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Locomotion elicited by MK801 in developing and adult rats: temporal, environmental, and gender effects

Kyle Frantz *, Carol Van Hartesveldt

Department of Psychology, University of Florida, Gainesville, FL 32611, USA

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Abstract

The effects of environmental novelty on locomotion elicited by an *N*-methyl-D-aspartate (NMDA) receptor antagonist, (+)MK-801 hydrogen maleate [(5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine], were investigated. Male and female rats aged 10, 20, 30 or 54–68 days were injected s.c. with MK801 and placed in activity monitors either immediately (no-delay) or after a 60 min delay (delay). In the no-delay condition, MK801 induced an inverse U-shaped dose–response effect on locomotion; peak activation occurred with 0.1 mg/kg and ataxia occurred with higher doses. The introduction of a novel environment 60 min after drug injection shifted the dose–effect function of MK801 to the left; i.e., in rats 20 days of age and older, the activity induced by 0.1 mg/kg MK801 was potentiated in the delay condition. For the 0.5 mg/kg dose, 20-day-olds showed activation in the no-delay condition but ataxia in the delay condition. This dose induced ataxia followed by activation in 30-day-olds and adult males or ataxia in adult females, regardless of delay condition. Age-, gender-, and novelty-dependent variations in MK801-induced locomotion may reflect differences in limbic–motor circuitry.

Keywords: Novelty; Ontogeny; NMDA receptor; Locomotion; (Rat)

1. Introduction

The excitatory amino acid neurotransmitter, glutamate, plays an integral role in locomotor behavior. In adult rats, a well-described motor syndrome results from blockade of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor with peripheral injections of the antagonist, MK801 (Ford et al., 1989; Tricklebank et al., 1989; Hargreaves and Cain, 1992; Ögren and Goldstein, 1994). MK801 is a non-competitive, use-dependent antagonist which binds to the phencyclidine (PCP) site inside NMDA receptor-associated ion channels to block the channels after they are opened by agonist activity at NMDA receptors. With increasing doses of the compound, the MK801-induced motor syndrome includes: (1) motor activity characterized by forward locomotion, sniffing, turning and rearing; (2) motor stereotypies such as stereotyped sniffing, head weaving and reciprocal forepaw treading; (3) ataxia, which consists of body rolling, a lack of limb coordination and decreased rearing, and (4) debilitating ataxia, which entails a loss of postural support, flattened posture, footsplay, akinesia, salivation and lacrimation. The locomotor activating effects of MK801 involve both glutamate and dopamine transmission; either glutamate or dopamine receptor antagonists can block MK801-induced locomotion (Willins et al., 1993; Ouagazzal et al., 1994).

Two reports have shown that, in developing rats, subcutaneous injections of MK801 produced an adult-like inverse U-shaped dose–response curve with regard to locomotor activity, but specific characteristics of the motor effects differed across ontogeny (Rajachandran et al., 1991; Scalzo and Burge, 1994). As in adult rats (see Hargreaves and Cain, 1992; Ögren and Goldstein, 1994 for examples), doses of 0.1 and 0.2 mg/kg MK801 increased locomotion, but higher doses of 0.5 and 1.0 mg/kg MK801 produced ataxia in both preweanling (3–4 or 12 days of age) and weanling rat pups (17–19 days of age). The magnitude of locomotor activation in weanling rats was higher than in preweanlings but still lower than in adult rats (Rajachandran et al., 1991). The response topography of rat pups also

* Corresponding author. Department of Neuropharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. Fax: +1-619-784-7405

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differed from that of adult rats in that pups did not exhibit sniffing, but adult rats do. Finally, Rajachandran et al. (1991) also reported an increase in grooming and a decrease in mouthing for milk in 3–4-day-old pups given 1.0 mg/kg MK801, findings which contrast those of akinesia in adult rats given the same dose (Tricklebank et al., 1989).

Further comparisons between rat pups of various ages are required to determine whether or not sensitivity to the motor effects of MK801 rises steadily into adulthood and how locomotor responses of developing rats change with time after drug injection. Thus, the first objective of the present study was to extend the developmental analyses of the locomotor effects of MK801 to include a wider age range, use a longer test session and record the same measure of activity in rats of various ages. MK801 was administered subcutaneously to 10-, 20-, 30-day-old and adult rats and locomotor responses were recorded for 2 h in automated activity monitors to which the animals were naive. Under these conditions, changes in locomotor activity over time after drug injection could be attributable to two main factors: (1) the distribution and subsequent metabolism of MK801 in the nervous system and (2) a decline in novelty of the testing environment as animals could explore and habituate to the testing arena.

Novelty is a major influence on locomotor activity. It elicits a locomotor response characterized in adult rats by a brief period of inactivity, or freezing, followed by exploratory behavior, consisting mainly of locomotion, sniffing and rearing, and ending with a decrement in activity, known as habituation (see O'Keefe and Nadel, 1978 for review). Novelty-induced locomotion in rat pups tested individually changes across ontogeny (Campbell et al., 1969; Spear and Brake, 1983). Exploration of a novel environment appears first at approximately 10 days of age, but in low magnitude. Levels of novelty-induced activation rise quickly to a peak at 15 days of age, decline by 25 days of age, and in some cases peak again during the periadolescent period. This response to a novel environment may be considered a stress response; in adult rats, it is accompanied by release of the stress hormones, adrenocorticotropin (ACTH) and corticosterone, in patterns similar to release in response to classic stressors, such as ether anesthesia (Brett et al., 1983). When animals are tested individually, the motor activation in a novel environment may also involve isolation stress (Randall and Campbell, 1976).

The role of glutamate in the ontogeny of novelty-induced locomotion is unknown. Therefore, the second objective of the present study was to analyze the interaction between MK801 and the novelty of the test conditions in eliciting locomotion in developing and adult rats. MK801 was injected subcutaneously as in the previous condition, but the rats were not placed in the activity monitors until 60 min after the injection. The novel environment was thus presented at the time of peak drug effect, defined as the time of highest locomotor activation under the condition in which animals were placed in the activity monitors immediately after the injection.

Previous research has shown that adult female rats exhibit higher hormonal and behavioral responses than male rats to environmental novelty (Campbell et al., 1969; Archer, 1975; Van Hartesveldt, 1997), stress (Kitay, 1961; Brett et al., 1983) and MK801 (Fleischmann et al., 1991; Blanchard et al., 1992; Hönack and Löscher, 1993; Haggerty and Brown, 1996). Therefore, both male and female rats were tested in the present studies.

