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Hyperactivity, hyper-reactivity, and sensorimotor deficits induced by low doses of the *N*-methyl-D-aspartate non-competitive channel blocker MK801

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Three doses of MK801 (0.05 mg/kg, 0.3 mg/kg and 1.0 mg/kg) were given systemically to adult male rats, which were then tested on a battery of previously learned, reactive and spontaneous behaviors. Hyperactivity, hyper-reactivity, reductions in rearing behavior and deficits in tongue extension were found at the 0.05 mg/kg dose. Similar, but more severe results were found at the 0.3 mg/kg dose, with the addition of difficulties in climbing, balancing on a beam, and abnormalities in orienting to tactile stimuli. A number of tasks could not be performed at the 1.0 mg/kg dose including tongue extension, orienting, balancing on a beam, and climbing. Additionally, abnormal postures, gaits, and swimming behaviors were observed at this dose. These results characterize the behavioral effects of MK801 as a syndrome of hyperactivity, hyper-reactivity, and sensorimotor deficits. Evidence of this syndrome was present at all three doses, including the 0.05 mg/kg dose, which previously has been claimed to induce deficits similar to hippocampal lesions. Learning literature employing MK801 is discussed in the context of the behavioral deficits found in this study.

INTRODUCTION

In 1984, Morris and Baker[22] outlined four different strategies employed in evaluating the relationship between learning and long-term-potentiation (LTP), an electrophysiological phenomenon that exhibits a functional similarity to the modifiable 'Hebbian' synapse. Of the four strategies evaluated they concluded that the 'Blockade/Facilitation' strategy was the most promising. This strategy entails seeking manipulations that either enhance or impede LTP. If parallel mechanisms underlie both LTP and learning then these manipulations should similarly affect learning. The converse should also be true such that any manipulations affecting learning should also affect LTP in the same way.

Since that time much work has focused on the 'Blockade' aspect of this strategy, particularly the pharmacological blockade of the *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptor subtype. The NMDA receptor has been shown to be critical for both the induction of a number of forms of LTP in the hippocampus[6,8,11] and for the acquisition of tasks that are sensitive to hippocampal damage[21,30].

The NMDA non-competitive channel blocker MK801 has become extensively employed in the current 'plasticity and learning' literature that employs the blockade strategy. MK801 has been shown to impair the induction of LTP in vitro[7] and in vivo[1,4]. Additionally, MK801 has been shown to impair the acquisition of tasks that are also impaired by hippocampal lesions[5,15,26,29,35].

In a re-appraisal of the NMDA receptor as a link between LTP and learning, Keith and Rudy[16] critique both studies that used D-amino-phosphono-valeric acid (APV), the competitive NMDA blocker[21,30], for not clearly ruling out sensorimotor impairments and behavioral abnormalities as the cause of the acquisition deficits. Morris[19] has expressed similar concerns about his own earlier work[21] and since done a number of experiments with AP5 to separate these effects[19]. Keith and Rudy[16] further critique the experiments done using MK801[26], for not being able to demonstrate that equivalent doses of MK801 block both LTP and learn-

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ing. However, since that time, reductions in the duration of LTP at doses previously claimed to selectively impair learning have been demonstrated[27].

Consequently it becomes appropriate to analyse the effects of MK801 for sensorimotor impairments and behavioral abnormalities. A number of recent studies using MK801 report that doses greater than 0.2 mg/kg result in gross behavioral abnormalities[12,35,39]. Additionally, several studies state that a number of doses lower than 0.1 mg/kg do not interfere with behavior[5,26,27,33,39]. However, two other studies note hyperactivity at 0.07 and 0.0625 mg/kg doses of MK801, but argue that this effect is also seen after hippocampal lesions and thus support the idea that MK801 acts as a functional hippocampal lesion[15,29].

Therefore, we undertook a behavioral assessment of MK801 using a battery of previously learned, reactive, and spontaneous behaviors. Specifically, we included a dose of MK801 as low as or lower than those doses claimed to uniquely induce 'hippocampal-like' learning deficits[5,15,26,29] and a dose high enough to effectively block in vivo LTP[1].

MATERIALS AND METHODS

Subjects

Twelve male adults rats (Long-Evans derived) were used. They were housed individually in suspended wire mesh cages, in a standard temperature-controlled colony room. The room was kept on a 12 h light/dark cycle, with lights out at 20.00 h and lights on at 08.00 h. All animals had free access to water and food (Purina lab chow).

Battery of behaviors

The test battery consisted of: two tests in the home cage, tongue extension[36] and somatosensory orienting[37]; a multivariate analysis of spontaneous locomotor behavior using a Digiscan automated activity monitor system[14,28]; a simple swim and vertical climbing task[34]; and systematic observations of table-top and edge walking and reactivity to handling. The tests making up this battery were chosen on the basis that they involved behaviors used in the execution of the radial-arm maze or Morris water maze, or that they examined behaviors that were particularly sensitive to sensory and motor deficits.

General procedure

Prior to testing the rats were extensively handled and habituated to the various apparatus, injection schedules and test procedures. For the swim and climb task and tongue extension task the animals were trained over a number of days to acquire the behaviors.

