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The expression of locomotor sensitization to apomorphine is dependent on time interval between injection and testing

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ABSTRACT

This study examined the onset of locomotor sensitization induced by apomorphine as a function of the temporal delay between drug injection and testing. In experiment 1, rats received three daily administrations of 2.0 mg/kg apomorphine or vehicle either immediately (0 min) or 20 min before being placed into the test environment for 20 min test sessions. Apomorphine given immediately before testing induced a stimulant effect during the first session and sensitization by the second session. However, when testing was delayed 20 min, apomorphine induced stimulant effects only after the third injection. In experiment 2, separate groups received a single 2.0 (mg/kg) apomorphine/vehicle injection immediately before being placed into the test environment for 60 min. In this experiment, apomorphine induced a stimulant effect at 0–20 and 20–40 min. However, the 20–40 interval increase in locomotion was relative to the low level of activity in the vehicle group and was not greater than the 0–20 min locomotion of the vehicle group. Thus, sensitization depends both on peak drug concentration and habituation state of the control group. The variable post-injection delays could be a useful method to study sensitization because it can avoid ceiling effects and changing baselines in the control groups.

1. Introduction

The development of locomotor sensitization to psychostimulant drugs is a well-established phenomenon (Carey and Gui, 1998; Damianopoulos and Carey, 1993; Kalivas et al., 1992; Mattingly et al., 1997; Robinson and Becker, 1986). Such sensitization can persist for a substantial period of time (Kuczenski et al., 1997; Robinson et al., 1988; Rowlett et al., 1997) and is considered to be an important contributor to psychostimulant drug abuse (Di Chiara et al., 1999; Robinson and Berridge, 1993; Vezina, 2007; Wolf, 2002). In a previous paper, we obtained findings that sensitization to apomorphine can occur even after one drug exposure (Bloise et al., 2007). In other instances of apomorphine sensitization, however, there appears to be a latent period even with a high dose (2 mg/kg) apomorphine treatment (Mattingly et al., 1997).

One variable, which may be critical for this sensitization effect, is the interval between drug injection and initiation of testing following an apomorphine treatment. In this report we present evidence that the detection of the onset of a sensitization effect is dependent upon the relationship between the time of the drug injection and time of testing. If a drug, such as apomorphine, which has a rapid rise to peak concentration (Acerbo et al., 2005; Martres et al., 1977; Smith et al., 1979) followed by steady decline, is administered immediately before testing, then, the drug effect reaches its maximum during the initial phase of testing. In behavioral testing with an open-field environment, the environment exerts an activation effect, which is maximal when the animal is initially placed into the test environment. Consequently, when animals are tested immediately after an apomorphine injection, the apomorphine stimulant effect is rising to a maximum level as the control group is undergoing habituation to environmental cues. Thus, the onset of peak apomorphine effect occurs when the environmental cues have passed the peak for eliciting locomotion in the control group. On the other hand, if the apomorphine drug test is delayed, and the testing begins after the apomorphine peak, then, under these circumstances, a diminishing apomorphine effect is being measured against the non-drug control group, which is being tested at its peak non-habituated state. To investigate this issue directly, we compared the effects of different delays (0, 20 min) between apomorphine injections and the start of behavioral testing. In order to detect the onset and time course of apomorphine behavioral effects in the 0 min delay treatment group, we recorded behavior every 2.5 min. In this way, we could monitor the temporal course of the onset and decline in the behavioral impact of apomorphine as well as the within-session habituation in the control group.