2. Materials and methods

2.1. Subjects

Subjects were derived from Sprague–Dawley dams and sires from Zivic-Miller Laboratories, (Portersville, PA). Female rats were placed in breeding cages with males and given daily vaginal lavage to check for sperm. Pregnant females were checked twice daily for litters, so that the time of birth was recorded within 12 h. The day of birth was recorded as day 0. On day 1, litters were culled to 10 pups with approximately equal numbers of males and females. On day 25, the rat pups were weaned, separated by sex and housed with at least one other littermate. Colony rooms were maintained at 21°C on a 10:14 light:dark cycle with lights on at 0800 h. Testing took place between 0800 and 1800 h in a room maintained at 26°C. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida and were in accordance with the principles of laboratory animal care established by the National Institutes of Health (Bethesda, MD, USA).

Rat pups were tested at 10, 20 and 30 days of age. Adult rats were between 54 and 68 days of age. Each dose-group consisted of 8 to 15 rats with approximately equal numbers of males and females. On the day of testing, a split-litter design was used so that at least three doses of the drug were tested in each litter. Each rat was tested only once.

2.2. Drug procedure

The glutamate NMDA receptor antagonist, (+)MK-801 hydrogen maleate ([(5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine], dizocilpine; Research Biochemicals International, MA, USA), was dissolved in saline, which was also used for control injections. Drug solutions were prepared on the day of testing. Subcutaneous injections were administered at the nape of the neck in doses of 0 (vehicle injection), 0.01, 0.10, 0.50 or 1.00 mg/kg. These doses were equivalent to 0, 0.0296,

0.296, 1.48 or 2.96 mol/kg. Injection volumes were 2.5 ml/kg for rat pups and 1.0 ml/kg for adult rats. Severe ataxia occurred in 10-day-old pups given 0.5 mg/kg MK801. The highest dose (1.0 mg/kg) was therefore not administered to this age group in any condition. Because the highest doses administered in the no-delay condition induced severe ataxia in animals of all ages, these doses (0.5 mg/kg for 10-day-olds and 1.0 mg/kg for older animals) were not tested in the delay condition.

2.3. Behavioral procedure

On the day of testing, rats were transported in their home cages from the colony rooms to the working laboratory. For the condition without a time delay (no-delay), injections were given in the testing room and recording began immediately after the injection. For the delay condition (delay), drug injections were given in a room separate from the testing room. After the injection, rats of all ages were returned to their home cages with littermates (and dam for preweanling 10- and 20-day-old pups) but were kept in the laboratory. One hour later, each rat was transported for the first time into the testing room and was placed in a randomly assigned activity monitor for a 2 h test session. One-way, sound-attenuated glass in the wall of the testing room enabled experimenter observation of locomotor behavior from an adjacent laboratory room. Thus, the quality of motor behavior was sampled.

Omnitech Digiscan Animal Activity Monitors recorded the locomotor activity of the rats. Each monitor was a $41.91 \times 41.91 \times 30.48$ cm^3 Plexiglas cage with a wire mesh floor. Photocell beams projected across the arena. They were spaced 2.54 cm apart such that 16 beams crossed side to side and 16 beams front to back, all 3 cm above the mesh floor. Solid flooring was added for the 10-day-old rats, so that beams crossed 1.5 cm above this floor board. The interruption of photocell beams was translated into various measures of locomotor activity by the Digiscan analyzer. Total distance travelled in cm was analyzed in 5 min intervals over a period of 2 h.

2.4. Statistics

Separate three-way analyses of variance (ANOVAs) with repeated measures (time factor) were carried out for each age group and each delay condition with drug dose, gender and time interval as the main factors. Additional analyses were conducted to compare groups in the delay and no-delay conditions during the 60–120 min post-injection period. Thus, four-way ANOVAs included delay condition, drug dose, gender and time (5 min intervals) as factors. Follow-up comparisons were made using three-way, two-way and one-way ANOVAs followed by Duncan's New Multiple Range Test to determine statistical significance at the $P < 0.05$ level.

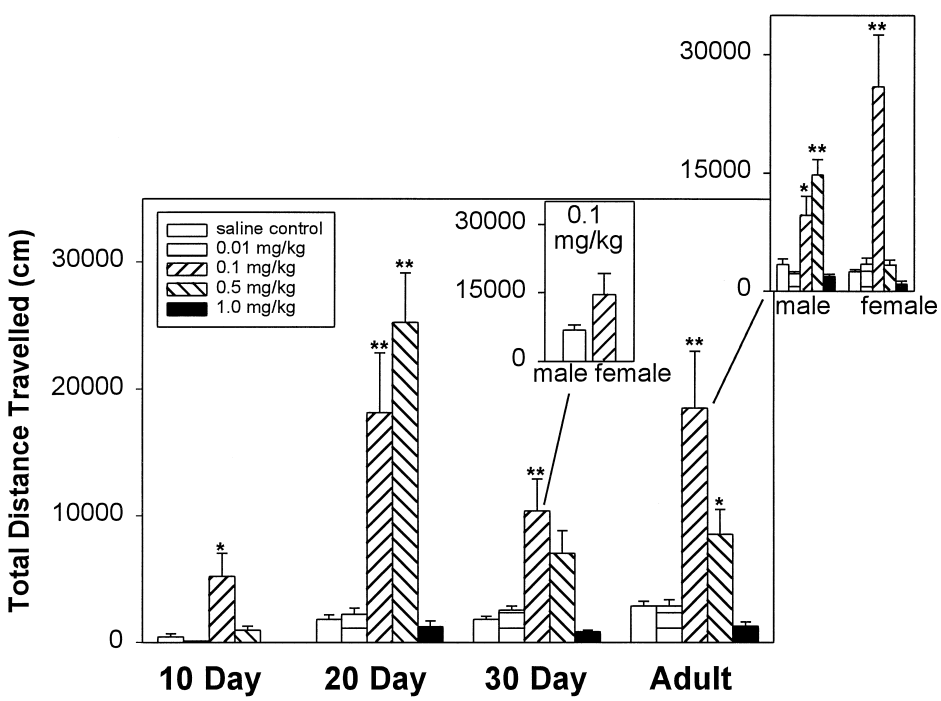

Fig. 1. Total distance (cm) travelled in a 2 h test session by rats of several different ages following s.c. injection with various doses of MK801. Significant differences from saline-injected controls are indicated ($^{**}P < 0.01$, $^{*}P < 0.05$). Error bars represent standard errors of the mean. Insets show data for males and females separately.

