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Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 799–807, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02075-6

A Dose–Response Analysis of the Behavioral Effects of (+)MK-801 in Guinea Pig: Comparison With CPP

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Received 7 May 1995; Revised 20 July 1995; Accepted 7 August 1995

JERRAM, A. H., P. F. SMITH AND C. L. DARLINGTON. *A dose-response analysis of the behavioral effects of (+)MK-801 in guinea pig: Comparison with CPP.* PHARMACOL BIOCHEM BEHAV **53**(4) 799–807, 1996.—The non-competitive *N*-methyl-D-aspartate (NMDA) antagonist, dizocilpine maleate {(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]-cyclohepten-5,10-imine maleate} [(+)MK-801], has attracted considerable interest because of its potential use as an anticonvulsant and neuroprotectant. However, its cognitive side effects in humans have limited its use in human pharmacotherapy. Although the behavioral effects of (+)MK-801 have been documented in mouse, rat, pigeon, and rhesus monkey, there are no available data on its effects in guinea pig. The objective of this study was to conduct a dose-response analysis of the effects of (+)MK-801 on stereotyped behavior, ataxia, locomotor activity, and righting reflex latency in guinea pig. In the dose range used (0.0625–0.5 mg/kg, IP), we found no significant differences between (+)MK-801 and vehicle in terms of stereotyped behavior and locomotor activity; however, at higher doses, (+)MK-801 caused significant ataxia and impairment of the righting reflex. By comparison, the competitive NMDA receptor antagonist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 5 mg/kg, IP), produced neither stereotyped behavior nor impairment of the righting reflex; at higher doses (10 mg/kg, IP), it produced only ataxia and an increase in locomotor activity.

NMDA receptors (+)MK-801 CPP NMDA antagonists Guinea pig

THE NONCOMPETITIVE *N*-methyl-D-aspartate (NMDA) antagonist, dizocilpine maleate {(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine maleate} [(+)MK-801)], has attracted considerable interest because of its potential use as an anticonvulsant and neuroprotectant (1–4,6,16, 20,22,23,31,32). Although its cognitive side effects in humans have limited its use in human pharmacotherapy (2,10,19,20, 23,24), they have, on the other hand, contributed to the development of the hypothesis that reduced NMDA receptor function may be implicated in the etiology of psychosis (10, 14,15,17,19). Because of its sedative properties, (+)MK-801 also causes considerable muscle relaxation and ataxia (7,11, 16,18,26,29,32).

To study the anticonvulsant, neuroprotectant, and other neural effects of (+)MK-801, it is important to have available a dose-response analysis of the general behavioral effects of the drug in the species being studied. Although the behavioral effects of (+)MK-801 have been documented in mouse, rat, pigeon, and rhesus monkey (3,11,18), to our knowledge there are no published studies of its effects in guinea pig. Nonetheless, (+)MK-801 has been used in guinea pigs to study the contribution of NMDA receptors to various forms of plasticity (7,26). The objective of the present study was to conduct a dose-response analysis of the effects of (+)MK-801 on stereotyped behavior, ataxia, locomotion, and righting reflex latency in guinea pigs, to provide a foundation for further studies of (+)MK-801 in this species. The competitive NMDA receptor antagonist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), was used for comparison (1,4,7, 8,12,22,25,26,29,30).

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METHODS

The subjects were 52 adult guinea pigs (316–410 g). They were housed in pairs in an animal holding room with a 12 L : 12 D cycle; food and water were available ad lib. One day prior to the beginning of the experiment, the animals were brought into the laboratory, housed individually, and allowed to adjust to the laboratory conditions in which the experiment would take place. Each animal was then housed individually in the experimental observation box (see below) 1 h prior to the beginning of the experiment.

The animals were randomly divided into seven experimental groups, each of which received a single IP injection of (+)MK-801 or CPP (RBI Inc., NJ). Group 1 (n = 4) received 0.5 mg/kg (+)MK-801; group 2 (n = 4) 0.25 mg/kg (+)MK-801; group 3 (n = 4) 0.125 mg/kg (+)MK-801; group 4 (n = 4) 0.0625 mg/kg (+)MK-801; group 5 (n = 4) 0.125 mg/kg (+)MK-801 + exposure to an auditory stimulus (see below); group 6 (n = 4) 5.0 mg/kg CPP; group 7 (n = 4) 10 mg/kg CPP. The (+)MK-801 and CPP doses were chosen on the basis of our previous studies in guinea pigs (7,25,26). In addition, six control groups (n = 4 animals in each), receiving vehicle injections in a way that corresponded to the first six experimental groups, were used to control for any day-to-day variability in laboratory conditions. These control conditions were run at the same time as the experimental groups, in exactly the same conditions; in all cases the vehicle injection consisted of a 1 ml/kg volume of distilled water. In the case of experimental group 7, the vehicle control group used for group 6 was used. All measurements were made using a double-blind protocol: the vehicle and drug solutions were colour-coded such that neither the person injecting the solutions nor the person observing the animals and making the measurements knew which animals received drug injections and which received vehicle injections.