MK801 ((+)-5-methyl-10,11-dihydro-5*H*-dibeno(*a*, *d*)cyclohepten-5,10-imine maleate; Merck Sharp and Dohme Research Labs) was delivered via i.p. injection, in a 2 ml/kg volume saline vehicle, at 0.05, 0.30 and 1.00 mg/kg doses, which were run in an ascending series to prevent residual effects from higher doses of the drug. The 1.00 mg/kg dose was chosen for its ability to block in vivo LTP[1,27], without the potentially lethal side effects of higher doses[1,35]. The 0.30 mg/kg dose was chosen on the basis of its reported ability to block exploration-dependent changes in the hilar evoked potential, without interfering with normal synaptic transmission[10]. As stated above, the 0.05 mg/kg dose was chosen as the lowest dose claimed to induce unique learning deficits[5,15,26].

The rats were split into two groups of six, which were run on alternate days during the testing period. Within each group, half the rats received MK801 first and the other half received the vehicle first. This application of treatments was reversed when the animals were run again 48 h later. In this way all animals were tested at the three doses of MK801, and underwent a saline control session for each dose of MK801. During a given test session animals were injected and tested 30 min later in the following sequence: sensorimotor orienting and tongue extension, followed by the Digiscan activity system, table-top walking, the swim and climb task, and edge walking.

Tongue extension. Rats learned to consume a chocolate chip cookie slurry (coarsely ground chocolate-chip cookies mixed with milk) by first presenting it in the home cage as a food supplement, and then on a spatula presented through the wire mesh of the cage. This was continued until the animals were reaching through the mesh to access the slurry. Finally, the chocolate chip cookie slurry was presented to the rats on the end of a ruler place against the wire mesh of their home cage. This procedure took eight days, at which time three baseline days were taken followed by drug testing. The portion of the ruler that was licked clean in millimeters was recorded as the amount of tongue extension[36].

Sensorimotor orienting. Cotton swabs were used to probe the portion of the rats' feet that protruded through the floor of the suspended wire mesh cage. This procedure was done to all four feet, and repeated two to three times. Care was taken to avoid having the animals observe the approach of the cotton swab. The vigorousness of each rat's response to the probe was recorded[37]. The animals were habituated to this procedure over a four day period prior to drug testing.

Assessment of spontaneous locomotor activity. This

assessment was accomplished using six Omnitech Digiscan Animal Activity Monitors (model no. RXYZCM [16]). The Digiscan activity monitor is an automated 40 × 40 × 30.5 cm open-field system with a grid of infrared beams mounted horizontally every 2.54 cm, and a second tier of beams mounted 11.5 cm above the floor to measure vertical (rearing) activity. The pattern of beam interruptions was recorded and analyzed by an Omnitech Analysis unit (model no. DCM-8) and then passed on to a microcomputer where it was stored on disk[28].

The rats were habituated to this apparatus over a period of ten days. Initially, rats were placed in the monitors and left alone for periods up to 2 h. On the last four days of habituation the data collection protocol for drug testing was followed, but no data were collected. During drug testing six samples of 5 min were collected from each animal per session. Nine locomotor activity variables were directly obtained or computationally derived from the obtained variables:

Number of horizontal movements (NM). Each time a cessation in horizontal activity occurred for a period of greater than one second, this variable was incremented by one. This indicated the number of separate horizontal movements executed by the animal in a given sample period.

Time spent in horizontal movement (MT). As long as the animal was moving, this variable was incremented. If the animal was stationary for more than one second, this parameter was no longer incremented. Thus, it corresponds to the amount of time the animal was in motion during a given sample period.

Time spent per horizontal movement (TM). This variable was derived from Number of horizontal movements and time spent in horizontal movement using the formula [MT/NM] = TM.

Total distance travelled (TD). The distance travelled was computed from the pattern of interruptions of infrared beams.

Average distance travelled per movement (AD). This variable was derived from Total distance and Number of movements using the formula [TD/NM] = AD.

Average speed per movement (AS). This variable was derived from Total distance and Time in movement using the formula [TD/MT] = AS.

Number of vertical movements (VM). Each time the animal reared up and interrupted the second tier of infrared beams, this variable was incremented by one. The animal was required to go below the level of the vertical sensors for at least one sec before the next rearing was registered.

Time spent in vertical movement (VT). When the animal activated the vertical tier of sensors by rearing, this variable started to increment and continued to increment until the animal went below the level of the vertical sensors.

Time spent per vertical movement (TV). This variable was derived from Number of vertical movements and Time spent in vertical movement using the formula [VT/VM] = TV.

Swim and climb task. A glass aquarium measuring 43 × 90 × 45 cm deep was filled to a depth of 25 cm with approximately 20 °C water. A wire mesh (13 mm squares) was folded over one end of the tank such that it formed a vertical wall with a horizontal resting platform at the top. A trial was started by placing a rat in a plexiglass holding box located at the end of the tank opposite to the wire mesh wall for 15 s. The rat was then released at that end of the tank and required to swim to the wire mesh and climb to the top, where it would be allowed to rest for another 15 s before being returned to the holding box for the start of the next trial. Rats were given five trials per day over a four day baseline period after which drug testing commenced. The time in seconds from release to touching the wire mesh was recorded as the swim latency, and the time in seconds from touching the wire mesh to placing both hind feet on the top of the platform was recorded as the climb latency. Rats that failed to complete a trial were given maximum latencies of 60 s[34].

