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Paradoxical effects of very low dose MK-801

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Abstract

Systemic injection of the noncompetitive NMDA (*N*-methyl-D-aspartate) receptor antagonist MK-801 (dizocilpine maleate) is known to cause increased locomotion and various stereotypic behaviors in rodents. However, the MK-801 dose ranges commonly examined usually begin at tenth of mg/kg and going higher, with the implicit assumption of lower doses being ineffective. We report here that very low dose MK-801, well below the commonly studied doses, exert distinct effects on rodent behaviors. In C57BL/6 mice, very low dose MK-801 (0.02 mg/kg) has strikingly different effects than higher doses commonly reported in the literature. Locomotion, rearing, grooming, and other behaviors are strongly inhibited, replaced by periods of immobility. This is in contrast to the mobility-enhancing effect of MK-801 at commonly reported dose ranges. The effects of very low dose MK-801 are qualitatively similar to those observed with moderate doses (0.1–0.2 mg/kg) of the typical antipsychotic haloperidol. These results highlight the complexity of the dose–response relation for MK-801-induced behaviors.

Keywords: Dizocilpine maleate; NMDA receptor antagonist; Locomotion; Immobility; Catalepsy; Haloperidol

1. Introduction

It is well known that systemic administration of the noncompetitive NMDA (*N*-methyl-D-aspartate) receptor antagonist MK-801 (dizocilpine maleate) causes enhanced locomotion in rodents and, at higher doses, stereotypic behaviors including head weaving and uncoordinated, ataxic gaits (Clineschmidt et al., 1982; Deutsch et al., 1997). The interest in behavioral effects of drugs such as MK-801 stems in part from the increasing use of NMDA receptor antagonists as animal models of schizophrenia. This is based on observations in humans that NMDA receptor antagonists mimic the symptoms of schizophrenia, including psychotic behaviors. In laboratory animals, both typical and atypical antipsychotics antagonize the behavioral effects of NMDA receptor antagonists. It has therefore been proposed that some aspects of schizophrenia may reflect a relative deficit in glutamatergic pathways (Kim et al., 1980; Javitt, 1987; Tiedtke et al., 1990; Carlsson and Carlsson, 1990; Moghaddam, 2003). The ability of various drugs to modulate MK-801-induced locomotion and stereotypy has been employed as a preclinical test for screening new antipsychotic compounds.

MK-801 enhancement of locomotion has been the focus of most studies reported in the literature. On the other hand, doses of MK-801 that are lower than those capable of stimulating locomotion are much less studied, and it is often assumed that such doses do not affect rodent behaviors. This view was justified by a lack of effect of MK-801 doses that are several times lower (e.g. 2–4 times lower) than the lowest

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dose required to stimulate locomotion. However, in our previous study that examined the commonly used dose range of MK-801 (Wu et al., 2005), we observed that rearing behavior in mice was suppressed by MK-801 at 0.02 mg/kg, a dose that was much lower than the doses at which MK-801 increased locomotion and produced stereotypy. Also surprising was the finding that the dose–response relation to suppress rearing was not monotonic: rearing was suppressed by 0.02 mg/kg MK-801, was not affected by a higher dose of 0.05 mg/kg, and was again suppressed by doses of 0.15 mg/kg and higher. Because doses lower than 0.02 mg/kg were not studied (Wu et al., 2005), it was not clear whether the rearing-suppressing effect of the very low dose MK-801 at 0.02 mg/kg was due to an aberrant data point, or whether it represented an intrinsic property of MK-801. The present study was aimed at characterizing in more detail the behavioral effects of very low doses of MK-801, i.e., doses that neither increase locomotion nor produce stereotypy. We examined a number of behavioral effects over a wider range of very low doses of MK-801 and report here several paradoxical behavioral effects that are reliably observed at MK-801 doses that are an order of magnitude lower than those commonly used to stimulate locomotion.

2. Materials and methods

2.1. Chemicals

MK-801 was obtained from Tocris Cookson Inc., Ellisville, MO. Stock solutions were made by dissolving MK-801 in saline, and aliquots were stored as a 4 mg/ml stock solution at −70 °C. Predilution of stocks with saline to the final injection concentrations was done at the beginning for each experiment, and these solutions were stored at 4 °C. Dilutions were designed to give injection volumes of 100 μl per 20 g of animal weight. Haloperidol was from Sigma-Aldrich, St. Louis, MO.