During testing, animals were placed in a large open box (61 × 61 × 22 cm) with a transparent perspex front panel. To facilitate the behavioral measurements, animals were videotaped using two video cameras (Panasonic NV-M7), each with a zoom lens; one was positioned directly above the animal, the other in front of the perspex window at the front of the box. The signals from the two cameras could be mixed using a Panasonic (Digital WJ-MX10) video mixer and were displayed on a Sony Trinitron color monitor using a split screen. Videotapes were replayed using a Mitsubishi E7 Black Diamond video recorder. Animals were videotaped for 20 min before the drug or vehicle injection and then for 155 min following the injection. With the exception of group 5 [i.e., 0.125 mg/kg (+)MK-801 + auditory stimulus], four behavioral variables were measured for all animals: 1) stereotyped behavior; 2) ataxia; 3) locomotor activity; and 4) righting reflex latency. For group 5, only the first three variables were measured. Stereotyped behavior, ataxia, and locomotor activity were measured using the Contreras et al. (5) modification of the rating scale developed by Sturgeon et al. (28) for the description of phencyclidine-induced behaviors in rats; these measurements were made at 10-min intervals for 20 min before the injection and for 2.5 h following the injection. Righting reflex latency was measured using a custom-made electronic device consisting of a semicylindrical platform positioned on a 2 kg load cell. The load cell transduced changes in load during a righting reflex into a signal that was amplified and displayed as a waveform on a MacClassic computer screen via a MacLab data acquisition system (Analog Digital Instruments). The MacLab system sampled from the amplifier at 20 Hz, giving a measurement resolution of 0.05 s. The latency to generate a righting reflex could be measured using cursors in the Chart program [see (9) for details]. Righting reflex latency measurements were made once prior to the injection and at 30-min intervals thereafter for 2.5 h.

We reasoned that an auditory stimulus might increase stereotyped behavior. In pilot studies, we began using tones of fixed amplitude and varying frequency to stimulate the guinea pig's behavior. However, we found that, in general, guinea pigs were more responsive to sounds that they associated with other guinea pigs. Therefore, we taped guinea pig vocalizations in the guinea pig holding room at feeding time and replayed them through a loudspeaker to group 5, before and following the (+)MK-801 or vehicle injection. The same 5 min excerpt from the tape was played every 15 min, at exactly the same volume.

Data were analysed using two-factor analyses of variance (ANOVA) with repeated measures on time (27). In all cases the significance rate was set at 0.05.

RESULTS

Figure 1D shows that 0.5 mg/kg (+)MK-801 produced no more stereotyped behavior than the control group ($p > 0.05$; Fig. 1D). Locomotor activity was nonsignificantly reduced ($p > 0.05$; Fig. 2D); however, 0.5 mg/kg (+)MK-801 resulted in a significant increase in ataxia ($p < 0.0001$; Fig. 3D) and righting reflex latency ($p < 0.0001$; Fig. 4D).

At a dose of 0.25 mg/kg (+)MK-801, ataxia was still significantly increased in the drug group ($p < 0.0001$), as was righting reflex latency ($p < 0.05$; Figs. 3 and 4). Neither stereotyped behavior nor locomotor activity was significantly different between the drug and control groups ($p > 0.05$; Figs. 1 and 2). At lower doses of (+)MK-801 (0.125 and 0.0625 mg/kg), neither ataxia, righting reflex latency, stereotyped behavior, nor locomotor activity was increased in the drug group ($p > 0.05$; Figs. 1-4). The introduction of an auditory stimulus did not increase stereotyped behavior or locomotor activity following a 0.125 mg/kg injection of (+)MK-801 ($p > 0.05$; Figs. 1E and 2E).

A 5-mg/kg injection of CPP caused no significant stereotyped behavior ($p > 0.05$) nor any increase in ataxia, righting reflex latency, or locomotor activity ($p > 0.05$; Fig. 5). However, a 10-mg/kg CPP injection caused a significant increase in ataxia ($p < 0.05$) and locomotor activity ($p < 0.05$), but no change in stereotyped behavior or righting reflex latency (Fig. 6).