Table-top and edge walking. Animals were induced to walk approximately one meter distance in front of the tank on the table top and also to walk the length of the tank on the top edge of the aquarium (width 15 mm). During both these events the rats were videotaped and examined for behavioral abnormalities, in gait, posture, balance, and overall behavioral directedness.

Statistical analyses

All data were analysed by SPSS/PC + using a series of within subjects repeated measures multivariate analysis of variance designs (MANOVAs), using the averaged *F*-ratios. Paired *t*-tests were subsequently employed, where appropriate, to further examine the significant main effects and interactions.

RESULTS

No statistical differences on any of the measures were found between the groups that were run on alternate days. Similarly, no differences were found between the animals that received the test drug first and the animals that received saline first.

The rats treated with the 0.05 and 0.3 mg/kg doses were hyper-reactive when handled compared to the

same animals injected with saline, as evidenced by more robust struggling and increased vocalization. Animals treated with the 0.05 mg/kg dose appeared to be more reactive than those treated with the 0.3 mg/kg dose. Rats treated with the 1.0 mg/kg dose were too motorically impaired to struggle at all. However, when these rats were first picked up it was noted that they quivered briefly, possibly indicating a reactivity that was largely occluded by motor deficits.

Tongue extension

An overall MANOVA found a statistically significant interaction between the drug and saline controls and the doses of MK801 ($F_{2,22} = 31.37$; $P < 0.0005$). Paired t-tests comparing each drug dose to the vehicle found significant differences at all three doses of MK801 ($t_{11} = -3.17$ $P = 0.009$, 0.05 mg/kg; $t_{11} = -7.79$ $P < 0.0005$, 0.3 mg/kg; $t_{11} = -15.37$ $P < 0.0005$, 1.0 mg/kg). MANOVAs comparing the three saline doses together, and the saline doses to the three baseline tests showed no significant differences. These results indicate that the animals were poorer at removing the cookie slurry from the ruler at all three doses of MK801 than when they were given saline. As Fig. 1 shows, this impairment occurred in a dose-dependent fashion. The lack of a difference between the ordering of the groups and the rats' consistent performance during the three saline treatments suggests that there were no residual effects of MK801 even at the 0.3 and 1.0 mg/kg doses.

None of the rats treated with the 1.0 mg/kg dose could perform the task and were scored as zero extension. All of these animals continuously circled around the inside perimeter of the home cages. Attempts to present the mash on the end of a spatula through the wire mesh had no effect. When the mash was smeared on the snout and whiskers, no attempts were made to clean it off. Animals showed obvious difficulty in performing this task at the 0.3 mg/kg dose. Abnormal behaviors noted during the 0.3 mg/kg dose were the head drifting out of position while continually licking at the slurry, and in initiating licking, which could be reinstated in some of the cases if the slurry was presented inside the cage on a spatula. None of these overt abnormal behaviors were observed in animals treated with the 0.05 mg/kg dose of MK801.

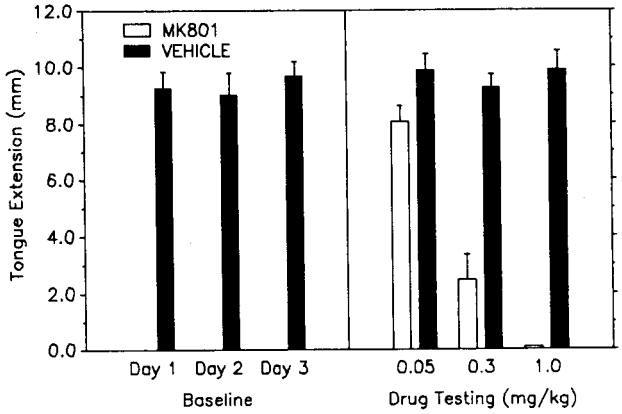

Fig. 1. Data from the tongue extension task. Tongue extension in mm from the three baseline days and drug testing period. In this and the following figures all rats received the 0.05, 0.3 and 1.0 mg/kg doses of MK801 and the saline control sessions in an alternating counterbalanced design. Animals were unable to perform the task at the 1.0 mg/kg dose, and subsequently given a minimal score of 0 mm. This and the following figures display means and S.E. of the means.

Sensorimotor orienting

At the 0.05 mg/kg dose 2/12 rats exhibited a slowness to orient that was limited to the hind feet. At the 0.3 mg/kg dose only 1/12 animals exhibited a normal orienting response. Of the remaining 11 animals at this dose, two displayed no orienting, four exhibited delayed and less vigorous orienting, two were hyper-reactive banging against the walls of the cage in response to the probe, two perseverated in their orienting, vigorously attending to the area where the probe had been for 3–5 s after its removal, and one animal oriented appropriately, but instead of biting at the cotton swab the rat attacked its own forefeet. At the 1.0 mg/kg dose only one of the animals successfully demonstrated orienting in any form. Most of the animals at this dose were observed to continually 'stagger' around the perimeter of their home cage (see table-top walking below).

Spontaneous locomotor activity

Data from the Digiscan activity monitors were analysed in variable clusters of three measures each: a movement characteristic cluster (TD, AD, AS), a horizontal movement cluster (NM, MT, TM) and a vertical movement cluster (VM, VT, TV). Overall MANOVAs were performed on each of these variable clusters, with subsequent univariate MANOVAs on the individual measures. These data can be seen in Figs. 2, 3 and 4.