3. Results

3.1. No-delay conditions

MK801 induced an inverse U-shaped dose–effect curve with respect to locomotion (Fig. 1). In rats of all ages tested, the lowest dose failed to alter the distance travelled relative to the saline-injected control group, mid-range doses increased activity and higher doses induced ataxic effects. The locomotor activation consisted of hyperlocomotion, stereotyped sniffing, 'frantic exploration' of the test monitor, hyperreactivity and hyperexcitability. The ataxic effects progressed from stereotyped head weaving into reciprocal forepaw treading, a lack of coordination, body rolling and decreased rearing. With higher doses, the motor response progressed into debilitating ataxia, involving a flattened posture, footsplay, immobility, akinesia, salivation and lacrimation. In terms of the dependent measure of the present study (distance travelled in centimeters), neither the lowest nor highest doses significantly changed the measure, but for different reasons. The lowest dose did not alter behavior as observed by the experimenters, whereas the highest doses elicited severe ataxia to the point of akinesia, bringing the distance travelled down to the level of vehicle-injected control rats.

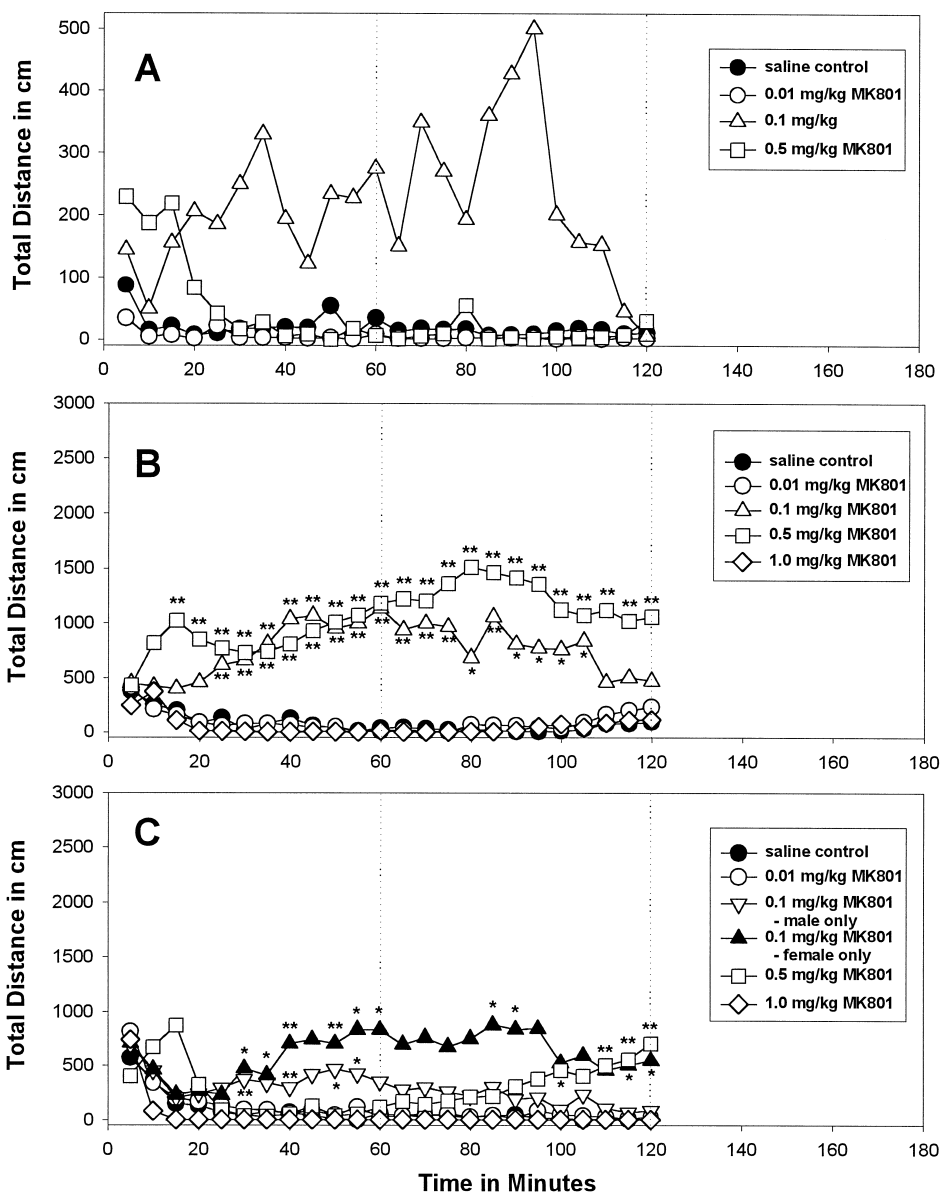

Fig. 2. Distance travelled (cm) immediately after s.c. injection with various doses of MK801 by 10- (A), 20- (B), 30- (C) day-old rat pups. Significant differences from saline-injected control groups are shown ($^{**}P < 0.01$, $^{*}P < 0.05$). Note the difference in range of the ordinate on graph A compared with graphs B and C. Data for male and female rats are combined for all groups except 30-day-old rats given 0.1 mg/kg MK801 (C). Error bars have been omitted for clarity.

3.1.1. Ten-day-old rat pups

In 10-day-old rat pups, MK801 produced the inverse U-shaped dose–effect curve, as confirmed by a significant main effect of dose [$F(3,35) = 5.49$, $P < 0.001$] (Fig. 1); only 0.1 mg/kg MK801 elicited significant locomotor activity relative to the saline-injected control group. The activity included sporadic bouts of motor activity, sometimes involving ataxic behaviors such as forepaw treading and body rolling, but the variability across individual pups obscured statistical significance of the dose × time interaction [$F(69,805) = 1.18$] (Fig. 2A).

3.1.2. Twenty-day-old rat pups

In 20-day-old rat pups, MK801 again induced an inverse U-shaped dose–effect curve with respect to the total distance travelled during the entire 2 h test session, resulting in a significant main effect of dose [$F(4,49) = 16.27$, $P < 0.001$] (Fig. 1). Both 0.1 and 0.5 mg/kg MK801 elicited significant differences in distance travelled relative to saline. There was also a significant dose × time interaction [$F(92,1127) = 2.786$, $P < 0.001$] (Fig. 2B). The 0.1 mg/kg dose increased the distance travelled from 25–105 min post-injection, whereas the 0.5 mg/kg dose increased the distance travelled with a shorter latency and longer duration, i.e., from the 15-min interval through the end of the test session. There were no noted differences between genders.

3.1.3. Thirty-day-old rat pups

In 30-day-old rat pups, the inverse U-shaped dose–effect curve showed that the 0.1 mg/kg dose significantly increased the distance travelled but the 0.5 mg/kg dose only tended to increase activity, according to a main effect of dose [$F(4,44) = 7.47$, $P < 0.001$] (Fig. 1). Neither the main effect of gender [$F(1,44) = 1.03$] nor the gender × dose interaction was significant [$F(4,44) = 1.46$], although females were slightly more active than males at the 0.1 mg/kg dose (Fig. 1, inset). There was, however, a significant gender × dose × time interaction [$F(92,1012) = 1.29$, $P < 0.05$] (Fig. 2C). The 0.1 mg/kg dose increased the distance travelled to a greater magnitude and longer duration in females than in males; the activity of males was increased from time interval 30–55 and the activity of females was increased at various intervals from 30 to 120 min post-injection, compared with the same-sex vehicle-injected controls. Females were more active than males at only three time intervals: 90, 115 and 120 min ($P < 0.05$). For both genders, the 0.5 mg/kg dose induced akinesia from approximately 15–60 min, but increased locomotion at the end of the session.