DISCUSSION

The results of the current experiment were surprising in view of the lack of stereotyped behavior and locomotor stimulation produced by (+)MK-801 across the dose range (0.0625–0.5 mg/kg) used. Because (+)MK-801 produced marked ataxia and increased righting reflex latencies at all but the lowest dose employed, it is unlikely that the drug solutions were inactive. Although previous studies have reported stereotyped behavior and locomotor hyperactivity with (+)MK-801 doses used in the present study (0.125, 0.25, and 0.5 mg/kg IP) (11,13), the intensity of the stereotyped behavior was reported to be less than that caused by phencyclidine and amphetamine (5,13). One possible explanation is that the ataxic effects of (+)MK-801 are greater in guinea pigs, thus obscuring the expression of stereotyped behavior and locomo-

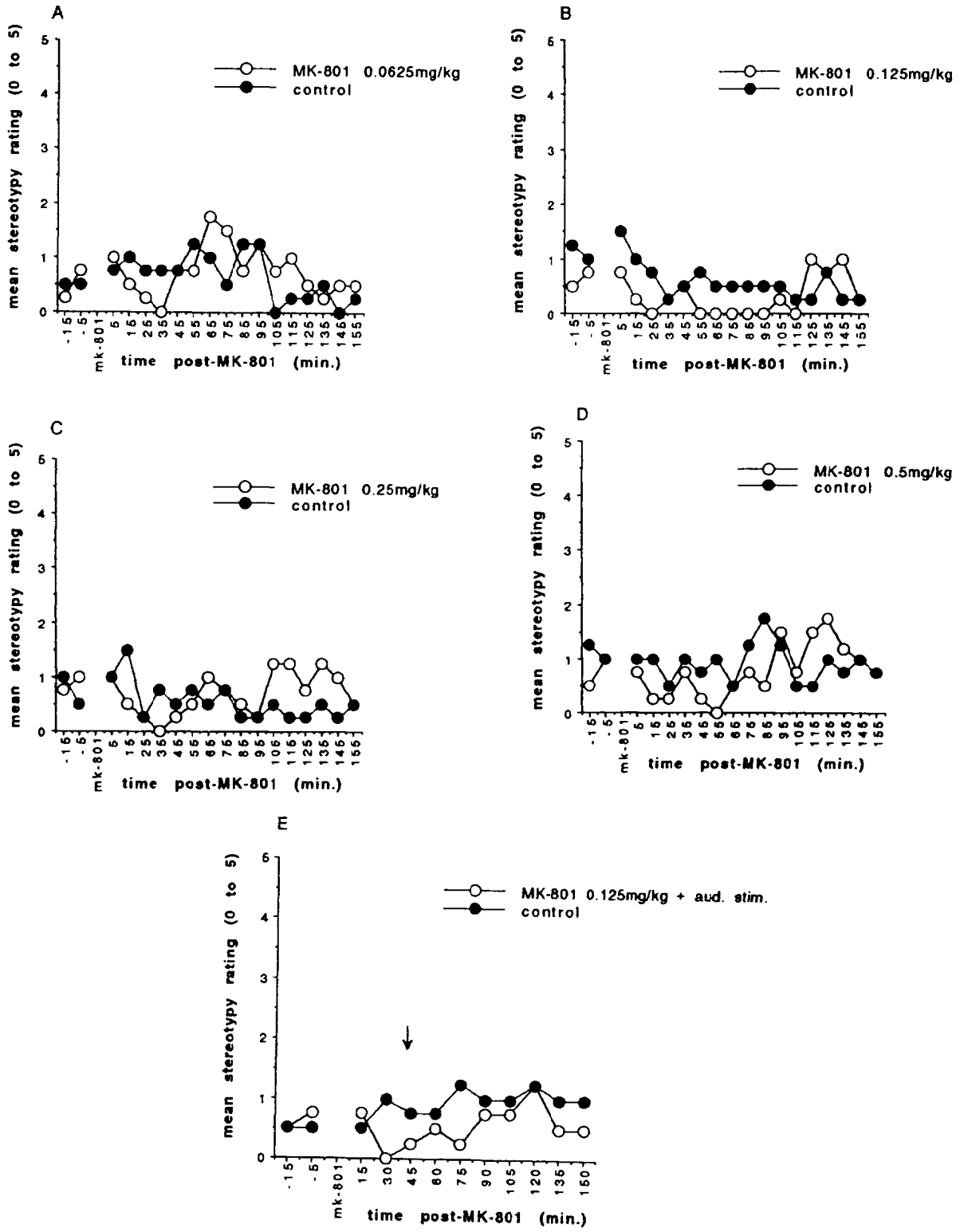

FIG. 1. Time course of effects of (+)MK-801 (IP) on stereotyped behavior at doses of (A) 0.0625 mg/kg, (B) 0.125 mg/kg, (C) 0.25 mg/kg, (D) 0.5 mg/kg, and (E) 0.125 mg/kg + auditory stimulus. Symbols represent mean ratings (0 to 5). Open symbols: (+)MK-801 groups (n = 4). Closed symbols: vehicle controls (n = 4). Arrow indicates the onset of the auditory stimulus.