2.2. Animals

Male C57BL/6 mice age 6 weeks, weighing 17–21 g, were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. Animals were housed in standard housing conditions (in plastic cages with sawdust bedding and free access to food and water, 12 h light–dark cycle) for 3–4 days before experiments were conducted. Principles of laboratory animal care were followed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996), the PRC National Standards for Laboratory Animal Quality, and Guidelines for the Use of Experimental Animals.

2.3. Automated locomotion measurements

Automated locomotion measurements were essentially the same as previously reported (Wu et al., 2005), using an automatic beam-crossing detection system from San Diego Instruments. Briefly, locomotion measurements were obtained by automated detection of infrared beam crossings. Animals were placed in a 48 × 24 × 20 cm rectangular chamber covered by glass. Horizontal locomotion was measured as transitions between eight equal areas of the chamber. Rearing behavior was detected by a second set of photodetectors mounted at 7.7 cm above the ground.

Animals were injected i.p. with saline or MK-801, then placed immediately into the recording chamber. Hence, the recordings include the initial period of adaptation to the novel environment. The recording period was 2 h. Data are presented as beam breaks per 5-min period unless otherwise indicated. Different mice were used to test each dose of MK-801.

To show the reproducibility of the results, and to minimize the potential effect from other confounding factors in light of the narrowness of the dose effect of MK-801 around 0.02 mg/kg, the entire experiment was repeated four times, over a period of 9 months. For each experiment, a new batch of mice were purchased from the supplier, and each mouse was tested only once. That is, each mouse was only administered one dose of saline or the drug for behavioral tests and was then sacrificed.

Haloperidol effects on mouse locomotion and immobility behaviors were examined in ways similar to those for MK-801. Each animal was injected i.p. with the appropriate dose of saline or haloperidol, then placed into a recording chamber. Data are presented as beam breaks per 5-min period unless otherwise indicated. Each mouse was used only once to test the effect of haloperidol.

2.4. Videotape analysis of specific behaviors

Animal behaviors in the locomotion measurement chamber were also videotaped. Hence, for each animal, locomotion data and other behaviors were obtained simultaneously, but independently of each other. No stereotypic or ataxic behaviors were observed at the MK-801 doses used in this study. Stereotypy was defined as previously described (Wu et al., 2005) and included behaviors such as head weaving, rotation, and other abnormally repetitive actions. Ataxia was defined as previously described (Wu et al., 2005) and included behaviors such as unsteady gait, hind limb spreading out, and body rolling or falling from time to time when moving.

2.5. Grooming

Grooming includes both the entire set of grooming activities (grooming from head to tail: beginning with grooming the head, then body, then licking the genital area, tail, and/or hind legs to end the individual grooming bout) as well as particular isolated grooming activities (e.g. “washing face” motion, licking foot or genitalia, and using mouth or foot to scratch the body). Mice were videotaped for 2 h in locomotion measurement chambers, and the videotapes were analyzed offline to measure grooming behaviors from 30 min to 90 min. The measured grooming parameters were the number of completed grooming bouts and

the cumulative time during 30–90 min spent on any grooming activities.

2.6. Immobility

Interspersed with locomotor activity, mice sometimes displayed periods of immobility. We recorded immobility behavior from 30 to 90 min after saline or drug injection and measured the cumulative duration of the immobility. For this measurement, timing of immobility started whenever the animal was immobile for more than 2 s. We also measured the time that elapsed before the mouse was first observed to be immobile for 1 min or longer. If during the 30–90-min measurement period, a mouse did not display the 1-min-long durations of immobility, then the elapsed time for this animal was recorded as 90 min (the measurement cut-off time value). These procedures were used for both MK-801 and haloperidol effects.

2.7. Holding bedding in mouth

We observed that mice would pick up pieces of bedding in their mouths and carry them around the chamber. We recorded the first time each mouse displayed the behavior of holding bedding in mouth. As for the other videotaped behaviors, this measurement was made during the 30–90-min time period, and if an animal did not display this behavior during this period, a cut-off value of 90 min was used.