Results from the first cluster (TD, AD and AS) indicated significant differences between the vehicle and MK801 for animals treated with the 0.05 mg/kg dose ($F_{3,9} = 6.07$; $P = 0.015$). The subsequent univariate F-ratios revealed similar significant differences for the three individual measures at this dose. Findings for the 0.3 and 1.0 mg/kg doses were similar. Thus, as can be seen in Fig. 2 the measures of TD, AD and AS revealed hyperactivity at all three doses, such that the rats travelled further overall, further per movement, and at a higher velocity per movement. The average speed during the 1.0 mg/kg dose revealed a slightly different

pattern of results such that the initial samples showed a lower speed than the saline condition and the later samples showed a greater speed. This can be explained by the standard scalloped shape of the saline control condition, where the initial average speed of the movements was high, and the later speed was low. This is in contrast with the 1.0 mg/kg dose of MK801, where the average speed was relatively constant throughout the six samples as can be seen in Fig. 2.

Results from the second variable cluster (NM, MT, and TM) also indicated overall differences between the vehicle and MK801 at the 0.05 dose ($F_{3,9}$ = 6.56; P = 0.012). The subsequent univariate F-ratios also revealed significant findings on all three measures. The higher doses also produced analogous dose dependent results, indicating that the rats treated with MK801 made more movements, and made those movements for a longer period of time (see Fig. 3).

Results from the third variable cluster (VM, VT, and

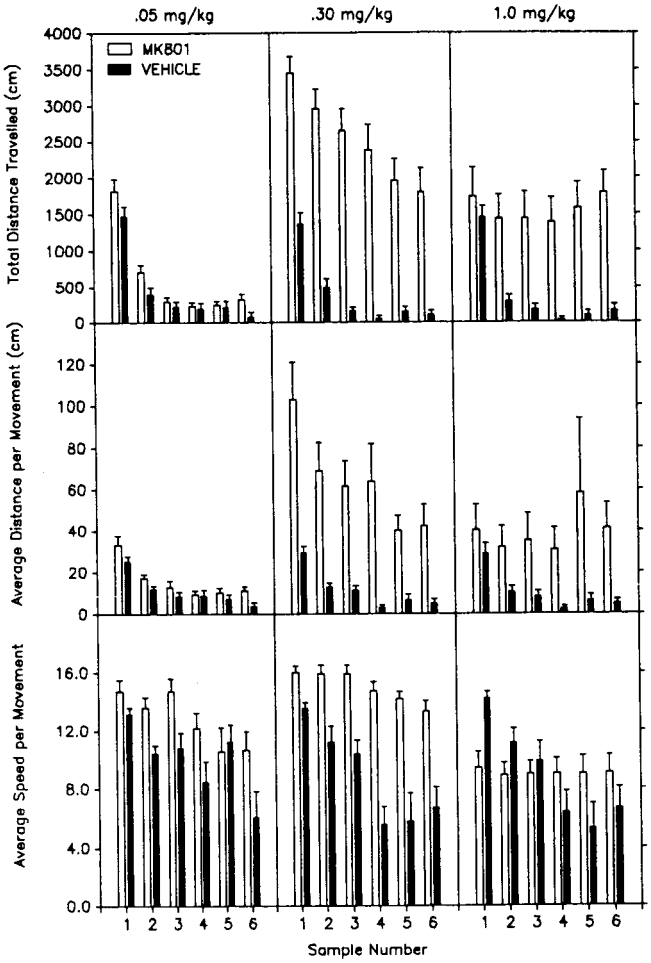

Fig. 2. Movement characteristics of the spontaneous locomotor activity data. Top, total distance travelled in cm (TD); middle, average distance travelled per movement in cm (AD); bottom, average speed per movement in cm/s (AS).

