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Behavioral sensitization is greater after repeated versus single chronic cocaine dosing regimens

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Abstract

Cocaine dosing regimens in animals are used to model behavioral and neurochemical changes in human cocaine abusers. Typically, rats are dosed for 5–14 days and assessed at some point during withdrawal. However, human cocaine bingers undergo multiple periods of several days of abuse. Here, we model the human binge pattern by giving rats two separate cocaine dosing regimens which results in greater behavioral sensitization than a single cocaine dosing regimen. This model also allows for the testing of drugs in reversal of a previously established sensitization. Multiple cocaine regimens may thus provide a better model for the human condition.

Keywords: Cocaine; Sensitization; Dosing regimen; Locomotion

1. Introduction

When modeling chronic cocaine abuse the drug can be given either non-contingently (e.g. experimenter delivered s.c. injections) or contingently (e.g. intravenous self-administration). There are differences in the outcome of these two models but the long-term effects, which may be important in craving and relapse, tend to be similar (Kalivas et al., 1998). In non-contingent models, rats are typically given a single cocaine dosing regimen involving s.c. injections for 5, 7 or 14 days (White et al., 1998; Kalivas and Duffy, 1998; King et al., 2000; respectively). Behavioral sensitization and neurochemical or electrophysiological changes are then examined at some point after withdrawal. Given that human cocaine bingers undergo repeated multi-day binges (Gawin and Ellinwood, 1988), a more approximate animal model would reflect the repeated chronic regimens and withdrawal consolidation phases. This would be especially true if one wanted to examine the effects of potential pharmacotherapies in *reversing* previously established cocaine sensitization rather than just inhibiting the induction or expression (i.e. the current episode) of sensitization. The ability to reverse previously established cocaine sensitization would provide a more realistic treatment paradigm. Here, we examine the differences in behavioral outcome between single and repeated cocaine dosing regimens in rats.

2. Materials and methods

Male Sprague–Dawley rats ($n=40$) weighing ~200 g at the start of dosing were kept two per cage on a 12:12 h light dark cycle (lights on at 7 a.m.) with water and rat chow available ad libitum. Animals were treated in accordance with the Guide for Care and Use of Laboratory Animals (NIH). After 7 days acclimatization to the vivarium, the rats were injected with 7.5 mg/kg i.p. cocaine and their ambulatory activity monitored for 60 min in Opto-Varimex 'minor' activity monitoring boxes as previously described (King et al., 2000). For the next 6 days, each animal received a 40 mg/kg s.c. cocaine injection in the home cage. All animals were then withdrawn for 7 days. This procedure was then repeated: a 7.5 mg/kg i.p. cocaine challenge injection with 60 min ambulatory monitoring followed by 6 days of 40 mg/kg s.c. cocaine. The animals were then withdrawn for 9 days after the second cocaine dosing regimen. On the 10th day of withdrawal, the animals were again challenged with 7.5 mg/kg i.p. cocaine. Ambulatory activity was monitored by IBM-compatible personal computer while a behavioral rating was given at 5-min intervals with 20 s observations for each rating as previously described (Ellinwood and

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Balster, 1974). Behavioral rating data were converted into 'percentage of time in behavior', i.e. the incidence of that particular behavior is given (Fig. 2A). This data were further divided (Fig. 2B) into (1) inactive behaviors (asleep, almost asleep and inactive (standing, crouching, etc. but not moving)); (2) active behaviors (normal active movement/exploration) and grooming behavior and (3) hyperactive behaviors (hyperactivity, patterned movements and stereotypy). Data were analyzed by one- or two-way analysis of variance (ANOVA) where appropriate and values are expressed as mean ± S.E.M. Only half of the animals were tested on the last day, the remainder were given drug treatments and these data are not reported here.

3. Results

3.1. Ambulatory activity

There was a significant effect of time after cocaine challenge, i.e. the cocaine challenge caused an increase in ambulations (Fig. 1A; $F(11, 1103) = 15.284$; $P < 0.001$). There was also an effect of cocaine pretreatment ($F(2, 1103) = 46.512$; $P < 0.001$), the first 7-day cocaine dosing regimen produced greater ambulatory activity versus naive rats ($P < 0.001$) while the repeated cocaine dosing regimen produced a greater increase in ambulatory activity than both the 7-day treatment ($P < 0.001$) and the naive rats ($P < 0.001$). There was no time by pretreatment interaction ($F(22, 1103) = 1.059$, $P = 0.387$). The total ambulations in 60 min (Fig. 1B) showed that the repeated cocaine regimen evoked greater ambulatory activity than both the single cocaine regimen ($P < 0.05$) and the naive rats ($P < 0.001$). There was no difference between the single cocaine regimen and the naive rats in this parameter ($P = 0.113$).