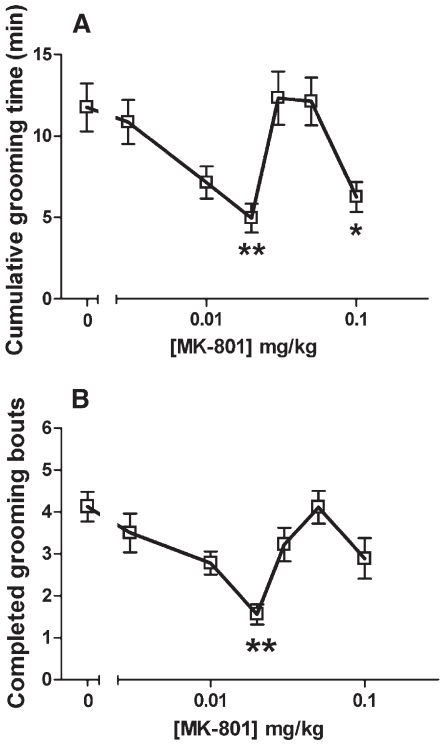

Fig. 1. Grooming behavior is attenuated by very low dose MK-801. (A) Cumulative grooming time from 30 min to 90 min after administration of saline or MK-801. (B) Number of completed grooming bouts from 30 min to 90 min after administration of saline or MK-801. *$P<0.05$ vs. saline (marked as "0" on abscissa for MK-801 doses); **$P<0.01$ vs. saline.

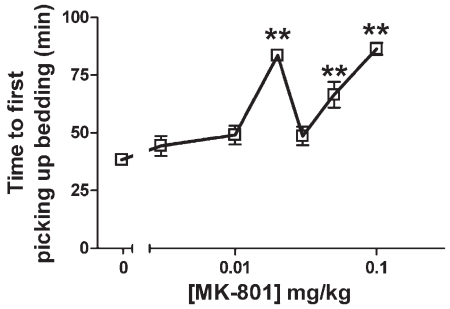

Fig. 2. The behavior of picking up bedding in mouth occurs later at very low dose MK-801. Observations were recorded 30 min to 90 min after administration of saline or MK-801. The cut-off time is 90 min. **$P<0.01$ vs. saline (marked as "0" on abscissa for MK-801 doses).

2.8. Catalepsy

Mice were gently handled and exposed to the testing environment for several times before measurements were taken. Catalepsy was evaluated as reported (Sanberg et al., 1988). The animal was positioned with both front paws over a horizontal bar (4 mm in diameter) elevated 4 cm from floor. The cumulative time that the animal maintained this position (either both paws or one paw on the horizontal bar) was recorded for 3 min. Catalepsy was considered finished either when the mouse removed both forepaws from the bar or when the mouse climbed onto the bar.

2.9. Data analysis

Averaged data are shown with error bars indicating the S.E.M. Effects of different doses of MK-801 or haloperidol were compared to saline controls using one-way analysis of variance (ANOVA) with Dunnett's post hoc test (Figs. 3–5). For comparison of the time course of locomotor effects at different doses, a repeated-measures ANOVA with Dunnett's post-test was used (Figs. 1 and 2). Significance is ascribed for $P<0.05$. Unless otherwise indicated, each experimental group had 8–10 animals.

3. Results

3.1. Behavioral changes by very low dose MK-801

In exploring MK-801 effects on mouse behavior, we tested very low doses of MK-801, using doses that are well below the commonly employed dose range. Animals were injected with saline or MK-801, placed individually in observation chambers, and were videotaped for behavioral analysis. As shown in Fig. 1, grooming behavior was attenuated at a dose of 0.02 mg/kg, for both cumulative time spending on grooming (Fig. 1A) and the number of completed grooming bouts (Fig. 1B). Interestingly, at a higher dose of 0.1 mg/kg MK-801 that is more commonly used and is known to impact rodent behavior, grooming time was also reduced (Fig. 1A). Doses intermediate between 0.02 mg/kg and 0.1 mg/kg did not show significant changes from saline (Fig. 1).