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2. Materials and methods

2.1. Subjects

Male Wistar albino rats provided by the State University of North Fluminense, initially weighing 250–300 g were housed in individual plastic cages (25×18×17 cm) until the end of experiment. Food and water were freely available at all times. The vivarium was maintained at a constant temperature (22+2 °C), and a 12/12 h light/dark cycle (lights on at 0700 h and off at 1900 h). All experiment occurred between 8:00 and 18:00 h. For 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. All experiments were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus and measurement of behavior

The behavioral measurements were conducted in a black open-field chamber (60×60×45 cm). A closed-circuit video-camera (DISISEC, model IR575M), mounted 50 cm above the arena was used to record behavioral data. The complete test procedure was conducted automatically without the presence of the experimenter in the test room. For locomotion (measured as number of crossings), the experimental arena floor was divided into eight equal-sized squares and the number of times that the rat passed from one square to another with its four paws was recorded. Locomotion was analyzed by a trained observer who was unaware of the treatment under test. All behavioral testing was conducted under dim red light to enhance the contrast between the white subject and dark background of the test chamber. Masking noise was provided by a fan located in the experimental room and was turned on immediately prior to placing the animal in the experimental arena and turned off upon removal of the animal from the experimental arena (i.e., test chamber).

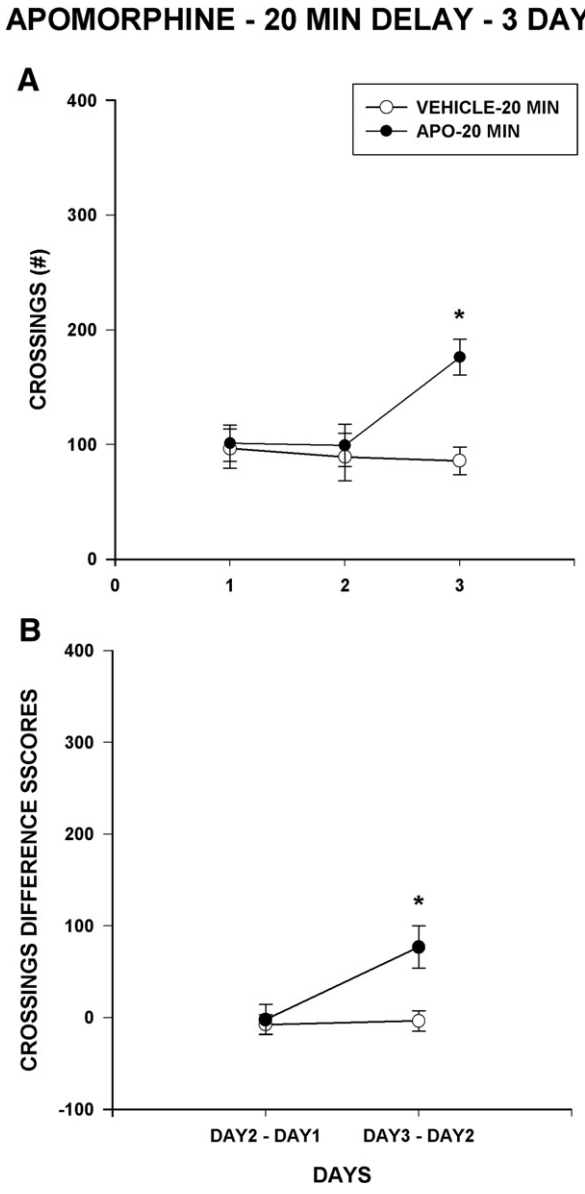

Fig. 1. Means and S. E. M. for locomotion (A) and locomotion difference scores (B) during the 3 days of the pharmacological treatment phase of the 20 min delay experiment. * denotes higher locomotor activity for apomorphine group than the vehicle group ($p<0.05$; two-way ANOVA and independent *t*-test).