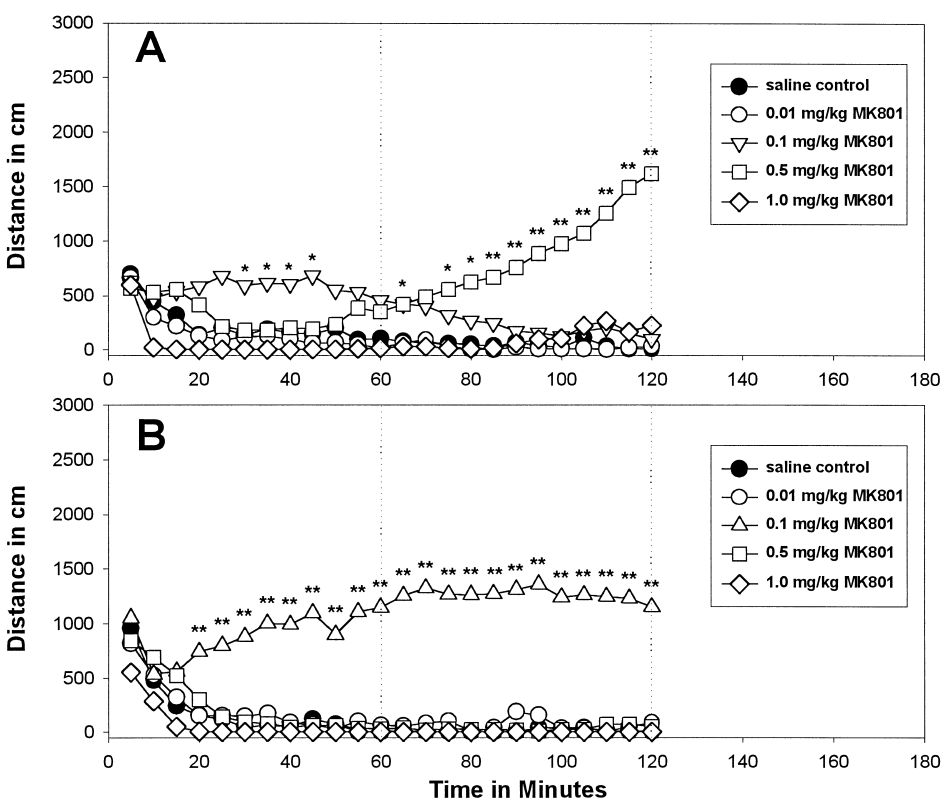

Fig. 3. Distance travelled (cm) immediately after s.c. injection with various doses of MK801 by adult male (A) and female (B) rats. Significant differences from saline-injected control groups are shown ($^{**}P < 0.01$, $^{*}P < 0.05$).

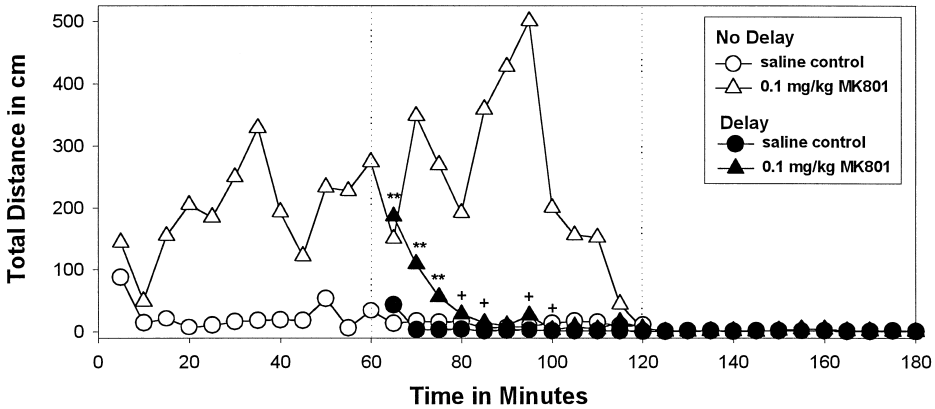

Fig. 4. Distance travelled (cm) by 10-day-old rats following injection of 0.1 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown ($^{**}P < 0.01$), as are differences between no-delay and delay groups ($^{+}P < 0.05$).

3.1.4. Adult rats

In adult rats, the inverse U-shaped dose–effect curve for the total distance travelled during the entire 2 h test session differed between genders, according to a significant main effect of dose [$F(4,41) = 16.846$, $P < 0.0001$] as well as a significant gender × dose interaction [$F(4,41) = 5.95$, $P < 0.001$] (Fig. 1). In males, the 0.1 and 0.5 mg/kg MK801 significantly increased the distance travelled, and 1.0 mg/kg MK801 induced ataxia. In females, 0.1 mg/kg substantially increased activity, but both 0.5 and 1.0 mg/kg induced ataxia. There was also a significant gender × dose × time interaction [$F(92,943) = 4.16$,