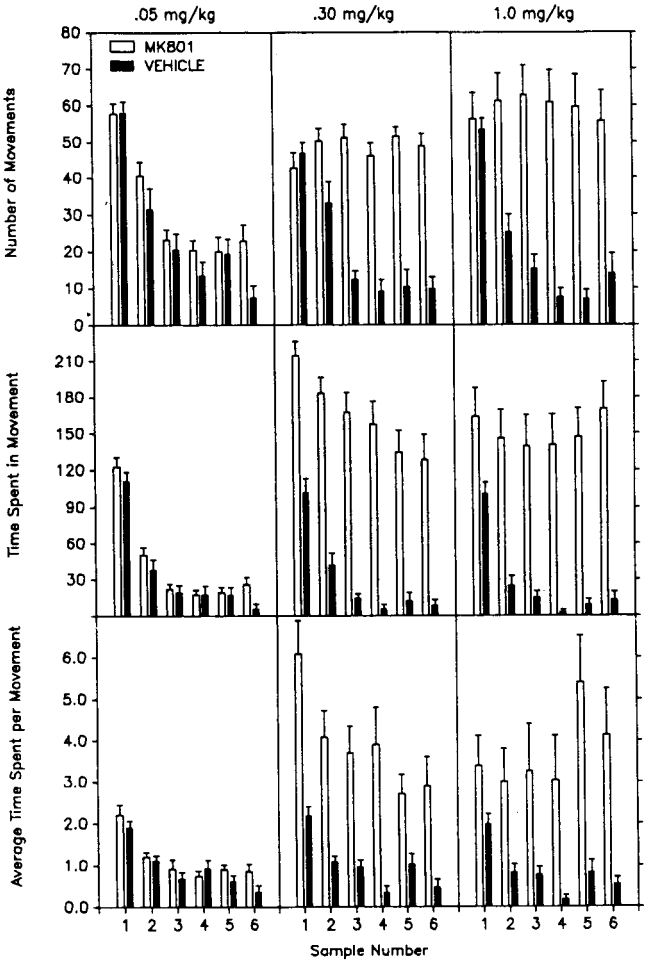

Fig. 3. Horizontal movements of the spontaneous locomotor activity data. Top, number of horizontal movements (NM); middle, time spent in horizontal movement in seconds (MT); bottom, average time per horizontal movement in seconds (TM).

TV) again indicated overall differences at the 0.05 dose ($F_{3,9}$ = 9.24; P = 0.004). The subsequent univariate F-ratios revealed significant findings for the variables VT and TV, but not for VM. The higher doses showed differences on all of the three measures, but not necessarily in the same direction. Thus, the rats treated with MK801 made the same number of vertical movements at the 0.05 mg/kg dose as the saline control sessions, more vertical movements at the 0.3 mg/kg dose, and a lower but constant number of movements, which were initially fewer than the saline control sessions at the 1.0 mg/kg dose (see Fig. 4). Further, rats spent less time overall making vertical movements after MK801 injections than after saline injections at all three doses, with the 1.0 mg/kg dose showing the greatest effect. Finally, rats under the influence of MK801 spent less time per vertical movement, in comparison to the saline control condition, which also decreased in a dose-dependent fashion.

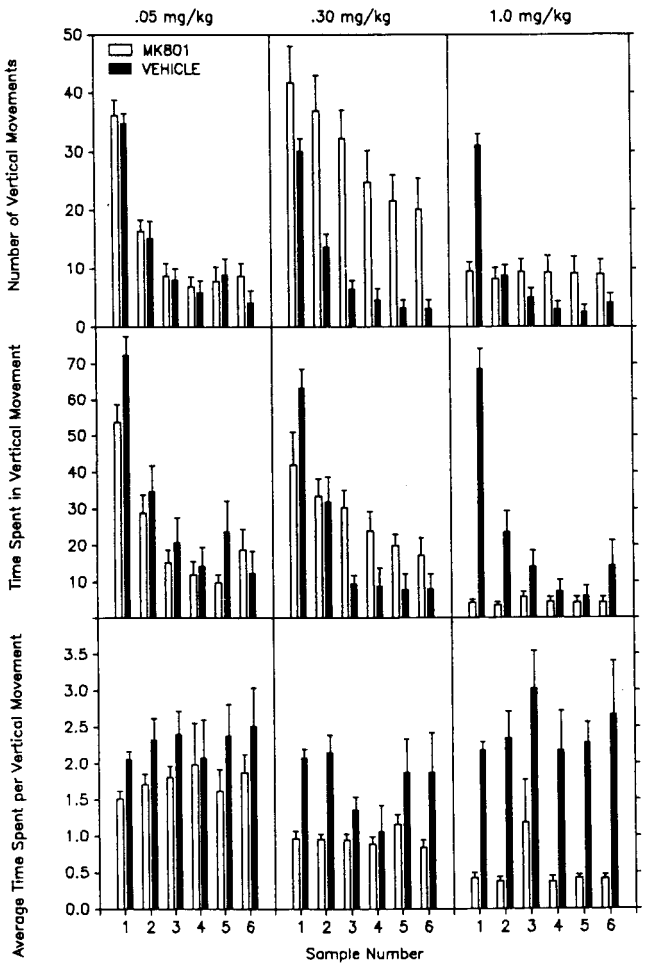

Fig. 4. Vertical movements of the spontaneous locomotor activity data. Top, number of vertical movements (VM); middle, time spent in vertical movement in sec (VT); bottom, average time per vertical movement in seconds (TV).

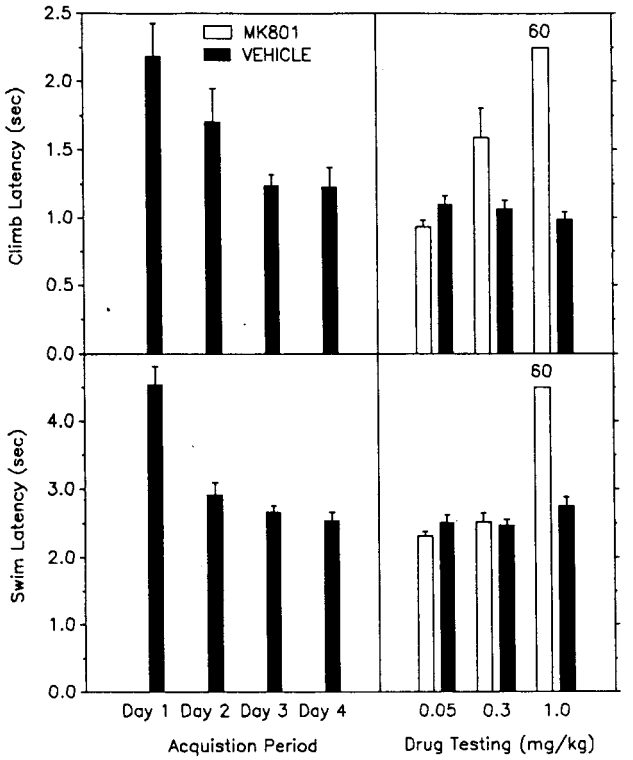

Fig. 5. Data from the swim and climb task. Top, climb latency in seconds; bottom, swim latency in seconds. Animals were unable to perform the task at the 1.0 mg/kg dose, and subsequently given maximal latencies of 60 s.