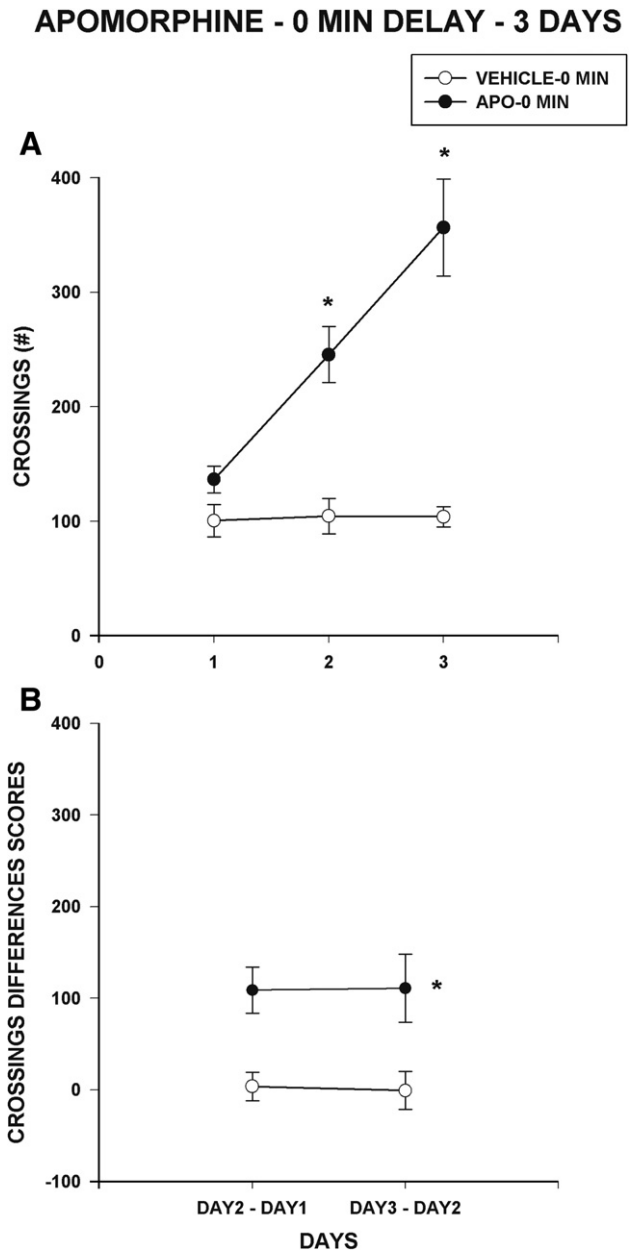

Fig. 2. Means and S. E. M. for locomotion (A) and locomotion difference scores (B) during the 3 days of the pharmacological treatment phase of the 0 min delay experiment. * denotes higher locomotor activity for apomorphine group than the vehicle group ($p<0.05$; two-way ANOVA and independent *t*-test).

2.3. Drugs

Apomorphine–HCl (Sigma, St. Louis, MO, USA) was dissolved in 0.1% ascorbate/saline (2.0 mg/ml) and was injected subcutaneously in the nape of the neck at a dose of 2.0 mg/kg using a volume of 1.0 ml/kg body weight. A 0.1% ascorbate/saline solution was used as vehicle. Drug solutions were freshly prepared before each experiment.

2.4. Design and procedures

Before starting the experiments, all rats received three 20 min habituation sessions (experiment 1) or a single 60 min habituation session (experiment 2) in which the animals were administered with saline and placed in the experimental arena for the allotted time**.** In experiment 1, the animals were randomly assigned to apomorphine (APO-20 MIN; $n=8$) and vehicle (VEHICLE-20 MIN; $n=5$) groups and received apomorphine (2.0 mg/kg) or vehicle 20 min before being placed in the test environment. The animals were observed for 20 min in the test environment and these treatments were administered for 3 days, with one trial per day. Another set of animals was randomly assigned to apomorphine (APO-0 MIN; $n=8$) and vehicle (VEHICLE-0 MIN; $n=11$) groups and received their respective pharmacological treatments immediately before being placed into the test environment. The animals were observed for 20 min in the test environment