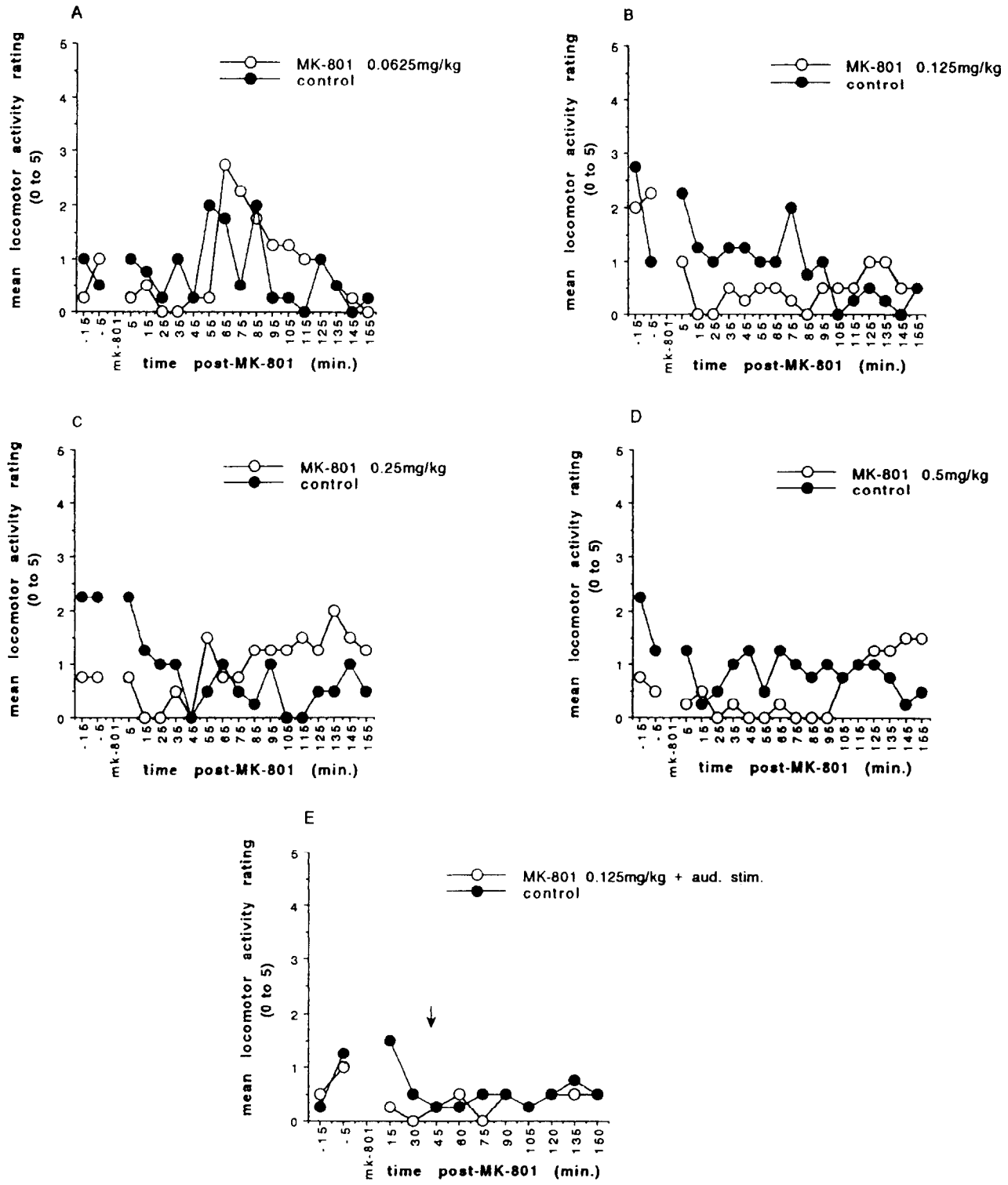

FIG. 2. Time course of effects of (+)MK-801 (IP) on locomotor activity at doses of (A) 0.0625 mg/kg, (B) 0.125 mg/kg, (C) 0.25 mg/kg, (D) 0.5 mg/kg, and (E) 0.125 mg/kg + auditory stimulus. Symbols represent mean ratings (0 to 5). Open symbols: (+)MK-801 groups (n = 4). Closed symbols: vehicle controls (n = 4). Arrow indicates the onset of the auditory stimulus.