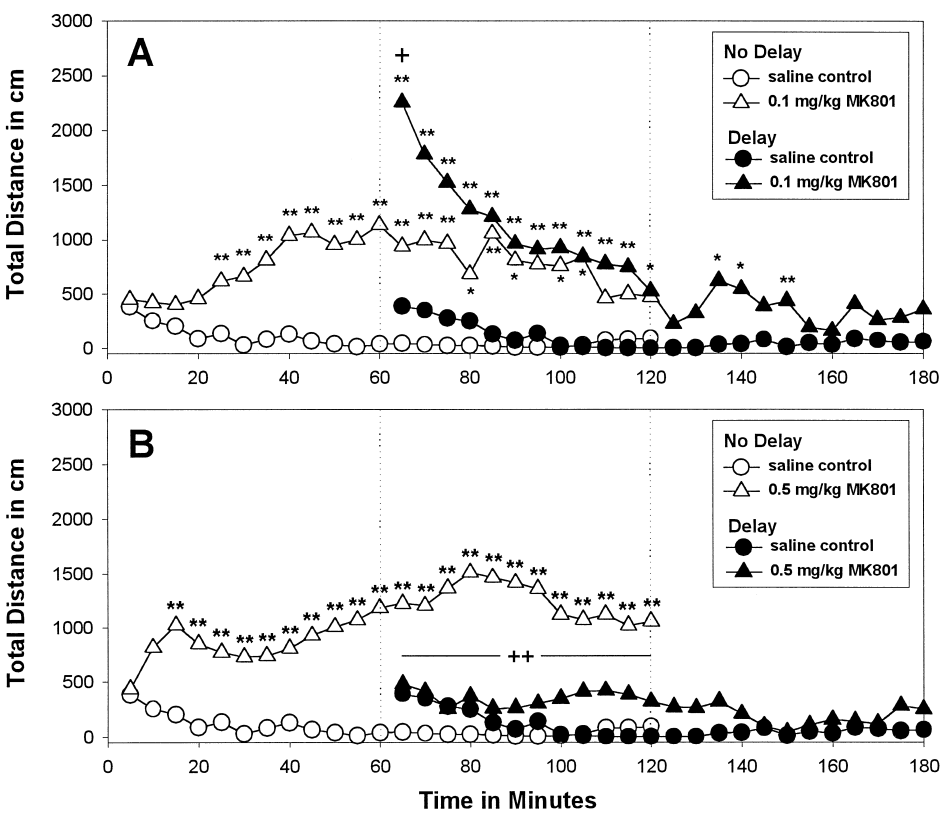

Fig. 5. Distance travelled (cm) by 20-day-old rats following injection of 0.1 mg/kg MK801 (A) or 0.5 mg/kg MK801 (B) or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown ($^{**}P < 0.01$, $^{*}P < 0.05$), as are differences between no-delay and delay groups ($^{++}P < 0.01$, $^{+}P < 0.05$).

$P < 0.0001$] (Fig. 3A and B). In males, the 0.1 mg/kg dose increased the distance travelled from time interval 30–45 and interval 65, compared with the male control group. The 0.5 mg/kg dose induced akinesia followed by activation in male rats, such that the distance travelled was increased from time interval 75 through the end of the session. In females, the 0.1 mg/kg dose of MK801 increased activity robustly from time interval 20 through the end of the session, compared with the female control group. The 0.5 mg/kg-induced akinesia was not followed by locomotor activation in females. Comparisons across gender showed that males and females did not differ from each other at any particular time interval after the 0.1 mg/kg dose, but males injected with 0.5 mg/kg MK801 were significantly more active than females given the same dose at interval 55 and from interval 65 through the end of the session ($P < 0.05$ at intervals 55 and 65–85, $P < 0.01$ at intervals 90–120).

3.2. Delay conditions

Imposing a delay between drug injection and placement of the rat in the novel environment altered the time course and severity of motor responses to MK801, as revealed by comparisons within the dose-groups in the delay condition as well as comparisons between the delay and no-delay conditions during the 60–120 min post-injection period (Figs. 4–8). However, the 0.01 mg/kg dose of MK801 did not significantly alter the distance travelled in either the no-delay condition (as noted above) or the delay condition (data not shown). Qualitatively, the locomotor responses were similar regardless of delay condition.

3.2.1. Ten-day-old rat pups

In the delay condition, the locomotor responses to MK801 (0.01 or 0.1 mg/kg) differed according to significant effects of dose [$F(2,40) = 8.335$, $P < 0.001$], time [$F(23,920) = 12.031$, $P < 0.0001$] and a dose × time interaction [$F(46,920) = 4.995$, $P < 0.0001$] (Fig. 4). The 0.1 mg/kg dose of MK801 significantly increased the distance travelled above saline-control levels at post-injection time intervals 65, 70 and 75 min.

Comparisons between delay and no-delay conditions revealed significant effects of delay condition [$F(1,67) = 5.053$, $P < 0.05$], dose [$F(2,67) = 8.84$, $P < 0.001$], time [$F(11,737) = 1.99$, $P < 0.050$] and a condition × dose × time interaction [$F(22,737) = 1.73$, $P < 0.05$]. Specifically, rat pups in the delay condition exhibited less activity than those in the no-delay condition at 80, 85, 95 and 100 min post-injection. Also, the activity levels of the pups

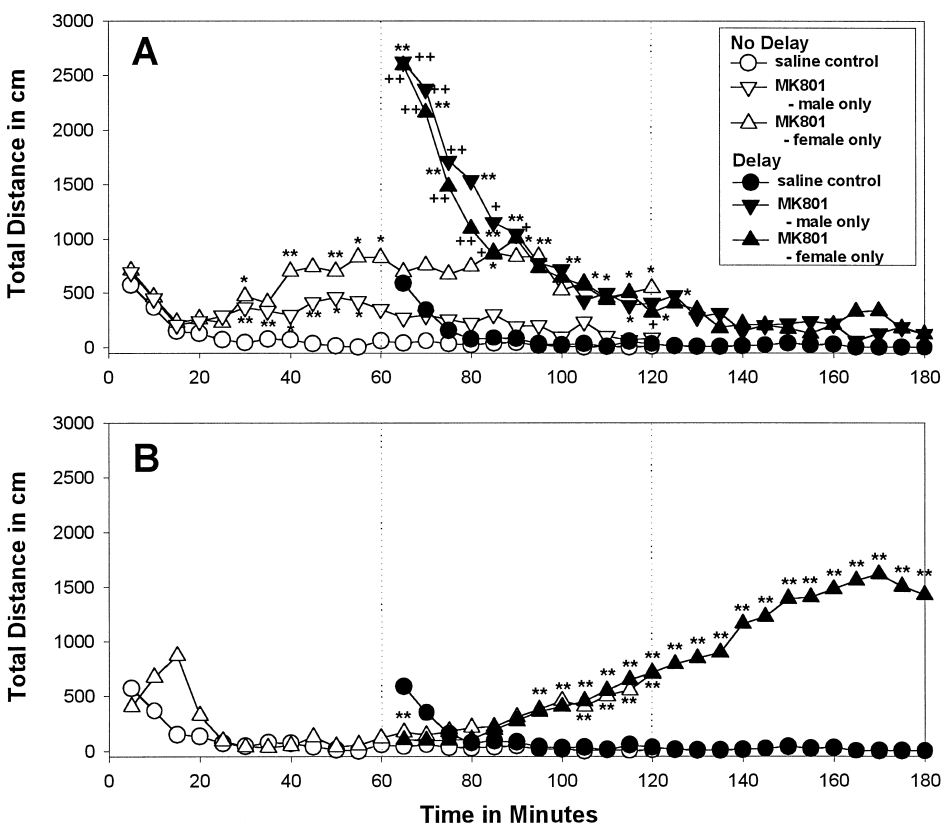

Fig. 6. Distance travelled (cm) by 30-day-old rats following injection of 0.1 mg/kg MK801 (A) or 0.5 mg/kg MK801 (B) or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown ($^{**}P < 0.01$, $^{*}P < 0.05$), as are differences between no-delay and delay groups ($^{++}P < 0.01$, $^{+}P < 0.05$). Male and female groups are shown separately in A but are combined in B.