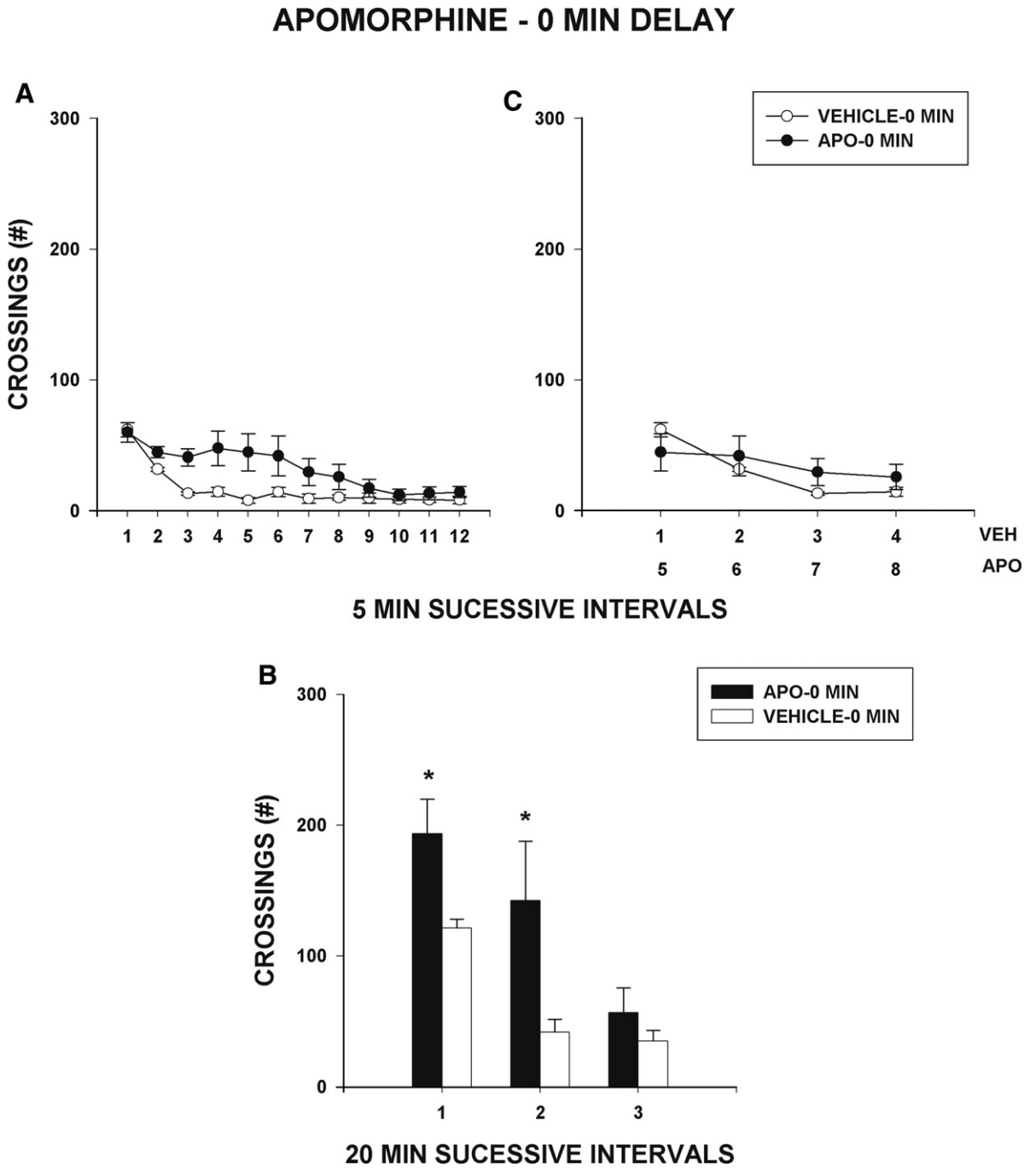

Fig. 3. Means and S. E. M. of locomotor activity for the 2.0 mg/kg apomorphine and vehicle groups tested immediately after injection during 60 min on day 1. The upper left panel (A) shows the within-session scores during 12 successive 5-min intervals for both groups. The bottom panel (B) shows the within-session scores during 3 successive 20-min intervals for both groups. The upper right panel (C) shows the successive 5-min intervals during the first 20 min for the vehicle group and during the second 20 min for the APO-0 MIN group. * denotes higher locomotor activity in the apomorphine group than the vehicle group ($p<0.05$; one-way ANOVA).

and these treatments were administered for 3 days, with one trial per day. In experiment 2, the animals were randomly assigned to apomorphine (APO-0 MIN; $n=8$) and vehicle (VEHICLE-0 MIN; $n=8$) groups and received their respective treatments immediately before being placed into the test environment. The animals were observed for 60 min in the test environment.

