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Differential effects of two NMDA receptor antagonists on cognitive–behavioral performance in young nonhuman primates II

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Abstract

The present experiment examined the effects of chronic exposure to remacemide (an NMDA antagonist that also blocks fast sodium channels) or MK-801 (which blocks NMDA receptors more selectively) on the acquisition of color and position discrimination and short-term memory behavior in juvenile rhesus monkeys. Throughout the 2-year dosing period, a conditioned position responding (CPR) task was used to assess color and position discrimination and a delayed matching-to-sample (DMTS) task was used to assess memory. Chronic exposure to high doses of either drug delayed the acquisition of accurate color and position discrimination without altering response rates. In the case of MK-801, these effects abated within 6 months of the start of treatment. In the case of remacemide, the effects persisted for 17 months of dosing. Neither compound significantly altered performance of the short-term memory task at any time point or at any dose tested. The fact that the effects of remacemide on behavioral performance were more persistent than those seen for MK-801 suggests that tolerance may develop to the behavioral effects of MK-801, which does not develop to the effects of remacemide. Alternatively, these results may suggest that the concurrent antagonism of NMDA receptors and fast sodium channels may have more profound consequences for behavior than does the antagonism of NMDA receptors alone.

Keywords: Remacemide; MK-801; *N*-methyl-D-aspartate (NMDA); Fast sodium channels; Operant behavior; Color and position discrimination; Short-term memory; Rhesus monkeys

1. Introduction

Remacemide hydrochloride is a relatively new compound that exhibits promising neuroprotective and anticonvulsant properties. In nonhuman animal models, remacemide hydrochloride can prevent seizures induced by the application of maximal [10] and subthreshold [20] electroshock, as well as those induced by *N*-methyl-D-aspartate (NMDA) [20] and kainic acid [5]. In human clinical trials, remacemide hydrochloride, administered as an adjunct to other antiepileptic drugs, has been proven effective in reducing the frequency of seizures [6].

The mechanisms that underlie remacemide's neuroprotective and anticonvulsant properties are thought to involve noncompetitive antagonism of NMDA receptors. The NMDA receptor is an important target for excitatory amino acid binding and is thought to play a critical role in the neural phenomenon known as long-term potentiation (LTP) [2]. LTP has been characterized as an increase in synaptic efficiency that can be induced by repeated tetanic stimulation [3,28]. It is generally believed that the mechanisms involved with the production and maintenance of LTP are intimately involved with learning and memory processes [12].

In addition to their purported role in learning and memory, the excitatory amino acids are also known to play an important role during development by regulating neuronal survival, axonal and dendritic structure, and synaptic genesis and plasticity [17]. Developmental observations in humans indicate that marked differences exist with respect to excitatory amino acid binding sites from the neonatal period through the 10th decade of life [4,9,15,27]. Consis-

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tent with these findings, there has been speculation that the infant brain may be more responsive to agents that affect NMDA receptor function than are adult brains [9].

The purpose of the present experiment was to examine the effects of chronic treatment with remacemide, and with the classical NMDA receptor antagonist, MK-801, on the acquisition and performance of complex operant behaviors in juvenile rhesus monkeys. Although both of these drugs bind to the noncompetitive channel site of the NMDA receptor, the affinity of MK-801 for the NMDA receptor is thought to be significantly greater than that of remacemide [18]. Furthermore, remacemide has the additional effect of blocking fast sodium channels, a property thought to contribute to its anticonvulsant action [19]. Concurrent investigation of these two compounds will enable direct comparisons to be made between the cognitive–behavioral effects of a relatively novel NMDA antagonist, remacemide, with those of the well-characterized NMDA receptor and antagonist, MK-801. Further, the results of these investigations may help to shed light on the respective roles of the NMDA receptor and sodium channel function in juvenile rhesus monkeys. Because remacemide and MK-801 are each known to inhibit the function of NMDA receptors, and because NMDA receptor function is thought to be critical for learning and development, we hypothesized that both MK-801 and remacemide would disrupt learning in our subjects. In addition to monitoring behavior during chronic treatment, behavior was also monitored during a two-step drug withdrawal procedure.