At the 0.3 mg/kg dose a number of the animals were observed to be circling around the perimeter of the monitors at the end of the session and at the 1.0 mg/kg all animals were observed to circle in this fashion. This was never seen in any of the animals given saline injections or treated with 0.05 mg/kg MK801. Additionally, it was not uncommon at these doses to observe the animals rear up in the activity monitors and fall over. Abnormalities in gait and posture were also observed at the 1.0 mg/kg dose as described below. Such overt abnormalities and impairments were not observed at the 0.05 mg/kg dose of MK801.

Swim task

An overall MANOVA found a significant interaction between the drug condition (MK801 vs. vehicle) and dose of the drug ($F_{2,22} = 105.21$; $P < 0.0005$). Subsequent MANOVAs found significant dose effects in the MK801 data alone for both swim ($F_{2,22} = 145.42$; $P < 0.0005$) and climb ($F_{2,22} = 105.53$; $P < 0.0005$) latencies. Paired t-tests comparing the 0.05 mg/kg dose to its vehicle control found significant differences ($t_{11} = -3.64$ $P = 0.004$ swim; $t_{11} = -3.61$ $P = 0.004$ climb). These significant differences were in the direction of shorter latencies as indicated in Fig. 5. Paired t-tests comparing the 0.3 mg/kg dose to its vehicle control found significant differences for the climb latency ($t_{11} = 2.45$; $P = 0.033$) but not for the swim latency ($t_{11} = 0.48$; $P = 0.640$).

Additionally, a number of the animals at the 0.3 mg/kg dose were observed to fall over when rearing, fall backwards off the wire mesh when climbing, exhibit continual circling in the holding box, and occasionally not pause on the resting platform, but continue over the opposite edge, where they would then scramble to remain on the platform. Only 1/12 animals treated with the 1.0 mg/kg dose were able to complete any of the swimming trials, and none of the animals were able to climb the wire mesh. Accordingly, no paired t-tests were run for the data at the 1.0 mg/kg dose. Abnormal swimming behaviors observed at this dose included fore-paw treading, underwater swimming, and synchronous hindfoot kicking which persisted for a number of seconds after removal from the water. MANOVAs

comparing the three saline doses together, and the saline doses to the last two acquisition tests showed no significant differences. These results indicate that the animals treated with the 0.05 mg/kg dose actually had shorter latencies than when they had been injected with saline. Results from the 0.3 mg/kg dose indicated no impairments in swimming, but did indicate impairments in climbing. The observations made at the 1.0 mg/kg dose indicated that, at this dose, the rats could not perform the task at all.

Table-top and edge walking

At the 0.05 mg/kg dose none of the rats had any obvious difficulty walking across the table-top. Similar results were found with the edge walking at this dose. However, the rats were noticeably hyper-reactive when handled and less reluctant to walk along the aquarium edge than during the saline condition. A number of the rats at the 0.3 mg/kg dose showed evidence of ataxia, particularly in their hindquarters. This was evidenced by a slight splaying of the hindquarters when table-top walking and a greater number of foot faults (of the hind feet) when edge walking. The rats at this dose also fell off the aquarium edge with greater frequency than during the saline condition. Finally, rats at this dose spontaneously walked out along the edge of the aquarium from the resting platform, a behavior not observed during the saline condition. At the 1.0 mg/kg dose rats were obviously impaired, with many of them walking off the table-top without any hesitation. Animals at this dose also did not truly walk, but crawled, with their abdomens placed against the ground. They also had great difficulty placing the bottoms of their feet on the ground, such that these animals were, in effect, walking on their knees and wrists. Related to this was a rolling of the shoulders and haunches such that the feet would be dragged upwards and forwards, instead of being properly placed. None of the rats at this dose could perform the edge walking task, as none were able to maintain themselves on the aquarium edge.

DISCUSSION

This study finds that MK801 produces a behavioral syndrome of hyperactivity, hyper-reactivity, and motor dysfunction. Symptoms of this syndrome were present in varying degrees at all three of the doses tested, including the low dose of 0.05 mg/kg.

No residual effects of MK801 were detected on any of the measures employed. This result indicates that four-day intervals between MK801 treatments did not affect saline control sessions, which were carried out 48 h after each drug administration.

Overt behavioral abnormalities and motor dysfunction were not apparent to casual observation at the 0.05 mg/kg dose. However, a number of the tests provided evidence indicating that the syndrome was present at this dose. Behavioral abnormalities and motor dysfunction were observable at the 0.3 mg/kg dose of MK801. At this dose all three aspects of the syndrome were observable. Obvious behavioral abnormalities and severe motor dysfunction were present at the 1.0 mg/kg dose. At this dose the motor impairments appeared to be the prominent aspect of the syndrome impinging upon both the hyperactivity and hyper-reactivity.