2.5. Statistics

For experiment 1, a repeated two-way analysis of variance (ANOVA) consisting of a between-subject factor group and a repeated-measurements factor day group. When an interaction group×day attained significance ($p<0.05$), independent *t*-tests were used to make specific group comparisons. For experiment 2, in order to make within-treatment assessments of the behavioral activity data, the total test time (60 min) was divided into 12 intervals of 5 min duration each and a repeated two-way ANOVA consisting of between-subject factor group and a repeated-measurements factor interval was used.

3. Results

Fig. 1 shows the scores of locomotor activity for the 2.0 mg/kg apomorphine and vehicle groups tested 20 min after injection during a 20 min period for 3 consecutive days. For the comparison between groups versus days (Fig. 1A), a repeated two-way ANOVA showed that there was an interaction effect, group×days ($F_{2,\ 12}=5.30$; $p<0.05$), a group effect ($F_{1,\ 12}=6.24$; $p<0.05$) but no effect of days ($F_{2,\ 12}=2.04$; $p>0.05$). In order to further analyze the interaction (group×days), independent *t*-tests were performed for each test day, comparing the APO-20 MIN versus VEHICLE-20 MIN groups. The results showed that on the first and second day, there were no differences between the experimental groups ($p>0.05$). However, on day 3, the APO-20 MIN group had higher locomotor activity than the VEHICLE-20 MIN group ($p<0.05$). In order to assess the onset of the sensitization effect, the different scores between test days for the APO-20 MIN and VEHICLE-20 MIN groups were calculated (i.e., day 2 minus day 1 and day 3 minus day 2). The increase in locomotion only begins to occur by day 3 (Fig. 1B), a repeated two-way ANOVA showed that there was an effect of group ($F_{1,\ 8}=5.07$; $p<0.05$), an effect of days ($F_{1,\ 8}=4.74$; $p<0.05$) but no interaction effect, group×days ($F_{1,\ 8}=1.63$; $p>0.05$). As can be seen in Fig. 1B, a sensitization effect was manifested by day 3.

Fig. 2 shows the scores of locomotor activity for the 2.0 mg/kg apomorphine and vehicle groups tested immediately after injection during a 20 min period for 3 consecutive days. To assess the effect of the 0 min delay on apomorphine induced locomotion (Fig. 2A), a repeated two-way ANOVA showed that there was an interaction effect, group×days ($F_{2,\ 21}=10.50$; $p<0.01$), an effect of group ($F_{1,\ 21}=63.0$; $p<0.01$), and an effect of days ($F_{2,\ 21}=13.50$; $p<0.01$). As can be seen in Fig. 2A, there was a progressive increase in locomotion with repeated APO treatments. To assess the magnitude and onset of sensitization, the scores from day 2 minus day 1 and day 3 minus day 2 for the APO-0 MIN group and the VEHICLE-0 MIN are shown in Fig. 2B. To assess the magnitude of this sensitization effect, a repeated two-way ANOVA was performed. The results showed that there was a group effect ($F_{1,\ 14}=11.70$; $p<0.01$) but no effect of days ($F_{1,\ 14}=0.040$; $p>0.05$) and no interaction group×days ($F_{2,\ 14}=0.007$; $p>0.05$). Thus, when expressed as an absolute change in locomotion, the rate of the sensitization effect was constant.