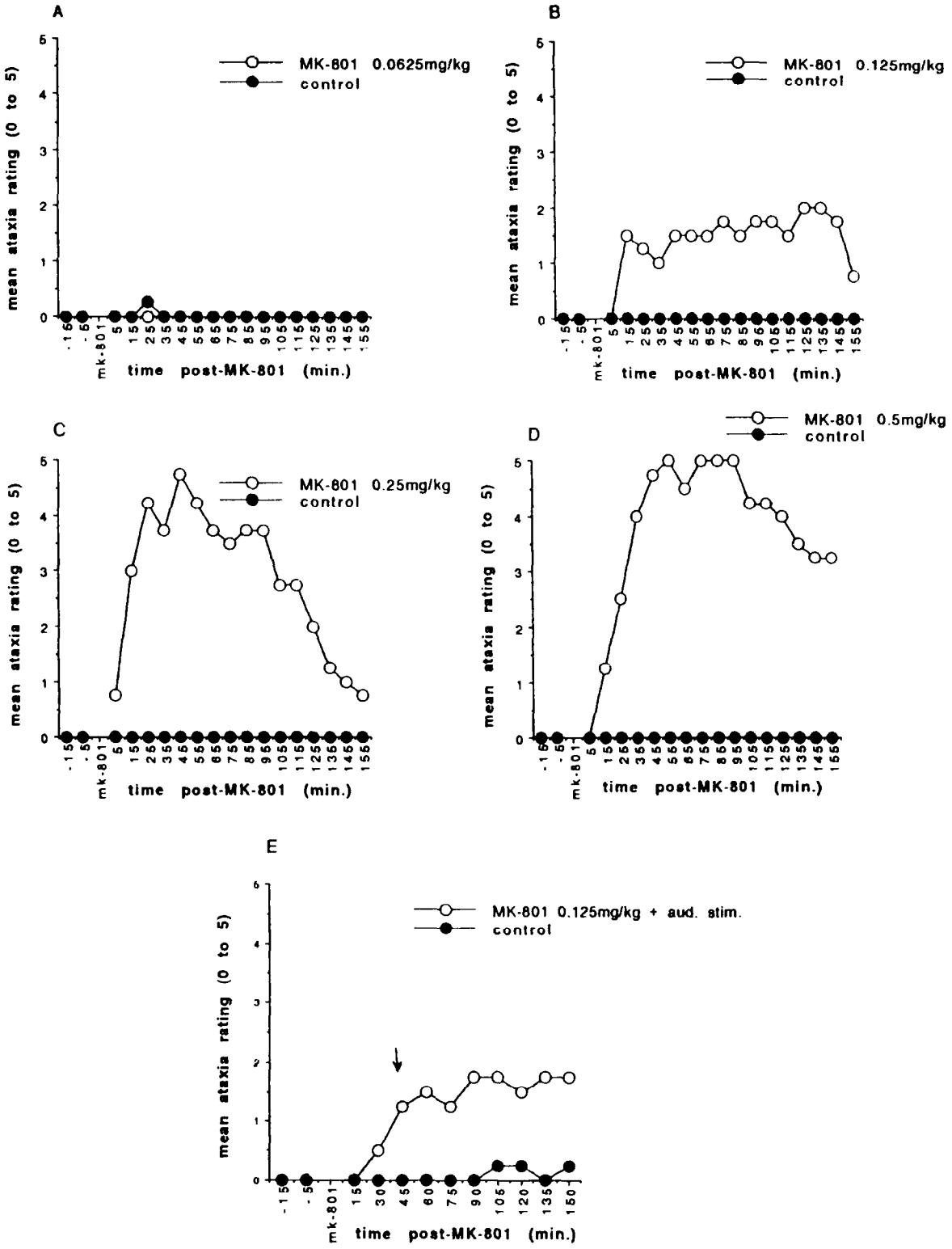

FIG. 3. Time course of ataxic effects of (+)MK-801 (IP) at doses of (A) 0.0625 mg/kg, (B) 0.125 mg/kg, (C) 0.25 mg/kg, (D) 0.5 mg/kg, and (E) 0.125 mg/kg + auditory stimulus. Symbols represent mean ratings (0 to 5). Open symbols: (+)MK-801 groups (n = 4). Closed symbols: vehicle controls (n = 4). Arrow indicates the onset of the auditory stimulus.

