15), however, a difference was seen in the effects of cocaine; the locomotor activity after cocaine administration was significantly higher (P < .05) in ethanol-treated animals than in controls (fig. 2C). On withdrawal day 21, the greater effect of amphetamine in ethanol-treated animals was again seen (P < .01), and there was again no difference between the effects of saline in controls and ethanol-treated mice (P > .1). Although the mean values for activity in the control mice after cocaine administration on days 15 and 21 were lower than those after the first administration, the differences were not significant (P > .05).

**Tests started 2 months after cessation of ethanol treatment.** In separate groups of mice from those used in the studies described above, the effects of amphetamine and cocaine were tested for the first time 2 months after cessation of the liquid diet treatment (fig. 3). Amphetamine caused a significant increase in locomotor activity in the group of mice previously given ethanol, compared with the locomotor activity after saline administration to ethanol-treated mice (P < .01), but no significant increase was seen in animals previously given the control liquid diet (fig. 3A). The difference in the activities between ethanol-treated animals and controls, each given amphetamine, failed to reach significance (P > .05). However, when the tests were carried out on withdrawal day 69, after the first set of repeated injections (fig. 3B), the effects of amphetamine were significantly greater in ethanol-treated mice than in controls (P < .001), and the difference between the activity after amphetamine and that after saline administration was significant (P < .001) for ethanol-treated animals but not for controls (P > .05). There was no difference on day 69 in between the activity of controls and that of ethanol-treated mice given saline (P > .1). When the activity was measured on withdrawal day 75 (fig. 3C), after the second sensitization phase (a total of 16 daily injections, including those on withdrawal days 61 and 69), the difference between the effects of amphetamine in controls and ethanol-treated mice was no longer significant (P > .1). On this test day, the locomotor activity after amphetamine was significantly greater in both controls and ethanol-treated animals, compared with the activity after saline injections (P > .1).

When given for the first time 2 months after the end of the ethanol treatment, cocaine caused a significant increase in locomotor activity in mice that had previously received ethanol, compared with the locomotor activity after saline administration (P < .05), but no significant increase was seen in animals previously given control liquid diet (fig. 3A). (Note that these studies were not carried out concurrently with the corresponding amphetamine experiments, so another set of controls was required). After the first set of repeated injections (fig. 3B), on day 69, a similar pattern was observed, with an increase in locomotor activity in animals previously treated with ethanol (P < .05) and no increase in animals previously treated with control diet. After the second sensitization phase (fig. 3B), however, the cocaine caused a significant increase in locomotor activity in control animals when compared with the activity of control animals after saline administration (P < .05). This effect was not significant in ethanol-treated animals, but the mean values after cocaine administration in ethanol-treated animals and controls were similar, the lack of significance of the effect of cocaine in ethanol-treated animals apparently being due to the higher
of cocaine were not significantly different in animals that had been on withdrawal days 61 and 69. The effects of amphetamine and total of 16 daily repeated injections of the psychostimulants, including withdrawal day 75, after the second phase of repeated injections (a required). Figure 3A shows the effects of the first administration. The locomotor activity after either amphetamine or cocaine administration required). Figure 3A shows the effects of the first administration, 2 months (with- out patterning) or second sensitization phase, on withdrawal day 61). The effects of amphetamine were greater in etha- nol-treated animals than in controls (P < .01). Figure 3A shows the effects of amphetamine or cocaine given on withdrawal day 61) after withdrawal from the ethanol treatment (these results of the handling score measurements on withdrawal day 1 are shown in table 2. The results for the two ethanol treatment schedules were obtained during the withdrawal phase, before the study of the effects of amphetamine or cocaine. The severity of the withdrawal hyperexcitability was significantly greater in the mice that received the longer ethanol treatment (P < .01), when comparison was made over the whole of the measurement period by two-way analysis of variance.

Brain amphetamine and cocaine levels. The concentra- tions of amphetamine, cocaine and cocaine metabolites are given in table 3. The locomotor activity measurements on withdrawal day 48 (for amphetamine) or withdrawal day 43 (for cocaine) for the animals from which the brains were taken for the concentration measurements are illustrated in figure 4C. Both amphetamine and cocaine had a significantly greater effect in the locomotor tests in animals that had previously had ethanol, compared with animals that were given the control diet (fig. 4C). However, it can be seen from table 3 that the control animals had significantly higher levels of amphetamine in the brain than the ethanol-treated animals (P < .01). The levels of cocaine and norcaine did not differ significantly between control and ethanol-treated animals, but those that had previously been fed the ethanol diet had significantly lower brain levels of methylecgonine than control animal (P < .05).