Fig. 3 shows the scores of locomotor activity for the 2.0 mg/kg apomorphine and vehicle groups tested immediately after injection during a 60 min period on day 1 of the treatment. Fig. 3A shows the scores for locomotor activity for the 2.0 mg/kg apomorphine and vehicle groups tested immediately after injection with activity monitored every 5 min during a 60 min period. Statistical analysis with a repeated two-way ANOVA indicated a significant interaction group×interval ($F_{11,\ 154}=3.24$; $p<0.01$), a significant effect of interval ($F_{11,\ 154}=16.83$; $p<0.01$), and a significant effect of group ($F_{1,\ 14}=48.34$; $p<0.01$). Fig. 3B shows the comparison between the APO-0 MIN and VEHICLE-0 MIN groups tested immediately after injection, where the total test time (60 min) was divided into 3 intervals of 20 min duration. A *t*-test comparison showed that during the first 20 min interval and the second 20 min interval, the apomorphine group had a higher level of locomotion activity than the vehicle group [t (14)= 2.65; $p<0.05$ and t (14)=2.16; $p<0.05$; for first and second 20 min intervals, respectively]. The results also showed that the first 20 min interval had a higher level of locomotor activity than the second and third intervals ($p<0.05$). While there were marked locomotion stimulant effects of apomorphine during the 0–20 and 20–40 min intervals, it is evident from Fig. 3B that the level of locomotion in the 20–40 apomorphine group was quite similar to and did not differ from the vehicle control group at 0–20 min ($p>0.05$). Thus, the locomotion stimulant effect of the apomorphine group was relative to the low habituated level of locomotion in the vehicle control group. Fig. 3C shows the scores for locomotor activity every 5 min during the first 20 min for the VEHICLE-0 MIN GROUP and during the second 20 min for the APO-0 MIN group. Statistical analysis with a repeated two-way ANOVA indicated that this was not a significant treatment group effect ($F_{1,\ 14}=0.20$; $p>0.05$).

4. Discussion

In the present study we found apomorphine sensitization effects expressed after one treatment as well as following a latent period. The difference depended upon whether the apomorphine treated animals were placed in the test environment immediately after injection or after a 20 min post-injection delay. With the 0 delay treatment, there was an apomorphine locomotion stimulant effect during the first treatment session; whereas, in the 20 min delay group, there was no locomotion stimulant effect either on the first or in the second apomorphine paired treatment session. By using a behavioral micro-analysis of locomotion; i.e., of changes within the first paired treatment session, we were able to show for the 0 delay groups that the difference between the paired apomorphine vs. paired non-drug treatments emerged between 7.5 and 10 min into the first 20 min interval. Furthermore, apomorphine did not induce an absolute increase in locomotion above the initial peak level of the non-drug group. Rather, the increase was manifested only when compared to the progressively declining baseline in the non-drug group during the test session. That is, the non-drug control group had a high level of locomotion following the initial placement into the test environment but, then, underwent a typical rapid within-session habituation in which locomotion decreased substantially. The apomorphine treated group also decreased from its initial level but, in contrast to the non-drug control group, it maintained a higher asymptotic level of locomotion, which was more than double the level of the non-drug group in this time period. Eventually by 40 min, the apomorphine treated group also underwent a steady decline in locomotion down to the level of the non-drug group. These findings suggest that the 20 min apomorphine delay group was tested during a transition phase in which the apomorphine locomotion stimulant effects were starting to wear off. While there is still a locomotion stimulant effect detectable in the 0 delay groups tested in the 20–40 min post-injection interval, the basis for this effect is that the locomotion of the comparison non-drug group is at a very low level at this time interval since it has already undergone a habituation to the test environment.