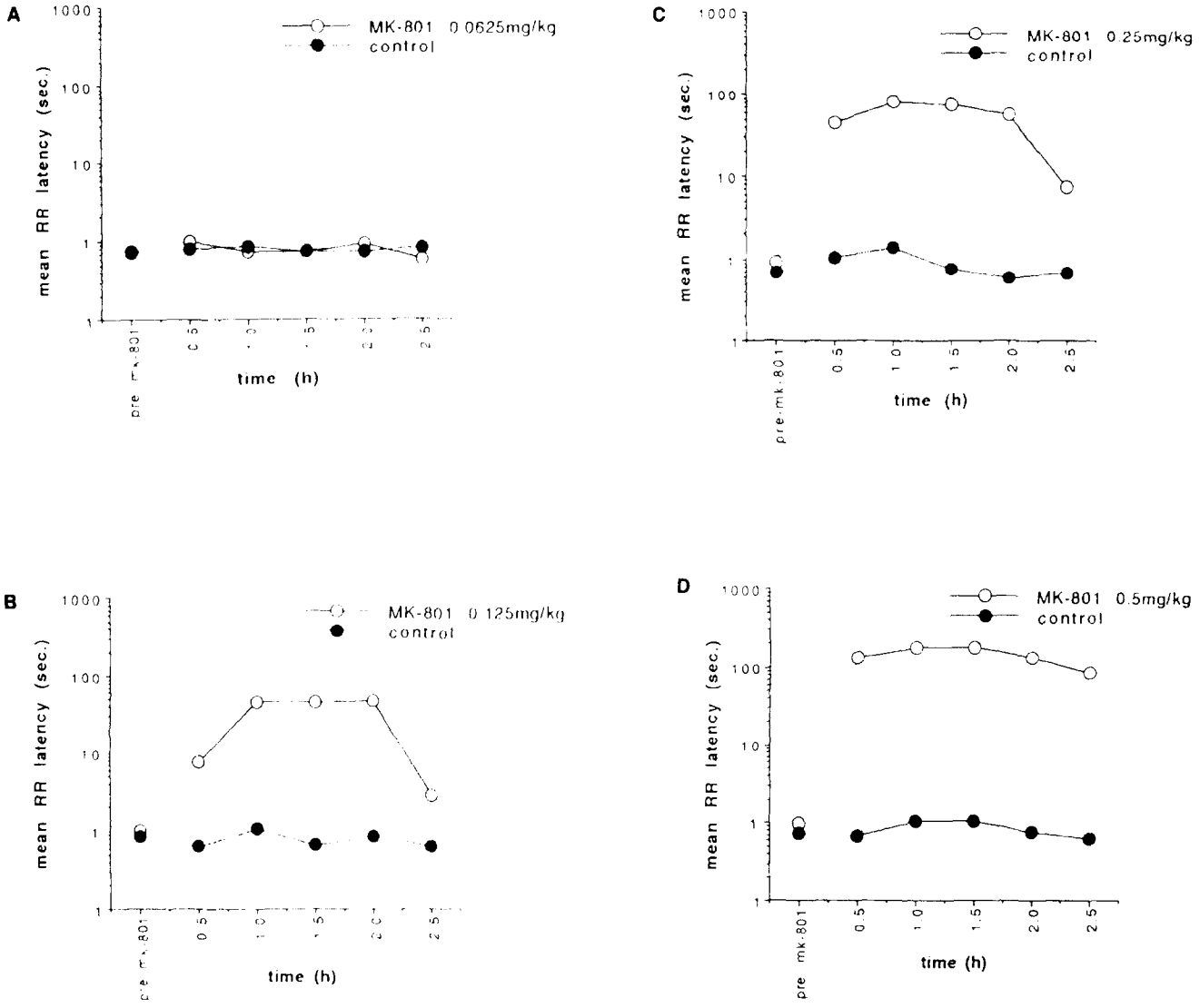

FIG. 4. Time course of effects of (+)MK-801 (IP) on righting reflex latency at doses of (A) 0.0625 mg/kg, (B) 0.125 mg/kg, (C) 0.25 mg/kg, and (D) 0.5 mg/kg. Symbols represent mean ratings (0 to 5). Open symbols: (+)MK-801 groups (n = 4). Closed symbols: vehicle controls (n = 4). RR: righting reflex. h: hours.

tor hyperactivity. However, even using 0.0625 mg/kg, which did not cause significant ataxia or increased righting reflex latency, stereotyped behavior was not evident. Similarly, 5 mg/kg CPP produced neither stereotyped behavior, locomotor hyperactivity, ataxia, nor impairment of the righting reflex. A 10-mg/kg CPP injection produced ataxia and an increase in locomotor activity but no stereotyped behavior.

We think it is unlikely that our methodology was responsible for the nondetection of stereotyped behavior in guinea pigs. Our use of a double-blind measurement protocol provided an additional experimental control in the application of the rating scales for stereotyped behavior, ataxia, and locomotor behavior. Furthermore, each experimental condition was compared with a new control group so that day-to-day variation in laboratory conditions could not influence the amount of stereotyped behavior or locomotion observed following the (+)MK-801 or CPP injections. One possible explanation of our data is that (+)MK-801 produces its cognitive effects in guinea pigs only at relatively high doses (e.g., 0.25–0.5 mg/kg, IP), and at such doses the ataxic and sedative effects of the drug may obscure stereotyped behavior and locomotor hyperactivity. However, the fact that, even at 10 mg/kg IP, CPP also failed to induce stereotyped behavior (but produced ataxia) suggests that antagonism of NMDA receptors by itself may not be sufficient to induce stereotypy; sigma receptor agonists such as (+)-SKF-10,047 may be more effective in inducing stereotyped behavior in the guinea pig (21). Alternatively, it may be that (+)MK-801-induced stereotyped behavior in guinea pig is not as pronounced as in mouse, rat, pigeon, and rhesus monkey (3,11,18). Because the rating scale that was used to quantify stereotyped behavior was based on that used by Contreras et al. (5) to rate phencycidine-induced behaviors in rats, it is possible that its application to guinea pigs was inappropriate. However, from our experience in applying