3.2. Behavioral rating

Fig. 2A shows the incidence of each behavior after cocaine challenge for each pretreatment group. These data were divided into three main categories (Fig. 2B; inactive, active and hyperactive) for statistical analysis. ANOVA showed that there was a main effect of behavior ($F(2, 281) = 4.452$; $P = 0.013$); that is, overall, there was a greater incidence of inactive versus hyperactive behaviors ($P = 0.012$) and a greater incidence of active versus hyperactive behaviors ($P = 0.031$) but no difference in the incidence of inactive versus active behaviors ($P = 0.477$). There was no effect of pretreatment group ($P = 1.00$) because in each of these groups the sum of behaviors equals 100% incidence. However, there was a behavior by pretreatment group interaction ($F(4, 281) = 14.863$, $P < 0.001$). Thus, the naive rats expressed a greater incidence of inactive behaviors than both the rats treated with a single cocaine regimen ($P < 0.001$) and those treated with a repeated cocaine regimen ($P < 0.001$), while the single regimen rats expressed inactive behaviors more often than the repeated regimen rats ($P = 0.034$). There was no difference in active behaviors between any of the pretreatment groups. However, the repeated ($P < 0.001$) and single ($P < 0.001$) cocaine regimen rats expressed a greater incidence of hyperactive behaviors than the naive rats. The difference in expression of hyperactive behaviors between the repeated and single cocaine regimen rats narrowly failed to reach significance ($P = 0.057$).

Within the naive rats there was a greater incidence of inactive versus active ($P < 0.001$), inactive versus hyperactive ($P < 0.001$) and active versus hyperactive ($P < 0.001$) behaviors. There was no difference between behaviors within the single cocaine regimen treated rats. However, within the repeated cocaine regimen rats there was a greater incidence of hyperactive versus inactive ($P = 0.016$) and active

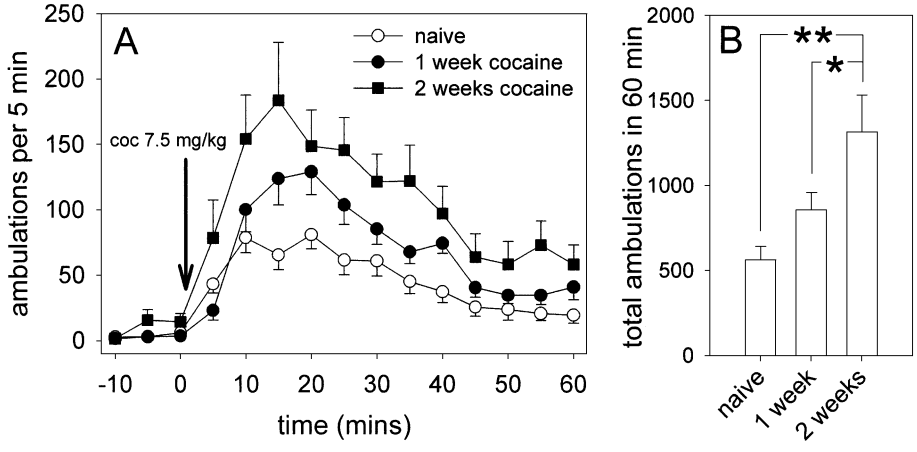

Fig. 1. The effect of different cocaine dosing regimens on the expression of locomotor sensitization. (A) Ambulatory activity was monitored every 5 min by computer, after a 15-min baseline period animals were injected with cocaine (7.5 mg/kg i.p.) and activity monitored for a further 60 min. Naive rats show a small increase in ambulations after cocaine challenge. The ambulatory response is increased on day 7 of withdrawal from a 7-day cocaine regimen (single regimen) and is further increased on day 10 of withdrawal from the repeated cocaine dosing regimen. (B) Shows the total ambulations in the 60 min following the cocaine challenge ** $P < 0.001$, 2 weeks of cocaine versus naive; * $P < 0.05$, 2 weeks cocaine versus 1 week cocaine.