In the measurement of the locomotion stimulant effect of apomorphine in the 0 and 20 min delay groups, it is important to recognize that the comparison vehicle control groups had similar levels of locomotion. Thus, differences were not related to some non-specific effect linked to temporal interval after injection. In fact, over the course of the three-apomorphine injections, for both groups, the baseline locomotion levels of the vehicle 0 and 20 min groups remained stable and

comparable. Thus, the effects observed in the apomorphine groups could not be accounted for in terms of changes in the comparison control groups. The 0 delay group effects are particularly revealing in that locomotion essentially tripled by the third treatment. Furthermore, the increase in locomotion generated by the second and third injections was equivalent. With further treatment, it is also evident that a ceiling effect would prevent the detection of additional sensitization effects. On the other hand, the fact that sensitization effects only begin to emerge in the 20 min delay group by the third treatment session suggest that it would take more apomorphine injections to achieve a ceiling effect with a 20 min delay protocol. Seemingly, this could be extended much further with a 40 min delay interval. The key advantage of this type of approach to sensitization effects is that the effects could be assessed against vehicle control groups that have similar baseline activity levels. This approach contrasts with a protocol in which the drug effects are measured starting immediately after injection for a prolonged interval. The problem with this approach is that the within-session habituation effects in the vehicle control groups can be profound so that drug effects are assessed against a changing baseline.

These considerations are relevant to the study of sensitization effects in which long intervals are used. In the Post and Rose (1976) study the rats were given an injection of either saline or cocaine (10 mg/kg, i.p.) and locomotor activity and stereotypic behaviors monitored for 90 min, once a day for 12 days. Acute cocaine administration resulted in a peak of locomotor activity early in the session (around 15 min), whereas chronic administration resulted in a later onset activity peak (around 90 min). Thus, a rightward shift in peak locomotor activity was found from acute to chronic administration of cocaine. Also, Ansah et al. (1996) replicated this experiment except that rats were given a higher dose of cocaine (20 mg/kg; i.p.) and locomotor activity was shifted rightward. On day 1, locomotor activity peaked at 15 min after drug administration. By day 12, the peak of the cocaine-induced response had shifted to 95 min. More recently (Geary and Akins, 2007), it has been reported that repeated cocaine treatments in Quail induced locomotor sensitization and that this locomotor sensitization included a temporal dimension as well as an amplitude dimension in the expression of locomotor sensitization.

In the studies cited above, the drug treatments were administered immediately prior to testing and the effects on locomotion were assessed for 60–120 min. The resulting data were evaluated in blocks of 15–20 min. Observed results from such studies, rather than being an indication of a rightward shift in efficacy, may indicate a sensitization effect in which ceiling effects occur later and later following drug injection giving the appearance of a rightward shift in efficacy. In the present study, only 0 and 20 min delays were used and the results indicate that a 20 min delay generated an apomorphine sensitization effect but requiring more apomorphine treatments than the same apomorphine treatment given with a 0 min delay to become manifested. This observation indicates that a broader range of delays between injection and testing would provide a new and effective protocol for characterizing the progression and temporal duration of an apomorphine locomotor sensitization effect (e.g., 0, 20, 40, 60 min delays). Conversely, challenge tests conducted after a 0 delay induction protocol could incorporate variable delays (e.g., 0, 20, 40, 60 min). Such a sensitization induction and expression protocol would provide a new approach to locomotor sensitization in which peak drug effect and temporal expansion of the locomotor sensitization effect could be experimentally investigated rather than being merely described. Furthermore, this approach would allow for the detection of ceiling effects and whether the rightward temporal shift in sensitization effects is simply a rightward shift in ceiling effects. A matter of fundamental importance in such studies is an adequate understanding of sensitization effects.

In summary, the present study examined the sensitization effects induced by apomorphine in terms of the temporal delay between drug injection and testing. The results showed that the 20 min delay generated a sensitization effect in which apomorphine did not induce locomotor stimulant effects until the third injection. In contrast, the same apomorphine treatment given with a 0 min delay induced a stimulant effect during the first 20 min interval of testing and a marked sensitization by the second injection. Thus, the sensitization results were dependent upon the time interval between injection and testing. The incorporation of the interval between injection and testing as an independent variable in sensitization protocols offers a new way to address important issues in the induction and expression of sensitization effects.

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