The operant behaviors monitored presently included: conditioned position responding (CPR) to assess color and position discrimination and delayed matching-to-sample (DMTS) to assess aspects of short-term memory. The CPR task requires subjects to associate a given stimulus (here, a color) with subsequent requirements for reinforcement [21,25,26]. This task has been described as a ''Symbolic'' matching-to-sample task [7], wherein specific visual or auditory stimuli serve as ''symbols'' for specific response locations. The DMTS task requires subjects to recognize a previously viewed geometric symbol (the sample) from a subsequently presented array. This type of operant task has been used extensively to measure aspects of memory and performance (e.g., Ref. [22]). Performance on each of these behavioral tasks has been shown to be positively correlated with IQ in children [23].

2. Methods

2.1. Subjects and housing

Subjects were 30 captive-bred female rhesus monkeys (*Macaca mulatta*) weighing approximately 1.8 kg (range: 1.3–2.3 kg) at the start of the experiment. Subjects ranged in age from 7.7–11.5 months at the start of the experiment to 31.7–35.5 months at the end of the experiment. This age range was selected to encompass the period corresponding to middle childhood to a time near the onset of puberty [11]. Daily access to food (High Protein Monkey Diet, PMI Nutrition International, Brentwood, MO) was supplemented with fresh fruit and chewable multivitamins (Select Brand Children's Chewables, Select Brand Distributors, Pine Bluff, AR) and was rationed to ensure that subjects gained between 0.05 and 0.1 kg body weight/month. This rate of weight gain was similar across treatment groups and was consistent with previous studies conducted in our laboratory [21–23,25,26]. Subjects were housed under a 12-h light/dark cycle (lights on at 6:00 AM CST) with temperature and relative humidity of $25 \pm 2^{\circ}C$ and $50 \pm 4\%$, respectively. All animal care procedures were in accordance with guidelines set forth by the American Association for Accreditation of Laboratory Animal Care and were approved by the NCTR Institutional Animal Care and Use Committee.

2.2. Drugs and dosing procedure

2.2.1. Treatment phase

Two days prior to the start of operant training, all 30 subjects (six subjects per treatment group) began an 18-month daily dosing regimen, which was followed by a 6-month, 2-step washout phase. Drugs were administered 7 days/week, within 1 h after daily (Monday–Friday) behavioral test sessions, and at the same time of the day on Saturday and Sunday. Administration of drugs immediately after daily behavioral assessment (rather than before) ensured that at least 23 h elapsed between the last drug treatment and the subsequent behavioral test session. Because both MK-801 and remacemide are known to be fully eliminated within 24 h [13] (unpublished observations), this dosing and testing procedure allowed analyses to focus on chronic rather than acute effects of exposure. During the 18-month initial treatment phase, doses of remacemide (20 or 50 mg/kg/day, free base) and MK-801 (0.1 or 1.0 mg/kg/day, HCl salt) were prepared in tap water and were administered via oral gavage. The low dose of remacemide was chosen to produce plasma levels that would be equivalent to the mean therapeutic plasma levels obtained during human clinical trails. The high dose of remacemide was chosen to produce plasma levels that would be equivalent to the highest plasma levels measured in human clinical trials (unpublished observations). The low and high doses of MK-801 were based on pilot studies in monkeys and represent a no-effect dose and the maximum tolerated dose, respectively. Subjects in the control condition received equivalent volumes of tap water alone administered via orogastric gavage. For the first 7 days of daily dosing, all remacemide-treated subjects received 20 mg/kg remacemide/day. After 7 days of treatment, half of these subjects began treatment with 50 mg/kg remacemide and continued to receive this dose for the remainder of the experiment. This ''ramping'' procedure allowed subjects to habituate to the transient emetic-producing effects that

sometimes accompany high-dose oral remacemide treatment. Each subject's daily dose was administered as a 5.0-ml bolus, which was immediately followed by a 5.0-ml H_2O flush to ensure that no test compound remained in the oral gavage tube. Each 48.8-cm orogastric gavage tube was cut from a length of plastic intravenous tubing (internal gauge = 0.64 cm). One end of the 48.8-cm gavage tube was trimmed at an angle and seared quickly with an open flame to remove sharp edges. A polypropylene Luer-lock connector was attached to the opposite end of the tube to allow attachment of a 10.0-ml dosing syringe. Gavage tubes and syringes were designated such that each syringe and a set of gavage tubes was used for a single subject, exclusively.