Discussion

The results show that 3 weeks of ethanol administration in mice can cause changes in the CNS that far outlast the phase of withdrawal hyperexcitability, as evidenced by changes in the actions of amphetamine and cocaine. The changes were given ethanol, compared with controls; cocaine caused a significant increase in control animals, compared with the effects of saline, tested concurrently. Key: Columns without patterning indicate results after saline injections: white columns = control diet; black columns = etha- nol diet. Stippled columns indicate results after amphetamine injec- tions, and striped columns indicate results after cocaine injections; in both cases, the lighter columns illustrate the results after control diet, and the darker columns illustrate the results obtained after ethanol treatment. A = significantly different from the effects of saline admin- istration on the same day and after the same liquid diet treatment. B = significantly different from the effects on the first administration, 2 months after withdrawal. C = effects significantly different in ethanol- treated animals than in controls.
always in the same direction: an increase in the locomotor stimulant actions of these compounds, seen either after the first administration or after repeated injections of the psychostimulant drugs. The results suggest that prolonged ethanol intake may increase the effects of amphetamine and cocaine in humans and may possibly increase the dependence liability of these drugs. A more important implication of these results is that they could be used as a model for the investigation of neuronal changes that might be responsible for the pattern of frequent relapse into drinking that is common in alcoholics who have gone through withdrawal.

The changes in the effects of amphetamine and cocaine were not seen when only 7 days of ethanol treatment were given. The severity of the withdrawal syndrome after the 3-week treatment was significantly greater than that after the 7-day treatment, so it is possible that the difference in the effects of amphetamine and cocaine after the two treatment schedules were due to differences in withdrawal severity. However, the withdrawal from the shorter ethanol schedule was very pronounced, the handling scores being consider-

\[ \text{Ethanol Concentration} = \frac{\text{Ethanol Intake}}{\text{Volume}} \]

\[ \text{Blood Alcohol Concentration} = \frac{\text{Ethanol Intake}}{\text{Weight}} \]

\[ \text{Brain Concentration} = \frac{\text{Ethanol Intake}}{\text{Brain Weight}} \]

\[ \text{Metabolite Concentration} = \frac{\text{Ethanol Metabolites}}{\text{Brain Weight}} \]

\[ \text{Psychostimulant Concentration} = \frac{\text{Psychostimulant Intake}}{\text{Brain Weight}} \]

\[ \text{Withdrawal Score} = \frac{\text{Handling-induced Hyperexcitability}}{\text{Time After Withdrawal}} \]

\[ \text{Relapse Probability} = \frac{\text{Number of Relapses}}{\text{Number of Subjects}} \]

\[ \text{Dependence Liability} = \frac{\text{Psychostimulant Intake}}{\text{Ethanol Intake}} \]

\[ \text{Therapeutic Index} = \frac{\text{Psychostimulant Efficacy}}{\text{Ethanol Toxicity}} \]

\[ \text{Sensitization Schedule} = \frac{\text{Number of Sensitization Days}}{\text{Wait Days}} \]

\[ \text{Behavioral Endpoint} = \frac{\text{Locomotor Activity}}{\text{Time After Sensitization}} \]

\[ \text{Blood Levels} = \frac{\text{Ethanol Metabolites}}{\text{Blood Volume}} \]

\[ \text{Brain Levels} = \frac{\text{Psychostimulant Metabolites}}{\text{Brain Volume}} \]

\[ \text{Withdrawal Syndrome} = \frac{\text{Handling-induced Hyperexcitability}}{\text{Time After Withdrawal}} \]

\[ \text{Relapse Behavior} = \frac{\text{Behavioral Score}}{\text{Time After Relapse}} \]

\[ \text{Dependence Index} = \frac{\text{Psychostimulant Intake}}{\text{Ethanol Intake}} \]

\[ \text{Therapeutic Ratio} = \frac{\text{Psychostimulant Efficacy}}{\text{Ethanol Toxicity}} \]

\[ \text{Sensitization Speed} = \frac{\text{Number of Sensitization Days}}{\text{Wait Days}} \]

\[ \text{Behavioral Observation} = \frac{\text{Locomotor Activity}}{\text{Time After Sensitization}} \]

\[ \text{Blood Measurement} = \frac{\text{Ethanol Metabolites}}{\text{Blood Volume}} \]

\[ \text{Brain Measurement} = \frac{\text{Psychostimulant Metabolites}}{\text{Brain Volume}} \]