We observed that mice sometimes picked up pieces of bedding in the mouth and carried them around the chamber. We thus analyzed the videotapes to determine when such behavior would first appear. In saline-injected mice, this behavior would first occur around 40 min (Fig. 2). A significant delay of this behavior was observed in mice injected with the very low dose MK-801 at 0.02 mg/kg (Fig. 2). The delay disappeared at higher doses, but was again evident at more "traditional" doses of around 0.1 mg/kg MK-801. Thus, these results suggest that the dose–response relation for MK-801 is rather complex, with very low dose MK-801 displaying behavioral effects previously not reported in the literature.

3.2. Locomotion is suppressed by very low dose MK-801

Since MK-801 is well known for altering rodent locomotion, we examined the effect of very low dose MK-801 on mouse locomotion. The effects on horizontal locomotion of various doses of MK-801 are shown in Fig. 3. As we previously reported (Wu et al., 2005), MK-801 doses around 0.1 mg/kg enhanced locomotor behavior throughout the 2-h recording period. Most of the lower doses had no significant effect on locomotion; however, MK-801 at the very low dose of 0.02 mg/kg resulted in marked reduction of locomotion (Fig. 3A), opposite of the locomotion-stimulating effect for commonly used higher doses (for example, see Wu et al., 2005). Combined data from the 30–90-min time period are shown in Fig. 3B. The locomotion data for this time period were shown because it was also the time period used to analyze videotaped behaviors (see above and below). However, similar results were observed when the entire 2-h recording period was analyzed (data not shown). To ensure reproducibility of the results in light of the narrowness of the dose effect of MK-801 around 0.02 mg/kg, we repeated the experiment four times over a period of 9 months, and the summary data from all four experiments were shown in Fig. 3C. The relative locomotion ratio represents the proportional ratio over the baseline locomotion, and such ratios from the four experiments were calculated to obtain the mean and S.E.M. for each MK-801 dose. A ratio of 1 would be expected if the locomotion at particular MK-801 dose did not differ from that of saline control. We observed consistent suppression of locomotion by 0.02 mg/kg MK-801 (Fig. 3C), with an average ratio for the four experiments of 0.64±0.08 (mean±S.E.M., $n=4$), which is significantly different from 1 ($P<0.01$). This supports the reproducibility of the locomotion suppressing effect by very low dose MK-801 at 0.02 mg/kg.

3.3. Rearing is suppressed by very low dose MK-801

When examining rearing behavior, we found that very low doses of MK-801 that reduced locomotion also suppressed rearing (Fig. 4A). Similar to the effects on locomotion, the dose–response relation of MK-801 on rearing was also non-monotonic: Rearing during the 30–90-minute time period was significantly suppressed at very low doses of MK-801 (0.02 and 0.03 mg/kg) as well as at the higher dose of 0.1 mg/kg compared with saline controls, but the intermediate dose of 0.05 mg/kg