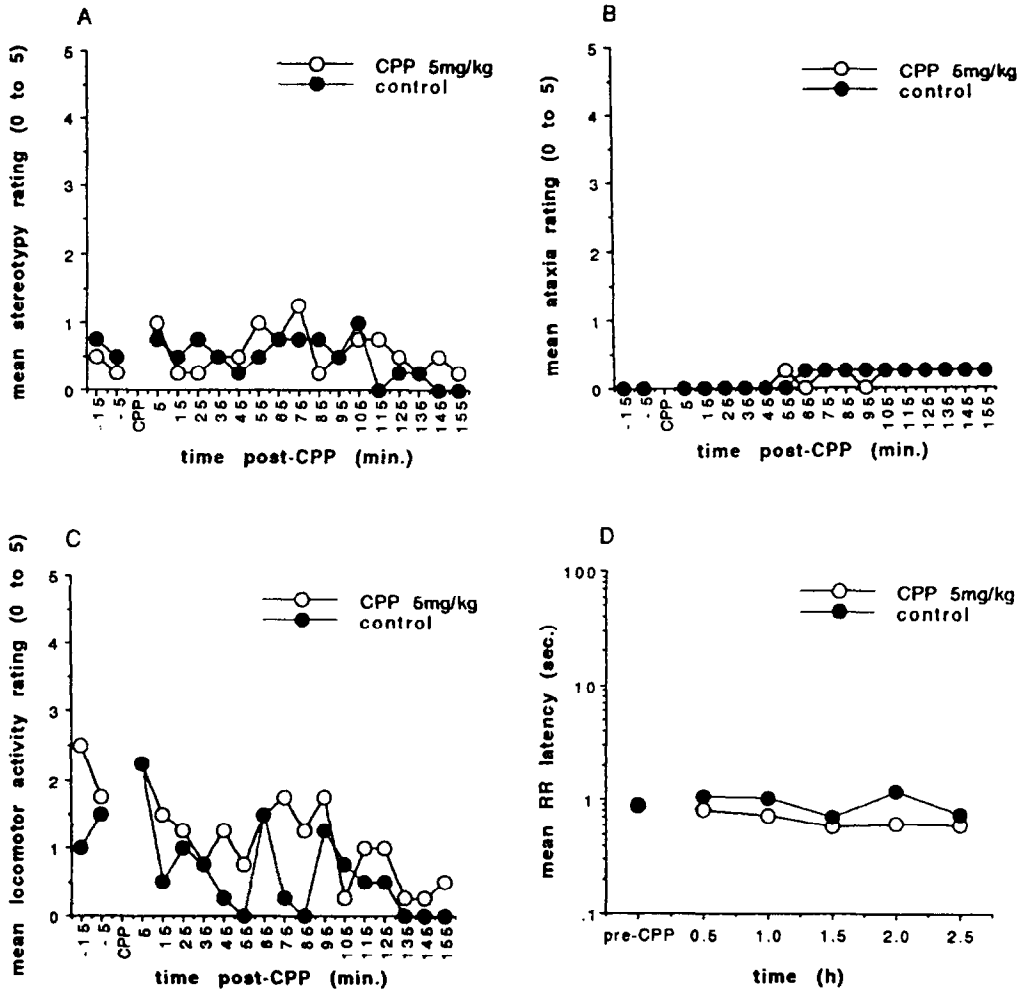

FIG. 5. Time course of effects of 5 mg/kg (IP) CPP on (A) stereotyped behavior, (B) ataxia, (C) locomotor activity, and (D) righting reflex latency. Open symbols: CPP group (n = 4). Closed symbols: vehicle controls (n = 4). Symbols on graphs (A) to (C) represent mean ratings (0 to 5). Symbols on graph (D) represent mean latencies in seconds, expressed on a log scale. RR: righting reflex. h: hours.

this scale it was not clear how it could be modified to be applied more effectively to guinea pigs.

The results of the present experiment emphasise the need to consider the ataxic and sedative side effects of (+)MK-801 when using it to evaluate the contribution of NMDA receptors to various forms of neural plasticity. For example, although it has been demonstrated that both competitive and noncompetitive NMDA receptor/channel antagonists disrupt vestibular compensation in response to deafferentation of the vestibular labyrinth [e.g., (7,25,26)], using higher doses of noncompetitive antagonists such as (+)MK-801 may induce a generalized ataxia that is not directly related to the loss of vestibular compensation.