\[ \text{Withdrawal Measurement} = \frac{\text{Handling-induced Hyperexcitability}}{\text{Time After Withdrawal}} \]

\[ \text{Relapse Measurement} = \frac{\text{Behavioral Score}}{\text{Time After Relapse}} \]

\[ \text{Dependence Measurement} = \frac{\text{Psychostimulant Intake}}{\text{Ethanol Intake}} \]

\[ \text{Therapeutic Measurement} = \frac{\text{Psychostimulant Efficacy}}{\text{Ethanol Toxicity}} \]

\[ \text{Sensitization Measurement} = \frac{\text{Number of Sensitization Days}}{\text{Wait Days}} \]

\[ \text{Behavioral Observation} = \frac{\text{Locomotor Activity}}{\text{Time After Sensitization}} \]

\[ \text{Blood Measurement} = \frac{\text{Ethanol Metabolites}}{\text{Blood Volume}} \]

\[ \text{Brain Measurement} = \frac{\text{Psychostimulant Metabolites}}{\text{Brain Volume}} \]

\[ \text{Withdrawal Measurement} = \frac{\text{Handling-induced Hyperexcitability}}{\text{Time After Withdrawal}} \]

\[ \text{Relapse Measurement} = \frac{\text{Behavioral Score}}{\text{Time After Relapse}} \]

\[ \text{Dependence Measurement} = \frac{\text{Psychostimulant Intake}}{\text{Ethanol Intake}} \]

\[ \text{Therapeutic Measurement} = \frac{\text{Psychostimulant Efficacy}}{\text{Ethanol Toxicity}} \]

\[ \text{Sensitization Measurement} = \frac{\text{Number of Sensitization Days}}{\text{Wait Days}} \]
ably, and significantly, higher than control values. We have previously demonstrated marked changes in the effects of convulsant drugs and hyperexcitability to audiogenic stimuli, as well as in the convulsive responses to gentle handling during the withdrawal syndrome after the shorter ethanol treatment (Watson and Little, 1995). We have also investigated the effects of intermittent alcohol treatment on the locomotor stimulant actions of amphetamine and cocaine, but no changes were seen in these studies (Manley and Little, 1995).

Various forms of stress have been shown to cause sensitization to the effect of amphetamine and cocaine (Antelman et al., 1980; Maccari et al., 1991; DeRoche et al., 1992; DeRoche et al., 1993). It is quite likely that the stress of the ethanol treatment and withdrawal contributed to the increases in the effects of cocaine and amphetamine. However, the lack of effect of the shorter ethanol treatment on the actions of amphetamine and cocaine suggests that the changes seen after the longer ethanol administration are unlikely to have been due entirely to stress. It is likely that greater, or different, neurochemical changes were produced by the longer ethanol treatment than by the shorter administration, which may have involved the dopamine pathways on which both amphetamine and cocaine are known to act. Sensitization to the effects of psychostimulant drugs due either to repeated administration or to stress has been linked to changes in the mesolimbic pathway arising from the VTA (Kalivas and Stewart, 1991; Kalivas and Duffy, 1993a,b; Vezina and Stewart, 1990).

When it was given for the first time 24 h after cessation of the ethanol treatment, the effects of amphetamine were unchanged, but they were increased when tested for the first time either 6 days or 6 months after the end of the ethanol administration. These results may reflect a series of alterations in neuronal function after the acute withdrawal hyperexcitability subsided. These results show some similarity to the progressive alterations seen over several days after withdrawal from chronic administration of amphetamine. Wolf et al. (1993) demonstrated that 3 to 4 days after cessation of repeated amphetamine injections, dopamine autoreceptor sensitivity was decreased in the VTA, with no change in extracellular dopamine concentrations in the nucleus accumbens after amphetamine administration. By 10 to 14 days after cessation of the treatment, the autoreceptor subsensitivity had disappeared, but the amphetamine-stimulated dopamine levels were increased.

The present results demonstrated increased effects of cocaine in ethanol-treated animals when this drug was given repeatedly, but not on the first administration, 24 h or 6 days after cessation of the ethanol treatment. This was in contrast to the increases in the effects of amphetamine when given for the first time. This different pattern of changes with amphetamine and cocaine may have been due to the doses used, because the dose of amphetamine was slightly higher on the dose-response curve for increases in locomotor activity than the dose of cocaine, or it may reflect differences in the mechanisms of action of the two psychostimulants. Full dose-response curves to the psychostimulants could not be established after the ethanol treatments because of the extremely large numbers of chronically treated animals this would have required. In control animals, some tolerance appeared to occur to the stimulant effects of cocaine after repeated ad-