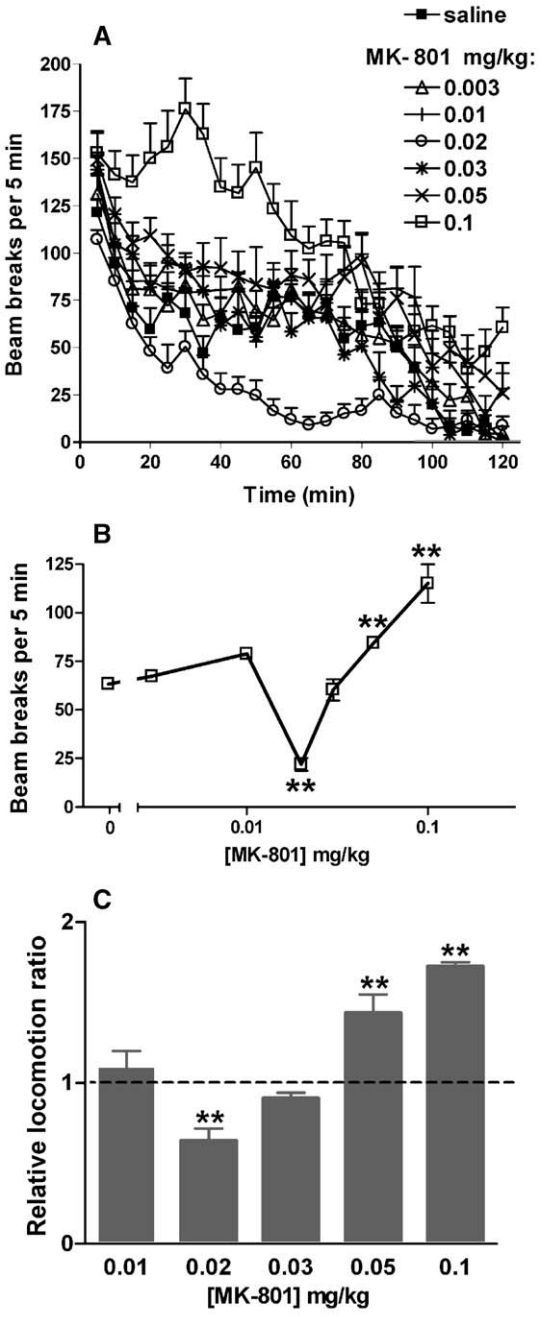

Fig. 3. Locomotion is suppressed by very low dose MK-801. (A) Beam breaks were binned into 5-min intervals. The doses of MK-801 are indicated. Data are shown as mean±S.E.M. (B) Dose effect of MK-801 on locomotion. Locomotion data from 30 min to 90 min after MK-801 administration were analyzed. **$P<0.01$ vs. saline (marked as "0" on abscissa for MK-801 doses). (C) Relative locomotion ratios show the summary data from four independent experiments conducted over a period of 9 months, to ensure the reproducibility of the MK-801 effect at 0.02 mg/kg. For each experiment, locomotion data at a given MK-801 dose was divided by the baseline locomotion data (saline-injected animals) to give a relative locomotion ratio, and such ratios from the four experiments were calculated to obtain the mean ratio and S.E.M. for each MK-801 dose. **Significantly different from a ratio of 1 ($P<0.01$).

MK-801 had similar level as saline control (Fig. 4B). Such complex dose–response relation was also present if the time period analyzed was the first hour of the experiment, when rearing was most evident (data not shown).

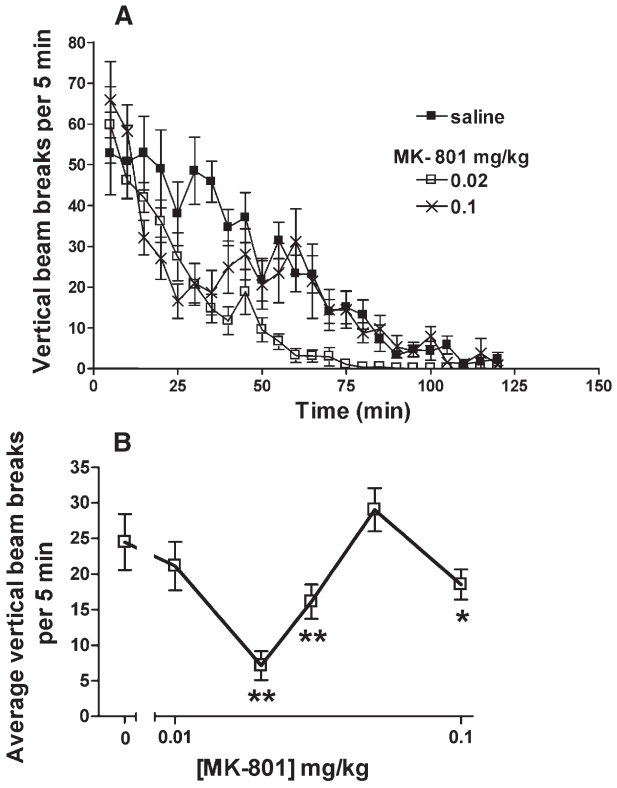

Fig. 4. Rearing is suppressed by MK-801 both at very low and higher doses. (A) Incidence of rearing was binned into 5-min intervals. Data are shown as mean± S.E.M. For clarity, only selected doses are shown; most other doses largely overlapped with the saline data. (B) Summary of dose effect on rearing behavior during the 30–90-min time period. *$P<0.05$ vs. saline (marked as "0" on abscissa for MK-801 doses); **$P<0.01$ vs. saline.