2.2.2. "Withdrawal" phase

The 18-month treatment phase was immediately followed by a 6-month, two-stage withdrawal phase. This length of time was deemed sufficient to detect evidence of "recovery" among subjects that had shown significant impairment during the chronic treatment phase of the experiment. During the first 3 months of withdrawal, subjects that had previously received the high dose of 50 mg/kg/day remacemide received the low dose of 20 mg/kg/day remacemide and subjects that had previously received the low dose of 20 mg/kg/day remacemide received water only. Similarly, subjects that had previously received the high dose of 1.0 mg/kg/day MK-801 received the low dose of 0.1 MK-801 mg/kg/day and subjects that had previously received the low dose of 0.1 mg/kg/day MK-801 received water only. Previous authors have reported that NMDA receptor upregulation, which might be expected following chronic antagonist treatment, is linked to increased seizure susceptibility in nonhuman animal models [29]. Therefore, in order to minimize the potential for withdrawal-induced seizures, doses were reduced in two stages for subjects that had received high doses of either MK-801 or remacemide. During the second 3 months of the 6-month withdrawal phase, all subjects received water only. Thus, all low-dose animals were gavaged using water only for the entire 6 months of withdrawal, whereas the high-dose animals were gavaged with the low dose for the first 3 months followed by water for the remaining 3 months. The purpose of the second withdrawal period was to determine the permanence of the behavioral effects observed during treatment and to assess the effect of total drug withdrawal in animals that had been chronically exposed to the high doses of these NMDA receptor antagonists.

2.3. Behavioral testing apparatus

Prior to operant behavior testing, subjects were placed into mobile restraint chairs (Primate Products, Redwood City, CA) and were held in place by a restraint collar. This method of restraint allows subjects free movement of fore and hind limbs and provides the ability to rotate 360° in three-dimensional space. Further, this method of restraint allows subjects to rest naturally on the haunches, while positioning the subjects' head in a manner optimal for viewing stimuli presented during behavioral testing. After placement in the restraint chairs, subjects were placed into one of three sound-attenuating operant test chambers (Model PCP-001, BRS/LVE, Beltsville, MD). Each test chamber measured $111.8 \times 68.6 \times 127$ cm and was equipped with a house light, a ventilating fan, and a food trough. The test chambers were additionally equipped with three model PPC-012 rear-projection press plates (BRS/LVE). Each operant test panel was controlled by a computerized input/output controller (developed at the FDA's National Center for Toxicological Research, Jefferson, AR) which administered the behavioral tasks and recorded behavioral responses. The software controlling the panel operation was written at the National Center for Toxicological Research.

2.4. Training and testing procedure

For 2 weeks prior to the start of drug dosing and behavior testing, subjects were conditioned to daily capture and chair restraint. During this time, subjects were also familiarized with the banana-flavored food pellets that were used as reinforcers during subsequent behavioral testing. At the end of the 2-week familiarization period, subjects were assigned to one of five treatment groups that were balanced with respect to age, place of birth, and body weight. On the third day of drug treatment, subjects began interacting with the behavioral test apparatus while performing an incremental repeated acquisition task. After five to seven consecutive test sessions of incremental repeated acquisition training, subjects began training on the CPR task on alternate days as described below. Data collected during performance of the incremental repeated acquisition task, as well as those collected using a progressive ratio task, are presented elsewhere [24].

2.5. Conditioned position responding (CPR)

Training for CPR consisted of five levels, each of which remained in effect until subjects received 100 reinforcers. If subjects did not complete all of the CPR training during a given test session, they began the next session at the point that they had reached previously. For example, if subjects completed level one of CPR training and earned 15 pellets on level 2, then that subject would begin the next test session on level 2 and would need to earn 85 more reinforcers to move to level 3 and so on. For CPR training level 1, each trial began with the illumination of each of the three press plates (white light) and a response to any of the three resulted in reinforcer delivery. The purpose of training level 1 was to familiarize subjects with the press plates and with the procedure for receiving reinforcers. At CPR training level 2, each trial began with the random illumination of two of the three press plates with the same one of four colors