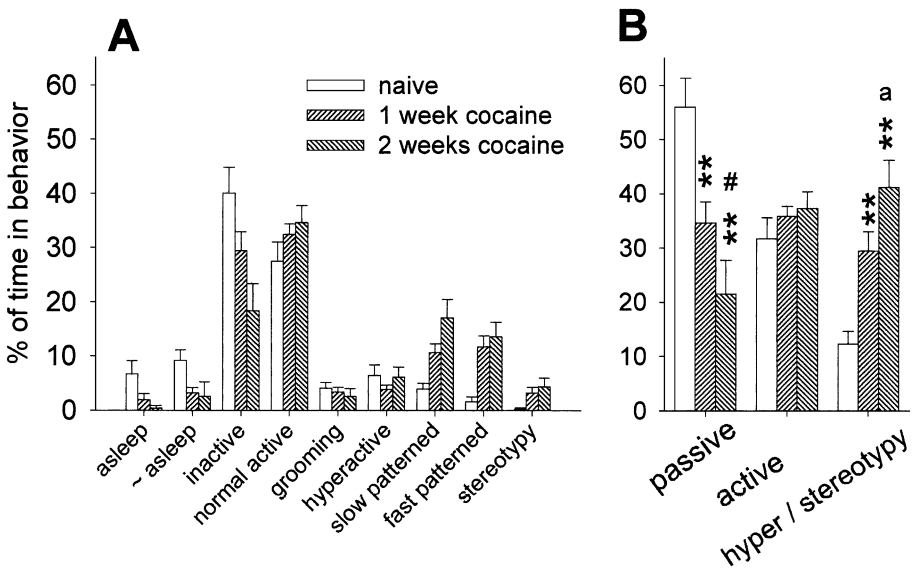

Fig. 2. The effect of single or repeated cocaine dosing regimens on behavioral ratings. While ambulatory behavior was being recorded by PC a trained observer rated the rats behavior at 5-min intervals using a modified rating scale (Ellinwood and Balster, 1974). (A) The percentage of time spent in each behavior is shown after single and repeated cocaine dosing regimens. (B) These behaviors were further divided into three main groups: (1) passive (asleep, almost asleep and inactive), (2) active (normal active and grooming) and (3) hyperactive (hyperactive, slow or fast patterned and stereotypy). ** $P<0.001$ versus naive; # $P<0.05$ versus 1 week cocaine; a $P=0.057$ versus 1 week.

versus inactive ($P=0.028$) behaviors but no difference in the incidence of hyperactive versus active behaviors ($P=0.582$).

4. Discussion

To more fully describe the behavioral changes after chronic cocaine, we have examined not only the increased ambulatory response to a cocaine challenge but also behaviorally rated the animals as previously described (Ellinwood and Balster, 1974). This is important as it (1) provides more detail of the animals behavior and (2) because stimulant-induced in-place stereotypies can sometimes inhibit ambulatory activity and mask sensitization (Segal and Mandell, 1974).

The single cocaine dosing regimen produced behavioral sensitization as previously shown (e.g. Kalivas et al., 1988; King et al., 1992). There was an increased ambulatory response to cocaine challenge although the total ambulations over the 60 min were not significantly changed. There was also evidence of sensitization from the behavioral rating data where animals showed decreased inactive behavior but increased hyperactive behavior after the single cocaine dosing regimen. The repeated cocaine dosing regimen produced an even greater sensitization. There was a further increase in ambulatory response and decreased incidence of passive behaviors when compared to the single cocaine regimen group. The increase in hyperactive behaviors in the repeated versus single cocaine dosing regimen narrowly failed to reach significance. To our knowledge, this is the first report showing that a repeated cocaine dosing regimen produces a greater sensitization than a single regimen. Thus, repeated cocaine dosing regimens not only have greater face validity to the human binge condition, where repeated cocaine binges occur (Gawin and Ellinwood, 1988) but also result in a more robust behavioral sensitization.

There is much evidence in the literature suggesting that by increasing the dose and/or duration of cocaine treatment one can cause increased sensitization (e.g. Post and Rose, 1976; Post et al., 1992). However, the idea that the longer the withdrawal period the greater the degree of behavioral sensitization is debatable. Thus, although Kalivas and Duffy (1993) found evidence of increasing locomotor sensitization in the weeks following cocaine withdrawal, others have found no augmentation of sensitization (Kilbey and Ellinwood, 1977) or a reduction in sensitization (Janak et al., 1997) during the withdrawal period. Further, it is known that stress (e.g. i.p. injections) can cause cross-sensitization with stimulants (Antelman et al., 1980; Robinson and Becker, 1986; Kalivas and Stewart, 1991); thus, the injections themselves (regardless of cocaine) during the second regimen could contribute to augmented sensitization. Therefore, as a caveat, we note that it is possible that the greater degree of sensitization seen at the third cocaine challenge (versus the sensitization seen at the second challenge) may not be wholly dependent upon the second cocaine dosing regimen.

Regardless, a further advantage of the repeated regimen is that it will allow the testing of putative pharmacotherapies for cocaine abuse in reversing previously established cocaine sensitization rather than just inhibiting the induction (King et al., 1997) or expression (King et al., 1994) of cocaine sensitization.

Given that this repeated cocaine dosing regimen provides a greater degree of sensitization, the question still remains as to whether this regimen will result in a different or more robust neurochemical or electrophysiological outcome from single cocaine regimens. If more robust changes were found then it would make the elucidation of these changes easier as they are often subtle (Kuhar and Pilotte, 1996 for review). In conclusion, we believe that the repeated cocaine dosing regimens are a better model for human cocaine binging and have advantages in examining putative pharmacotherapies for cocaine and other stimulants.

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