3.4. Very low doses of MK-801 cause immobility in mouse that resemble haloperidol effect

In addition to reduced locomotion, very low dose MK-801 also resulted in periods of total immobility, with episodes lasting from a few seconds to over 1 min. Latency to the first episode of 1-min (or longer) immobility was measured, and the latency was significantly shorter for very low dose MK-801 at 0.02 mg/kg (Fig. 5A). When the cumulative time of immobility (for episodes of 2 s or longer) was tabulated, very low doses of MK-801 (0.02 and 0.03 mg/kg) showed significantly longer time of immobility (Fig. 5B).

Since MK-801 effect in rodents is considered a model of psychosis, we compared the behaviors by very low dose MK-801 with those by haloperidol, a typical antipsychotic. Both locomotion and rearing were inhibited by haloperidol, and the inhibitory effect was progressive as the doses of haloperidol increased (Fig. 6A and B). Haloperidol also elicited immobility (Fig. 6C and D), quite similar to that caused by very low dose MK-801 at 0.2 mg/kg (levels indicated by dotted lines in Fig. 6). However, unlike MK-801, haloperidol at higher doses caused even further increases in immobility, which occurred earlier than with lower doses of haloperidol. In addition, haloperidol was also able to induce overt catalepsy, as measured by the conventional bar test (Sanberg et al., 1988) (data not shown). No cataleptic effects were observed for any doses of MK-801. Rather, as shown in previous figures, increasing doses of MK-801 led to behaviors generally opposite to those seen at very low doses of MK-801.

4. Discussion

We report here the effects of very low doses of MK-801 (in the range around 0.02 mg/kg) that paradoxically inhibit locomotion and increase immobility in C57BL/6 mice, as well as altering other behaviors. Previous studies have shown that MK-801 stimulates horizontal locomotion and inhibits rearing at the lower end of the commonly used doses (in the range of 0.1–0.5 mg/kg in mice), while at higher doses, ataxia and stereotyped behaviors predominate (Clineschmidt et al., 1982; Deutsch et al., 1997; Wu et al., 2005). However, in a previous study (Wu et al., 2005) we also observed that rearing behavior in mice was suppressed by MK-801 at a very low dose of 0.02 mg/kg. The present study was aimed at determining whether the very low dose effect represented an intrinsic property of MK-801, and characterizing in more detail the behavioral effects of very low doses of MK-801, i.e., doses that neither increase locomotion nor produce stereotypy. We examined a number of behavioral effects over a range of very low doses of MK-801 and found that very low doses of MK-801 inhibited both locomotion (Fig. 3) and rearing (Fig. 4) and caused prolonged periods of immobility

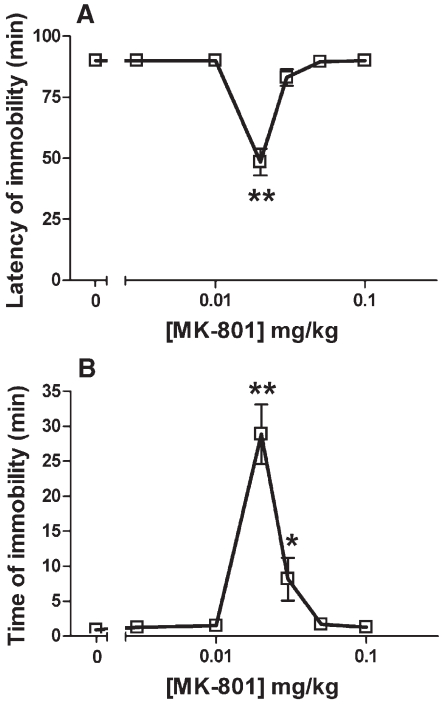

Fig. 5. Immobility occurs sooner and occupies longer cumulative time at very low dose MK-801. (A) Latency of immobility: the time from injection (saline or MK-801) to first appearance of immobility. Observation cut-off time was 90 min. (B) Time of immobility: cumulative time for immobile periods of 2 s or longer were tabulated. Observations were from 30 min to 90 min after administration of saline or MK-801. *$P<0.05$ vs. saline (marked as "0" on abscissa for MK-801 doses); **$P<0.01$ vs. saline.