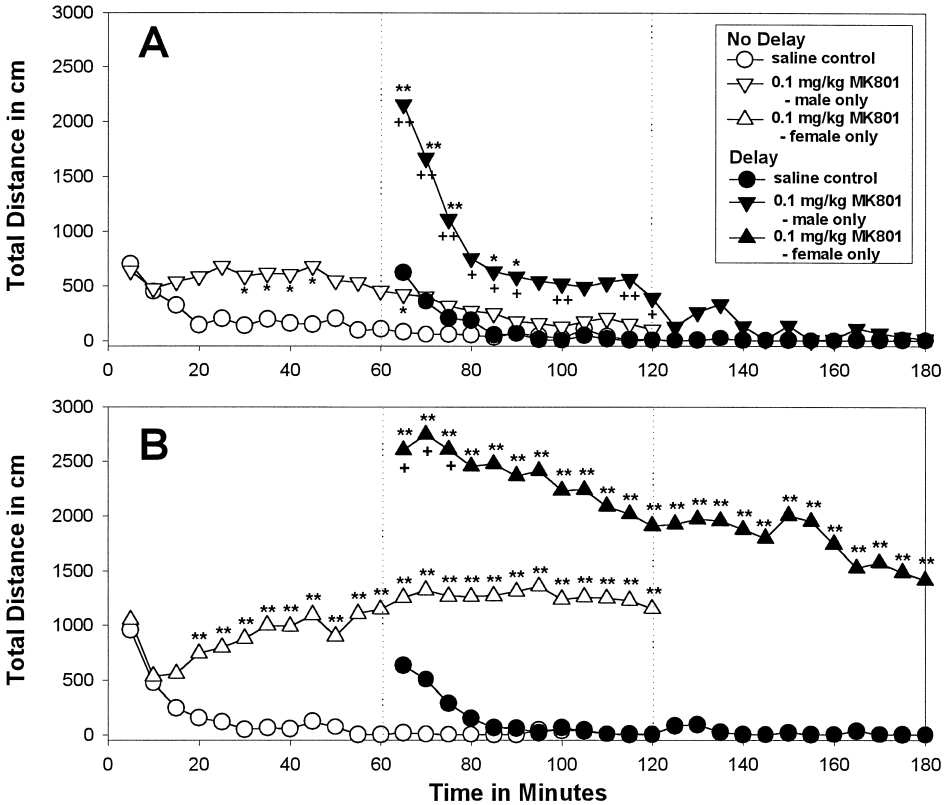

Fig. 7. Distance travelled (cm) by adult male (A) or female (B) rats following injection of 0.1 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown ($^{**}P < 0.01$, $^{*}P < 0.05$), as are differences between no-delay and delay groups ($^{++}P < 0.01$, $^{+}P < 0.05$).

appeared to be less variable in the delay condition than in the no-delay condition both across animals at each time interval and in individual pups over time.

3.2.2. Twenty-day-old rat pups

Comparisons within the delay condition revealed significant main effects of dose [$F(3,40) = 11.74$, $P < 0.0001$], time [$F(23,920) = 19.27$, $P < 0.0001$] and a dose × time interaction [$F(69,920) = 5.30$, $P < 0.0001$] for 20-day-old rat pups. The 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the delay condition, from approximately 65–150 min post-injection (Fig. 5A). There were no significant gender effects in the delay condition.

There was also a significant interaction between delay condition × dose × time [$F(33.891) = 2.16$, $P < 0.001$] (Fig. 5A and B). Following 0.1 mg/kg MK801, the delay condition × time interaction was significant [$F(11,209) = 3.45$, $P < 0.001$], such that the distance travelled in the first 5 min interval of the delay condition (65 min post-injection) was significantly higher than the distance travelled in the no-delay condition at the same post-injection time (Fig. 5A). On the other hand, following 0.5 mg/kg MK801, the two-way ANOVA of delay condition × time showed only a main effect of condition [$F(1,20) = 15.872$, $P < 0.001$] because the delay condition produced ataxia consistently throughout the test session while pups in the no-delay condition were consistently activated through that time period (Fig. 5B).

3.2.3. Thirty-day-old rat pups

Within the delay condition, there were significant effects of dose [$F(3,44) = 19.09$, $P < 0.0001$], time [$F(23,1012) = 18.99$, $P < 0.0001$] and a dose × time interaction [$F(69,1012) = 37.70$, $P < 0.0001$]. The 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the same delay condition, from approximately 65–125 min post-injection. The 0.5 mg/kg dose suppressed the initial increase in distance travelled during the 65 min interval (the first 5 min of exposure to the activity monitor) but steadily increased distance travelled from the 95 min interval through the end of the session.

As in the 20-day-old pups, a potentiation of the initial levels of locomotor activity occurred following injection of 0.1 mg/kg MK801 in the delay condition (Fig. 6A), but in 30-day-olds, the delay condition did not change the time course of effect for 0.5 mg/kg MK801 (Fig. 6B). Although there was no difference between genders in the delay condition, the data were analyzed separately for

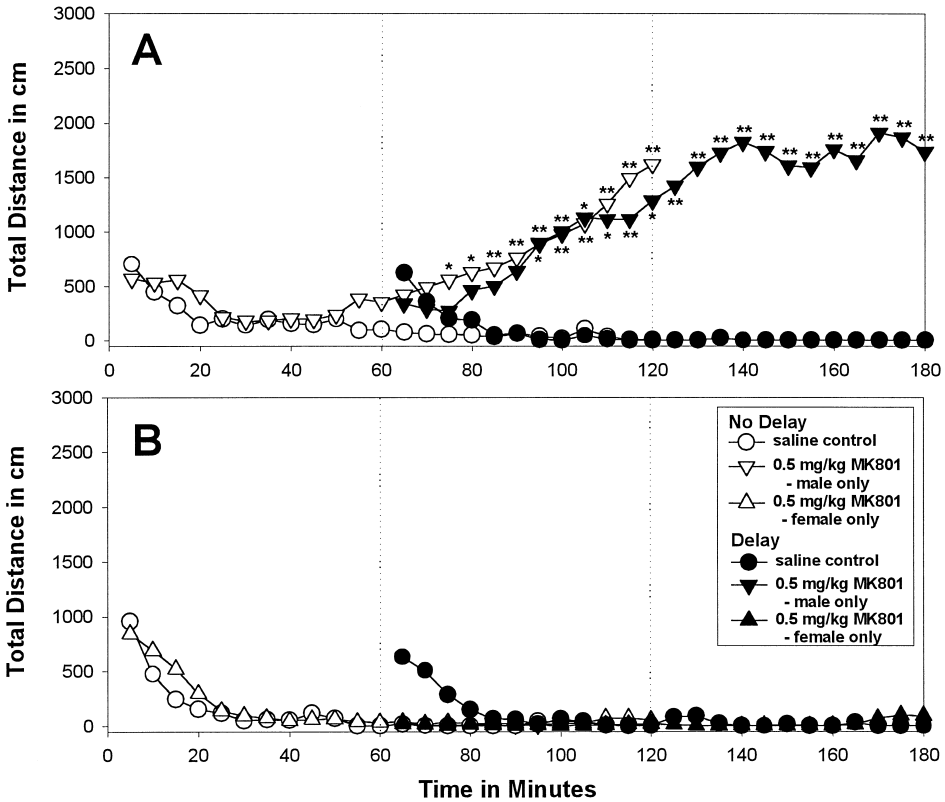

Fig. 8. Distance travelled (cm) by adult male (A) or female (B) rats following injection of 0.5 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown ($^{**}P < 0.01$, $^{*}P < 0.05$).