Our findings of a dose-dependent hyperactivity are in agreement with those found by others[5,33,35]. Simple increases in activity levels of animals treated with MK801 over animals treated with saline have also been reported[12,15,29]. Although Butelman[5] found a dose-dependent increase at 0.3 and 0.4 mg/kg, he did not find similar results at doses of 0.1 and 0.2 mg/kg. Similarly, another study[33] found a dose-dependent increase at 0.125 and 0.5 mg/kg, but only a trend towards an increase at 0.0625 mg/kg. However, this latter study counted the number of 5 × 5 cm squares that mice entered and Butelman[5] employed a monitor with two photocells, where the number of beam interruptions served as activity counts. Neither of these methods may have been sensitive enough to detect the increased activity at the lower doses. We and others[35] have employed similar beam breaking methods, but with a minimum of sixteen photocells in each apparatus. Video tracking, another method sensitive in detecting activity levels, has also been employed[15]. These latter studies all found increased activity levels at doses below 0.2 mg/kg. Further, a re-analysis of Butelman's[5] efficiency and latency scores derived from the radial-arm maze revealed that animals treated with 0.1 and 0.2 mg/kg doses of MK801 spent less time per arm than did saline-treated animals. This re-analysis parallels comments that MK801-treated animals entered the arms of the radial maze more quickly than saline controls[29], and supports a conclusion of hyperactivity at these doses.

The close match between the pattern of results obtained from Ford and associates[12] and our own are especially pertinent, since a Digiscan activity monitor system was also employed in their study. The specific behavioral topography of MK801 at 0.5 mg/kg is a pattern of increased movements, duration of such movements, average speed, and subsequently distances travelled, paired with deficiencies in vertical activity[12]. The number of vertical movements is the only measure that does not consistently fit the profile[12]. In the present study at the 0.05 mg/kg dose no differences were found between MK801 and saline treated animals, at the 0.3 mg/kg dose the number of vertical movements was

increased over that of the saline treated animals, and at the 1.0 mg/kg dose the number of vertical movements were greatly reduced, when averaged across the full session. Ford and associates[12] found the number of vertical movements reduced at a dose of 0.5 mg/kg. This difference, however, can probably be attributed to the difference in dosage between these two studies.

Wozniak and colleagues[39] have reported that rats injected with a 0.2 mg/kg dose of MK801 exhibited deficits on four sensorimotor tests, and that half of the animals treated with this dose fell off an elevated radial-arm maze during an initial trial. However, similar motor impairments were not found at 0.05 or 0.1 mg/kg doses of MK801[39]. Tricklebank and colleagues[33] have also described a number of abnormal motor behaviors in mice after receiving 0.1–0.3 mg/kg MK801 i.v., including wide-angle lateral head movements and side-to-side movements of the hindquarters.

It has also been reported that animals treated with 0.08 mg/kg MK801 exhibited abnormal swimming paths as analysed by videotracking techniques, during a habituation trial in a Morris water maze[26]. Additionally, these animals required longer latencies in a simple swim to visible platform task. However, rats treated with 0.05 mg/kg did not exhibit these abnormal patterns or longer latencies[26].

These results are at odds with our findings of a decreased latency at 0.05 mg/kg and normal swim latencies at 0.3 mg/kg. We interpret the former to be a hyper-reactive response to being placed in the cold water, since it was frequently accompanied by vocalization. Thus, the discrepancy between results might be accounted for by differences in the temperature of the water between the two swim tasks. The temperature of the water used in our simple swim task was approximately 20°, whereas the temperature of the water in the other task[26] was 27°. Possibly, the warmer water may have permitted the display of the abnormal swimming paths, while the cold water in our task may have inhibited this behavior, simultaneously inducing a hyper-reactive response. An alternative explanation is that our animals were well-experienced swimmers, in addition to being well-trained in the task demands prior to testing with MK801. In the case of the habituation trial, the animals were not well-experienced swimmers, and in the case of the swim to visible platform were not well-trained in the task demands[26]. This possible explanation is supported by observations of increased escape latencies in 0.0625 mg/kg treated animals during cued visible platform training, which attenuated over a six-day period[3]. Others have found similar differences between well-trained and naive animals in a rotating platform version of the Morris water-maze[32]. The deficits in swimming behavior observed by Whishaw and Auer[35] at a dose of 0.25 mg/kg, however, are also at odds with our results, even though the animals in their study received training prior to MK801 administration and the temperature of the water was 18°. The only notable differences between the two procedures were that female rats were used and MK801 was delivered intravenously[35]. Presumably the drug administration method would not affect these results, since adequate lag time was allowed between injection and testing in both studies. However, sex and/or strain differences in behavioral sensitivity to MK801 have been reported by others[15,29].

Dose-dependent deficits in tongue extension were also present in our data. Possibly related to this deficit were the observations that 0.1 mg/kg doses resulted in a suppression of drinking from a water bottle spout[26] and that doses of 0.125 and 0.15 mg/kg resulted in transient aphagia[29]. All three difficulties in consummatory behavior may result from deficits in the control of fine motor movements or deficits in the initiation of such movements.