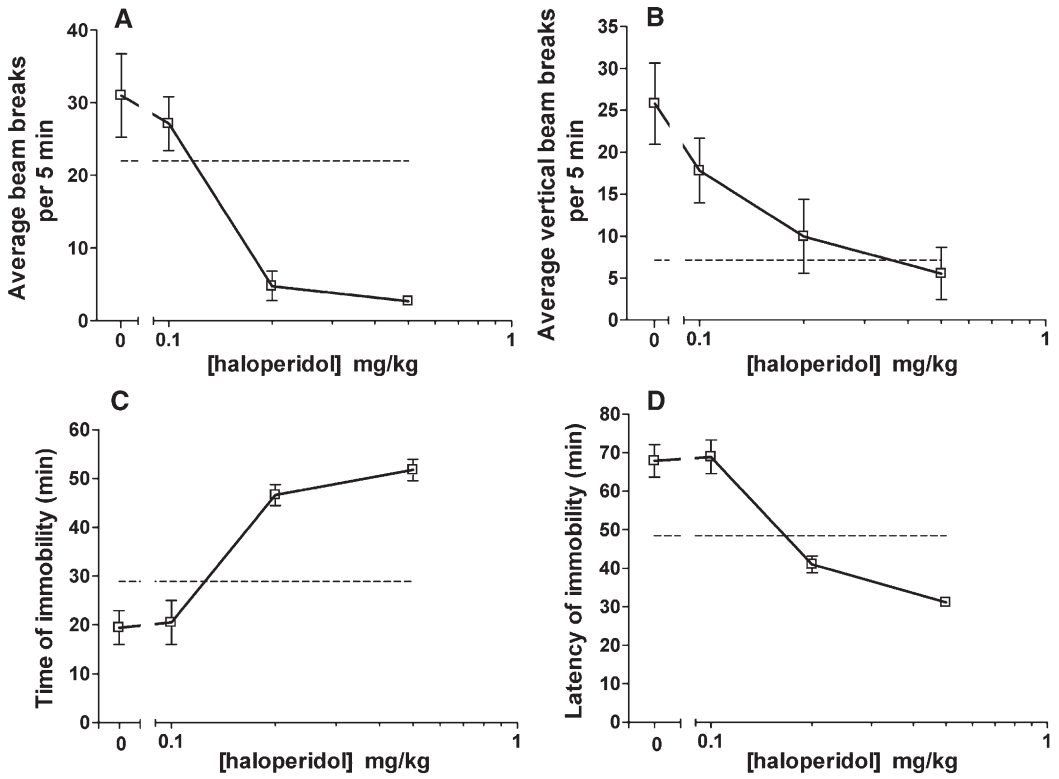

Fig. 6. Comparison of haloperidol-induced behaviors with very low dose MK-801 effects. Effects of haloperidol (open squares) at various doses on (A) locomotion (analyzed from 30 to 90 min, as in Fig. 3); (B) rearing (analyzed from 30 to 90 min, as in Fig. 4); (C and D) immobility (analyzed as in Fig. 5). For comparison, the values observed for 0.02 mg/kg MK-801, taken from the corresponding figures, are indicated by the dashed lines in each panel of haloperidol data. Saline is marked as "0" on abscissa for haloperidol doses.

(Fig. 5). In separate experiments, immobility in a forced swim test was also seen to be lengthened by very low dose MK-801 (unpublished observations). The suppression of locomotion was very robust, and the dose–response relationship correlated well with the effects of very low dose MK-801 on a number of other behaviors, including reduction in grooming (Fig. 1) and a delay in picking up bedding in mouth by the animals (Fig. 2). The dose–response range for these effects was rather narrow, centering around 0.02 mg/kg. To ensure reproducibility of the results in light of the narrowness of the dose effect of MK-801 around 0.02 mg/kg, the entire experiment was repeated four times over a period of 9 months, and we observed the 0.02 mg/kg MK-801's effect in all four experiments; thus, we felt rather confident about the reliability and reproducibility of the findings. This very low dose of MK-801 in our experiments is about an order of magnitude lower than the commonly used doses of MK-801 that effectively stimulate locomotion in this strain of mice (Wu et al., 2005). Our results thus suggest that the effects of very low dose MK-801 represent a previously unknown aspect of MK-801-induced behavioral modulation. In particular, the opposite effects on locomotion by very low dose MK-801 vs. the commonly used dose ranges suggest the complexity of the dose–response relation for MK-801-induced effect.