males and females in order to compare the data with the same-sex groups in the no-delay condition (which were slightly different from each other). For males, the delay condition × dose × time interaction was significant [$F(33,407) = 19.89$, $P < 0.0001$], such that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from 65–90 min post-injection (Fig. 6B). Similarly for females, the delay condition × dose × time interaction was significant [$F(33,429) = 22.54$, $P < 0.0001$], such that the 0.1 mg/kg dose increased activity more in the delay than in the no-delay condition from 65–85 min post-injection (Fig. 6A). In addition, females given 0.1 mg/kg MK801 in the delay condition travelled less than females in the no-delay condition at 120 min post-injection. On the other hand, the 0.5 mg/kg dose of MK801 induced ataxia followed by activation that did not differ between conditions or genders (Fig. 6B).

3.2.4. Adult rats

Comparisons within the delay conditions revealed significant effects of dose [$F(3,27) = 25.79$, $P < 0.0001$] and time [$F(23,621) = 16.88$, $P < 0.0001$] as well as a significant gender × dose × time interaction [$F(69,621) = 6.58$, $P < 0.0001$]. For males, the 0.1 mg/kg dose increased the distance travelled at intervals between 65 and 90 min post-injection (Fig. 7A). For females, the 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the delay conditions, at every time interval in the session (Fig. 7B). The distance travelled by females given 0.1 mg/kg MK801 was even greater than that of males in the same delay conditions from 70 min post-injection through the end of the session. After the 0.5 mg/kg dose, males exhibited ataxia at the beginning of the session but activation from 95 min post-injection through the end of the session (Fig. 8A), whereas females were severely ataxic throughout the test session (Fig. 8B). Thus, the activity in males was greater than in females from time interval 85 through the end of the session.

The potentiation of the initial levels of locomotor activity also occurred for adult rats when a delay was imposed between the injection of 0.1 mg/kg MK801 and placement in the activity monitor (Fig. 7A and B). For males, the delay condition × dose × time interaction was significant [$F(33,319) = 2.871$, $P < 0.0001$], such that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from approximately 65–120 min post-injection (Fig. 7A). For females, the delay condition × dose × time interaction was also significant [$F(33,374) = 2.089$, $P < 0.001$] and follow-up tests revealed that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the

no-delay condition from 65–75 min post-injection (Fig. 7B). Not only did the females exhibit potentiated levels of activity in the delay condition but also they continued to locomote at high rates throughout the 2 h test session.

4. Discussion

MK801 induced an inverse U-shaped dose–response function with respect to locomotion in rats of all ages. The low dose had no effect; mid-range doses induced hyperlocomotion and stereotypies; and higher doses induced ataxia and even akinesia. The quality of these effects was similar in rats 20 days of age and older, but 10-day-old rats showed a different behavioral topography. In addition, the relative dose-responsivity to MK801 varied across age and gender such that 20-day-old pups were the most active overall and females were more active than males at 30 and 60 days of age. Introduction of a novel environment 60 min after the MK801 injection altered the drug effects in a dose-, age- and gender-dependent manner.

4.1. Ten-day-old rat pups

The motor responses of 10-day-old rat pups to MK801 and environmental novelty differed in several ways from those of older rats. First, 10-day-old saline-injected control rats exhibited lower levels of novelty-induced locomotion and habituated to the test monitor more quickly than older animals. This effect is in accordance with reports that exploration in response to novelty is first apparent at 10 days of age but only at a low level (Campbell et al., 1969). Second, in response to 0.1 mg/kg MK801, 10-day-old pups demonstrated low levels of activation that occurred in short bouts with varying times of onset and offset for each animal. This time course of MK801-induced activation in 10-day-old rats is reported here for the first time, but the low level of activation and an observed lack of sniffing confirm previous findings on MK801-induced activity in rat pups (Rajachandran et al., 1991; Scalzo and Burge, 1994). Ten-day-old pups do have the ability to exhibit consistent high levels of locomotion, however, as demonstrated in response to the D_2/D_3 dopamine receptor agonist, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (Frantz et al., 1996). In fact, bouts of activity were no longer prevalent in 10-day-olds under the present delay condition; rather, the locomotion was consistently increased above control levels by 0.1 mg/kg MK801 for the first 15 min after placement in the activity monitor and declined at a consistent rate across animals. The pups were placed back in their home cages during the delay, so some aspect of the interactions with their dam and littermates in that time apparently 'organized' the motor response or decreased the variability in the behavior across the group of young pups. Finally, introducing a novel environment 60 min after the injection of 0.1 mg/kg MK801 did not potentiate the initial levels of locomotion in 10-day-old rat pups, as it did in older rats.

4.2. Rats 20 days of age and older

In rats 20 days of age and older, the most interesting present finding is that the initial locomotor response to novelty was greatly potentiated when a delay was imposed between the injection of 0.1 mg/kg MK801 and placement of the rat in the activity monitor. Therefore, blockade of glutamate NMDA receptors and introduction to a novel environment interact to increase locomotion, such that the level of activity is greater than that induced by either the NMDA receptor blockade as the predominant activating stimulus (as shown by the MK801-injected groups in the no-delay condition) or environmental novelty as the predominant activating stimulus (as shown by the saline-injected control groups). A possible interpretation of this finding is that the delay condition shifts the dose–effect function for MK801 slightly to the left, such that the same dose of MK801 exerts a slightly greater motor effect.