A majority of the studies discussed thus far have also shown that MK801 impaired the acquisition, but not the retention, of a number of tasks that have been similarly disrupted by hippocampal lesions[5,15,26,29,35]. Two of these studies argue that at appropriate doses of MK801, impairments in learning can be recorded without observing alterations in ongoing behavior[5,26]. We disagree with Butelman's[5] conclusions for the reasons discussed above. Whether the data presented by the latter study are free from such alterations in behavior is not possible to evaluate, since no report on behavior was presented[26]. However, it has been shown that the initial swimming behavior of non-experienced swimmers can be affected by doses as low as 0.05 mg/kg[3,33]. Additionally, data from the present study and elsewhere[35] indicate that hyperactivity can be present at doses of MK801 as low as the 0.05 mg/kg.

Two other studies also reported that animals treated with MK801 were hyperactive at similarly low doses (0.07 and 0.0625 mg/kg, respectively)[15,29]. However, this was interpreted as being similar to animals with hippocampal lesions, which therefore argued that parallel mechanisms disrupt the behavior and learning in both preparations[15,29].

While it is true that at low doses MK801 induces a hyperactivity reminiscent to that of hippocampally lesioned animals, the hyperactivity induced by MK801 is dose-dependent and at higher doses appears to exceed the hyperactivity observed in hippocampally lesioned animals[23]. Further, it appears that hippocampal lesions reduce the number of rearings[23,31].

MK801 in the present study did reduce the time spent rearing, but did not similarly affect the number of rearings. At the 0.05 mg/kg dose the number of rearings was the same as the saline control sessions. The number of rearings increased at 0.3 mg/kg, and decreased comparatively at 1.0 mg/kg, but not consistently below the level of the saline control sessions. A reduction in rearing time and a minor decrease in the number of rearings was also reported to occur at a 0.5 mg/kg dose of MK801[12]. This dose-dependent non-linear effect presumably does not occur in hippocampally lesioned animals. Thus, the hyperactivity observed in the two preparations is possibly of different origins, and therefore also re-opens the question of the origin of the learning deficit seen in MK801 treated animals.

In support of this criticism there is evidence that the taste-potentiated odor aversion task used by one of the studies[29] can also be disrupted by lesions to the amygdala[17], specifically the basolateral nucleus[2], another area that has a high density of NMDA receptors[9,18]. This evidence suggests an alternate site of action for MK801 in the taste-potentiated odor aversion.

Similarly, the failure to demonstrate, in MK801-treated animals, a dissociation between working and reference memory[29] – after experimental manipulations that demonstrate this dissociation in hippocampally lesioned animals[24,25] – suggests that this learning impairment may also be of different origins.

Finally, four of the six studies employing the Morris water maze concluded that MK801 did not induce spatial learning deficits[3,13,15,26,32,35]. All five studies reporting escape to platform latencies indicated increases in MK801 treated animals over controls[3,13,15,26,35]. However, one study[15] reported significant correlations between escape latencies and open-field activity in MK801 and APV treated animals, and Bohbot and associates[3] specifically showed that prior swim to visible platform training under the influence of MK801 decreased subsequent motor impairments in hidden platform trials, although latencies were still increased over those of saline controls. Similarly, Sutherland and co-workers[32] found that extensive pretraining on a shifting platform version of the Morris water maze enabled the animals to acquire the new platform location regardless of being treated an hour earlier with saline or MK801. Subsequently, a number of naive animals were tested on a stationary platform, where impairments in acquiring the platform's location were evident[32]. No systematic differences other than prior swimming experience and results appear to exist among these six studies[3,13,15,26,33,35]. The exception is Halliwell and Morris[13], who gave the animals no prior swimming experience and also found no effects of MK801 on platform acquisition as tested by probe trials 24 h later. It has been suggested, however, that this finding is the result of the shortened interval between MK801 administration and testing[20].

In summary, our results have shown that MK801 produces a behavioral syndrome of hyperactivity, hyper-reactivity, and motor dysfunction. Evidence of this syndrome was observed to be present at doses as low as 0.05 mg/kg. Further, upon a review of the literature a number of studies give similar evidence for behavioral abnormalities at 0.05, 0.0625, 0.07, 0.08, 0.1 and 0.15 mg/kg doses of MK801[3,5,13,15,26,29,35,39]. Thus, it appears that the critique made by Keith and Rudy[16] that learning studies using NMDA antagonism to impair acquisition have not adequately ruled out behavioral deficits, can also be extended to those studies employing MK801, even at doses as low as 0.05 mg/kg.

We further suggest that the MK801 behavioral syndrome, as we have defined it, does not have a unitary underlying cause, or target structure, since the strength of the various components of the syndrome vary with the dose of MK801. This is also complicated by the use-dependent nature of MK801, which requires the activation of NMDA receptors before blocking effects can occur[15,20,37]. This may further increase the variability of the syndrome observed under different behavioral situations, and different latencies after drug administration.

As such, from the evidence presented above, it is clear to us that the effects of MK801 are not identical to those of hippocampal lesions nor are the effects of MK801 understandable on this basis alone. However, the results do not rule out the hippocampus as a target site nor do they rule out an interference with some forms of learning as one of the effects of MK801.

In conclusion our results support Whishaw and Auer's[35] contention that the behavioral abnormalities observed after MK801 administration prevent an unambiguous interpretation of the learning deficits, and we extend this now to include doses as low as 0.05 mg/kg.

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