In contrast to horizontal locomotion, rearing was suppressed at both the very low doses of MK-801 and the more commonly used low dose (Fig. 4). A number of studies suggest that different neural systems mediate the two behaviors of horizontal locomotion and vertical rearing (Miyamoto et al., 1984; Masuo et al., 1995), and our findings are consistent with this notion of two behaviors being mediated differently. However, although MK-801 only suppressed, and never enhanced, rearing behavior, the dose–response curve was biphasic. Rearing was suppressed at 0.02 and 0.3 mg/kg, as well as at 0.1 mg/kg, but not at the intermediate dose of 0.05 mg/kg (Fig. 4). This suggests that different mechanisms are likely to be mediating the suppression of rearing at very low dose vs. low to high MK-801 doses.

Our findings are somewhat paradoxical in that MK-801 is commonly used as an animal model to study the effect of antipsychotics, yet at very low doses, it affected animal behaviors in an opposite manner. The immobility induced by very low dose MK-801 was qualitatively similar, albeit less pronounced, to the overt catalepsy induced by the typical antipsychotic haloperidol. Catalepsy is sometimes used as an animal model to predict extrapyramidal side effects of potential antipsychotics and in a number of rodent studies MK-801 at higher doses actually antagonizes the catalepsy induced by haloperidol (Schmidt and Kretschmer, 1997; Yanahashi et al., 2004). Dopamine antagonists (such as haloperidol) and MK-801 generally have opposing effects on locomotion when MK-801 is administered in the commonly used range of 0.1 to 0.5 mg/kg, which stimulates locomotion

(Irifune et al., 1995; Schmidt and Kretschmer, 1997; Adriani et al., 1998; Leriche et al., 2003). Our results of inhibitory effect on locomotion by very low dose MK-801 highlight the complexity of this drug's effects in animals.

For behavioral effects other than locomotion, two other studies have reported unexpected effects at very low doses of NMDA receptor antagonists (Abel et al., 2003; Jackson et al., 2004), even though at doses normally studied the drugs are considered a good model for many features of schizophrenia. It has been reported that low doses of ketamine, another NMDA receptor antagonist, increased pre-pulse inhibition in normal human subjects (Abel et al., 2003). In another study, in which MK-801's negative effects on working memory were studied in rats (a model of cognitive deficits, a negative symptom of schizophrenia), the very lowest dose of MK-801 studied paradoxically improved working memory (Jackson et al., 2004). Thus, it appears that very low doses of NMDA receptor antagonists may exert behavioral effects that are different from, and sometimes opposite of, the effects by more commonly used drug doses.

Even considering only the behavior of horizontal locomotion as commonly measured, the dose–response relationship for MK-801 is quite complex, ranging from no effect at the very lowest doses we tested, to suppression with immobility, to no apparent effect, to stimulation, and to suppression with stereotypy and ataxia. One plausible explanation for the complexity and wide range of the drug's dose–response curve is the fact that most neurons in the brain express NMDA receptors. The behavioral effect of blocking these receptors will depend on their exact location in particular neural circuits, the degree to which these receptors are activated at the time the blocker is given, and whether the receptors are pre- or post-synaptic. In addition, the sensitivity of NMDA receptors to MK-801 may vary substantially: in NMDA-evoked release measurements (in brain slices), the IC_{50} values for MK-801 have a rather wide range (100-fold or more), depending on the transmitter being studied. This very wide range correlates roughly with anatomical localization of various NMDA receptor subtypes and their MK801 sensitivity in vitro (Nankai et al., 1998). Hence, the lowest doses of MK-801 may block a unique subset of the most sensitive receptor subtypes, whose anatomical location accounts for the decreased locomotion; increasing doses will block an increasingly larger subset of receptors, including some whose effects override the inhibition of locomotion to yield stimulation, then stereotypy, then ataxia. Such diversity in behaviors is likely in part due to the heterogeneity of NMDA receptors at the molecular level, which arises from a large number of genes and splice variants for each subunit and a large number of ways for various subunits to combine into a functional NMDA receptor channel.

In conclusion, we report here that a number of behaviors in mouse, including locomotion, rearing, grooming, and other behaviors, are affected by very low doses of MK-801, well below the commonly studied doses in the literature. These findings highlight the complexity of the dose–response relation for MK-801-induced behaviors.

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