When the higher dose of 0.5 mg/kg MK801 was tested in 20-day-old pups, the introduction of the novel environment 60 min after the injection completely changed the drug effect from robust activation to debilitating ataxia in 20-day-old pups. Again, the delay condition shifted the dose–effect function to the left. In other words, increasing the dose of MK801 from 0.5 mg/kg to 1.0 mg/kg in the no-delay condition switched locomotor activation to akinesia, and adding environmental novelty to the 0.5 mg/kg dose of MK801 in the delay condition also switched activation into akinesia. In 30-day-olds and adults, however, the delay condition did not change the effect of 0.5 mg/kg MK801, perhaps indicating that the drug effect (akinesia) was maximal at 60 min post-injection and could not be increased further by environmental novelty. The locomotion exhibited by these rats at approximately 90 min post-injection was probably induced by the interaction between what was essentially a lower dose of MK801, as the drug was metabolized, and what was essentially a novel environment, because the rats had been incapable of exploration while akinetic.

4.3. Hypothesized neural substrate

Novelty-induced locomotion and MK801-induced locomotion are modulated by neural activity in the nucleus accumbens of adult rats (Hamilton et al., 1986; Svensson and Carlsson, 1992; Willins et al., 1993; Wu et al., 1993; Maldonado-Irizarry and Kelley, 1994; Svensson et al., 1994; Al-Khatib et al., 1995; Burns et al., 1996). Therefore, a low and sporadic locomotor response to novelty or MK801 in 10-day-old rats may occur because some aspect of the limbic–motor circuitry in the brain is not yet fully functional. Glutamate projections to the nucleus accumbens arrive from the prefrontal cortex and hippocampus

(see Heimer et al., 1993 for review). These two brain regions mature relatively late in ontogeny (Angevine, 1975; Alexander and Goldman, 1978; O'Keefe and Nadel, 1978; Benes, 1989), making it conceivable that their projections modulate locomotion differentially across ontogeny. Alternatively, sufficient levels of glutamate may be released in young rat pups, but NMDA receptors may not yet be associated with the appropriate intracellular mechanisms or may not be present on the appropriate output neurons. Wall-climbing and locomotion elicited by dopaminergic drugs in rat pups also occur in low-level bouts (Barrett et al., 1982; Van Hartesveldt et al., 1994; Frantz and Van Hartesveldt, 1995), perhaps demonstrating other manifestations of nervous system function which is still developing.

Twenty-day-old pups may exhibit a high magnitude of MK801-induced locomotion because of their high levels of glutamate release, high densities of NMDA receptors or supersensitive receptors for glutamate or other neurotransmitters in the motor circuitry during this stage of intense neuron growth and glutamate release (see McDonald and Johnston, 1990 for review). The low magnitude of locomotion in 30-day-old pups could reflect programmed cell death, synaptic pruning or receptor elimination after the growth spurt at 20 days of age. The adult levels of locomotion induced by MK801 most likely reflect the stabilization of glutamate transmission. Intra-accumbens injections of MK801 produce similar ontological changes in sensitivity to the drug, supporting the role of nucleus accumbens glutamate in these effects (Frantz and Van Hartesveldt, in press). Although MK801 also alters the transmission of dopamine, another major influence on locomotion, the ontogenetic changes in response to MK801 do not correlate well with known changes in nucleus accumbens dopamine transmission (Andersen et al., 1997; Stanwood et al., 1997; Andersen and Teicher, submitted).

4.4. Gender differences

The greater responsiveness of female rats compared with males to the motor effects of MK801 has been well-quantified (e.g., Fleischmann et al., 1991; Blanchard et al., 1992; Hönack and Löscher, 1993; Haggerty and Brown, 1996) and may be attributable to peripheral effects of the drug. Female rats have less efficient liver breakdown of PCP than males (Nabeshima et al., 1984) and given that PCP and MK801 bind to the same site in the NMDA receptor-associated ion channel and exert similar behavioral effects, a similar biotransformation for the two drugs is likely. MK801 may therefore be active in the nervous system of female rats at a higher concentration for a longer period of time and consequently may exert greater behavioral effects in the form of higher and longer activation in response to 0.1 mg/kg MK801 and longer periods of akinesia in response to 0.5 mg/kg MK801. The time of ontological onset for these gender differences between 20 and 30 days of age supports a role for gonadal hormones in modulating the response to MK801 because this developmental stage approaches 'periadolescence' in the rat pup, i.e., the initiation of sex hormone circulation in young rats and the stage of vaginal opening for females (Andrews et al., 1981; Spear and Brake, 1983; Losada et al., 1993). Furthermore, a novel environment increases plasma levels of ACTH and corticosterone to higher levels for a longer period of time in female rats as opposed to males (Kitay, 1961; Brett et al., 1983), so stress hormones could also contribute to a more robust response in females. Mediation of gender differences by peripheral mechanisms is supported by a lack of gender-dependent responses to centrally-injected MK801 (Frantz and Van Hartesveldt, in press). However, Hönack and Löscher (1993) point out that gender-dependent behavioral differences can occur within minutes of drug injection, undermining the importance of metabolic differences.

4.5. A role for stress hormones

Insofar as exposure to novelty is considered a mild stressor, responses to novelty and their modulation by MK801 can also be contemplated in relation to stress hormones. In adult rats, both novelty and MK801 can increase plasma levels of stress hormones (Brett et al., 1983; Pechnick et al., 1989) and all three of those stimuli, novelty, MK801 and stress hormones, can potentiate the locomotion induced by each stimulus alone (Oitzl et al., 1994; Wedzony and Czyrak, 1994; Sandi et al., 1996). In rats 20 days of age and older, introducing the novel environment simultaneously with the peak MK801 effect (the delay condition) may have potentiated the initial levels of locomotion by potentiating corticosterone release. On the other hand, 10-day-old rats may have been in a 'stress hypo-responsive period' which occurs during the first two postnatal weeks and involves decreased hypothalamic–pituitary–adrenal axis responses to noxious stimuli (Walker et al., 1986). If 10-day-old rat pups do not exhibit increased levels of stress hormones in response to novelty or to MK801 either, then none of the interactions between novelty, stress hormones and MK801 would occur. Thus, levels of locomotion would be lower overall and no potentiation of locomotion would be exhibited.

4.6. Clinical significance

Research on MK801-induced locomotion has clinical implications for schizophrenia and Parkinson's disease, two human conditions in which abnormal interactions between glutamate and dopamine in mesocorticolimbic and basal ganglia circuitry produce cognitive, emotional and motor dysfunction. While dopamine has long been a target for pharmacological treatment of these disorders, glutamate has become a new target for pharmacotherapies (Carlsson and Carlsson, 1990; Wachtel and Turski, 1990; Moghaddam, 1994; Halberstadt, 1995; Olney and Farber,

1995a,b; Carlsson et al., 1997). The gender-dependent effects of MK801 caution against the assumption that glutamatergic drugs will be equally effective in male and female patients, especially if females are in simultaneous estrogen replacement therapy. The analysis of ontological changes in novelty- and MK801-induced locomotion may help to define normal limbic–motor maturation against which abnormal development, as in the case of schizophrenia, can be compared.

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