ACKNOWLEDGEMENT

This research was supported by a Project Grant (to P.F.S. and C.L.D.) from the Health Research Council of New Zealand.

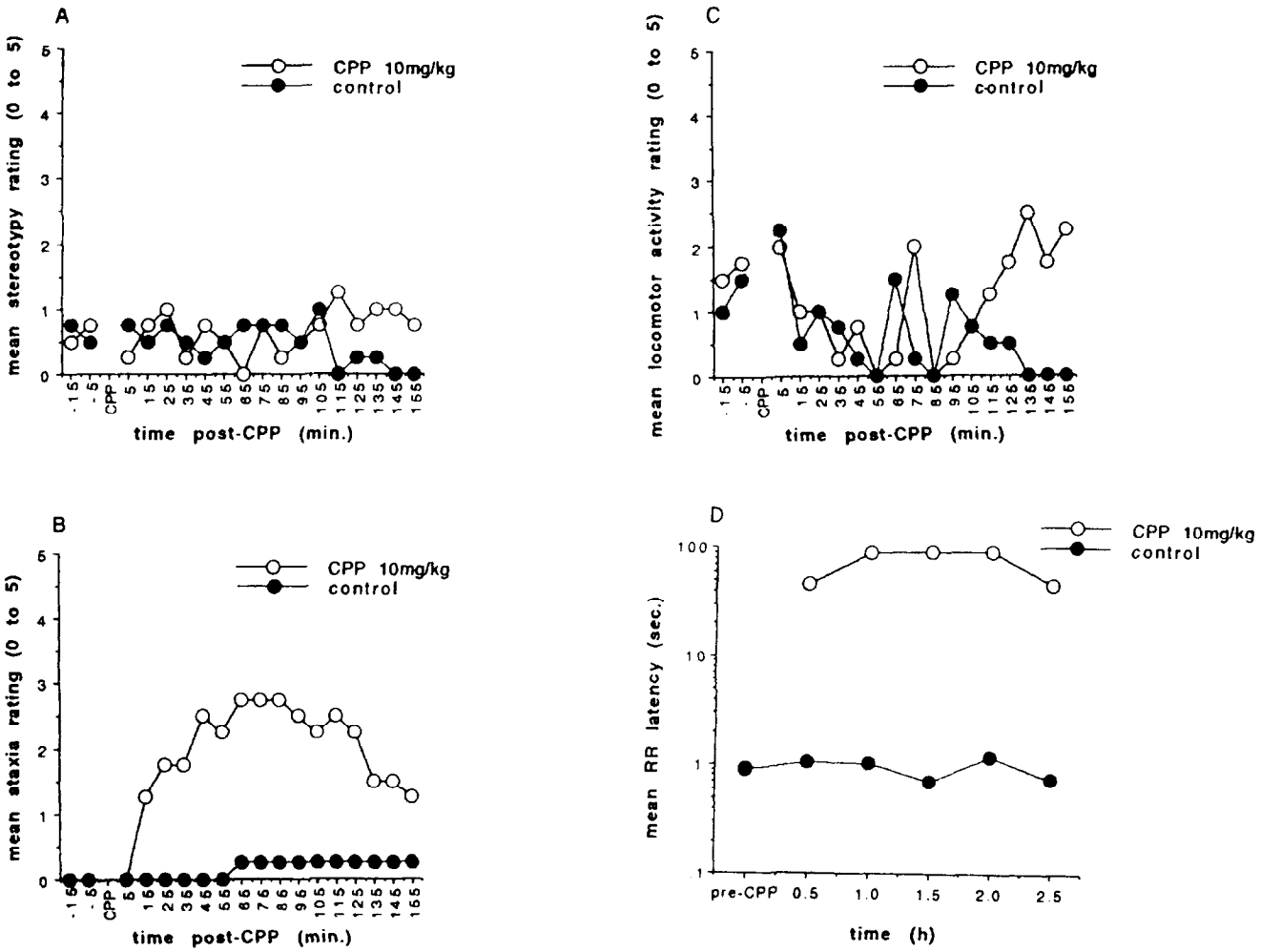

FIG. 6. Time course of effects of 10 mg/kg (IP) CPP on (A) stereotyped behavior, (B) ataxia, (C) locomotor activity, and (D) righting reflex latency. Open symbols: CPP group (n = 4). Closed symbols: vehicle controls (n = 4). Symbols on graphs (A) to (C) represent mean ratings (0 to 5). Symbols on graph (D) represent mean latencies in seconds, expressed on a log scale. RR: righting reflex. h: hours.

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