ministration, whereas the opposite pattern was seen after the ethanol treatment. Tolerance to the reinforcing effects of cocaine (Katz et al., 1993; Li et al., 1994), to its effects on dopamine uptake (Izenwasser and Cox, 1992) and to its cardiovascular actions (Johansson et al., 1992) has been reported. Whether tolerance or sensitization is seen may be determined by the dose used and the treatment regime, but results have not been entirely consistent, and the mechanisms of the changes are not fully understood. When cocaine was given 2 months after cessation of ethanol treatment in the present study, an increase in activity was seen in ethanol-treated animals, but not in controls. This was evident on first administration of cocaine and after the first, but not after the second, set of repeated injections; significant tolerance was not seen in these results. Greater effects of cocaine were also seen in ethanol-treated animals when tests were made 26 days after the second set of repeated injections (fig. 4C).

The changes in the measured effects of cocaine and amphetamine on locomotor activity were unlikely to be influenced by the development of stereotyped behavior. Throughout all the studies, the mice were observed very carefully before, after and during the measurements of locomotor activity. No stereotyped behavior was seen at any of the doses used of either cocaine or amphetamine, either on first administration or after repeated injections. Prior studies in control animals showed that stereotypy was seen in this TO strain of mice at doses of 10 mg/kg and above of amphetamine and at doses of 50 mg/kg and above of cocaine. No other abnormal behavior was seen in the animals, and they appeared quite normal on each occasion before the administration of the psychostimulants. No differences in body weight were seen between the treatment groups at any time.

In rats, sensitization can be associated with changes in the timing of stereotypy and locomotor stimulant phases (Leith and Kuczynski, 1982). However, experiments carried out in parallel that examined the time course of activity in amphetamine-sensitized animals, over a 1-h period after amphetamine administration, revealed that in ethanol-treated animals the time of maximal effect of the psychostimulant was between 20 and 30 min after administration (results not illustrated), the same time at which maximal effects had been seen in the preliminary studies in naive animals.

The similar effects of psychostimulants and ethanol on dopamine release in the mesolimbic system were in the Introduction. Results from dopamine receptor binding studies after prolonged ethanol administration have not been consistent, several groups finding no changes (e.g., Hietala et al., 1990), but the great majority of these studies investigated changes soon after ethanol withdrawal. One study, however (May, 1992), demonstrated an increase in the affinity of the high-affinity state of the D1 receptor in striatal membranes 7 months after the cessation of ethanol treatment. Studies of the effects of dopamine agonists after chronic ethanol administration have reported both decreases (Tabakoff et al., 1978) and increases (Lai et al., 1980) in behavioral responses, but again these studies were made within a short time of cessation of ethanol treatment. Fahlke et al. (1995) reported that amphetamine had a greater locomotor stimulant effect in rats with a high ethanol intake than in those with a low intake, when tested 3 weeks after the cessation of ethanol drinking. Repeated amphetamine administration increased...
ethanol intake in rats (Fahlke et al., 1994). Experiments are under way in our laboratory to determine whether the changes in the effects of amphetamine and cocaine seen in the present study were due to alterations in the terminal areas, such as the nucleus accumbens, causing changes in dopamine release or to alterations in the firing of the neurons in the VTA.

Analysis of the brain psychostimulant concentrations revealed lower amphetamine levels in the brains of animals previously treated with ethanol, but behaviorally, these animals were more sensitive to the locomotor effects of amphetamine. Although cocaine also had a greater effect on animals previously treated with ethanol than on control animals, the brain concentrations did not differ significantly between the two treatment groups, although levels of methylecgonine were decreased in ethanol-treated animals. It is unlikely that the latter difference was involved in the differences between control and ethanol-treated animals, as methylecgonine has been reported to decrease the effects of cocaine (Schuelke et al., 1996). It appears, therefore, that although the ethanol treatment did cause some long-term changes in the pharmacokinetics of the psychostimulants, such changes do not account for the behavioral changes observed in these experiments.

In conclusion, the results demonstrate that chronic ethanol treatment can cause increases in the effects of amphetamine and cocaine that last considerably longer than the withdrawal hyperexcitability. The appearance of these changes was dependent on the duration and pattern of ethanol intake. These results are of relevance to the problem of relapse and the development of tolerance to the psychostimulants. The evidence indicates that the ethanol withdrawal syndrome may contribute to the pathophysiology of drug dependence and relapse.


Send reprint requests to: H. J. Little, Psychology Department, Durham University, Science Laboratories, South Road, Durham DH1 3EL, United Kingdom.