(red, yellow, blue, or green). The far left press plate was only illuminated red or yellow. The far right press plate was only illuminated blue or green. Responses to either of the illuminated press plates resulted in reinforcer delivery. Responses to the third (darkened) press plate had no programmed consequences. At CPR training level 3, each trial began with the illumination of the center press plate using one of the four colors listed above. A press to the center press plate resulted in the immediate illumination of one of the side keys with the same color. Therefore, if the center key had been illuminated red or yellow, then the left press plate was illuminated with the same color. If the center key had been illuminated blue or green, then the right press plate was illuminated with the same color. A response to the illuminated side key resulted in reinforcer delivery. Responses to the darkened press plates had no programmed consequences. At CPR training level 4, each trial began with the illumination of the center press plate using one of the four colors listed above. A response to this (center) press plate resulted in the immediate illumination (white) of the appropriate side press plate. If the center key had been illuminated red or yellow, then the left press plate was illuminated white. If the center key had been illuminated blue or green, then the right press plate was illuminated white. A response to the illuminated side key resulted in reinforcer delivery. Responses to the darkened keys had no programmed consequences. At CPR training level 5, each trial began with the illumination of the center press plate as described for level 4, but responses to the center plate resulted in the illumination of both of the side press plates (white). If the center press plate had been illuminated red or yellow, then a response to the left press plate resulted in reinforcer delivery. If the center press plate had been blue or green, then a response to the right press plate resulted in reinforcer delivery. Responses on the incorrect press plate resulted in a 10-s timeout followed by another presentation of the same behavioral problem (i.e., the center press plate was reilluminated with the same color as it had been on the previous trial). The same behavioral problem was repeated indefinitely until the subject responded correctly. After 300 reinforcers were earned at CPR training level 5, the full CPR task was presented. The full CPR task was identical to training level 5 with the exception that no error correction was permitted. That is, behavioral problems were presented randomly on each trial regardless of the subject's performance on the previous trial. After subjects earned a cumulative total of 1000 reinforcers while performing the full CPR task, the CPR task duration was shortened from 50 to 10 min and the total number of reinforcers available was reduced from 100 to 60. Training on the DMTS task began at this time.

2.6. Delayed matching-to-sample (DMTS)

The DMTS task lasted 40 min and was presented 1 min after the termination of the 10-min CPR session. Training for DMTS was comprised of six levels. Each of the first four levels were in effect until subjects earned 100 reinforcers. Each of the last two levels was in effect until subjects earned 500 reinforcers. As was the case for the other behavioral task, subjects that did not complete DMTS training within a given session began the following session at the point that they had ended previously. For DMTS training level 1, each trial began with all three press plates illuminated with the same one of seven geometric symbols (+, −, |, □, Δ, O, or X) and a response to any of the three press plates resulted in reinforcer delivery. At training level two, each trial began with the center press plate and one of the side press plates (chosen randomly) illuminated with an identical geometric symbol (randomly). A response to either illuminated press plate resulted in reinforcer delivery. A response to the darkened press plate had no programmed consequences. At DMTS training level 3, each trial began with the random presentation of a symbol on the center press plate. A response to that press plate extinguished the presentation of the symbol and resulted in the immediate presentation of the same geometric symbol on one of the side press plates. A response to this side press plate resulted in reinforcer delivery, whereas a response to one of the darkened press plates had no programmed consequences. At training level 4, each trial began with the illumination of a geometric symbol on the center press plate. A response to this center press plate extinguished the presentation of the symbol and resulted in the immediate illumination of both side press plates. At this level, one of the side press plates was illuminated with a "matching" symbol and the other side press plate was illuminated with a "nonmatching" symbol. A response to the press plate that was illuminated with the "matching" symbol resulted in reinforcer delivery. A response to the "nonmatching" symbol resulted in a 10-s timeout followed by the representation of the same behavioral problem. The same behavioral problem was repeated until subjects responded correctly. At training level 5, each trial began with the presentation of a randomly selected geometric symbol as described for training level 4. A single response to this illuminated press plate resulted in the immediate presentation of different geometric symbols on each of the three press plates. Two of the press plates presented "nonmatching" geometric symbols, whereas the third press plate presented a "matching" geometric symbol. Responses directed toward the matching symbol resulted in reinforcer delivery. Responses directed toward the nonmatching symbol were followed by a 10-s timeout (all press plates dark) and the subsequent presentation of the same behavioral problem. DMTS training level 6 was identical to training level 5, but no error correction was permitted — after an incorrect choice was made a new, but not necessarily identical, problem was presented. After subjects received 6000 reinforcers on DMTS training level 6, delays of varying duration were systematically inserted between the presentation of the initial geometric symbol

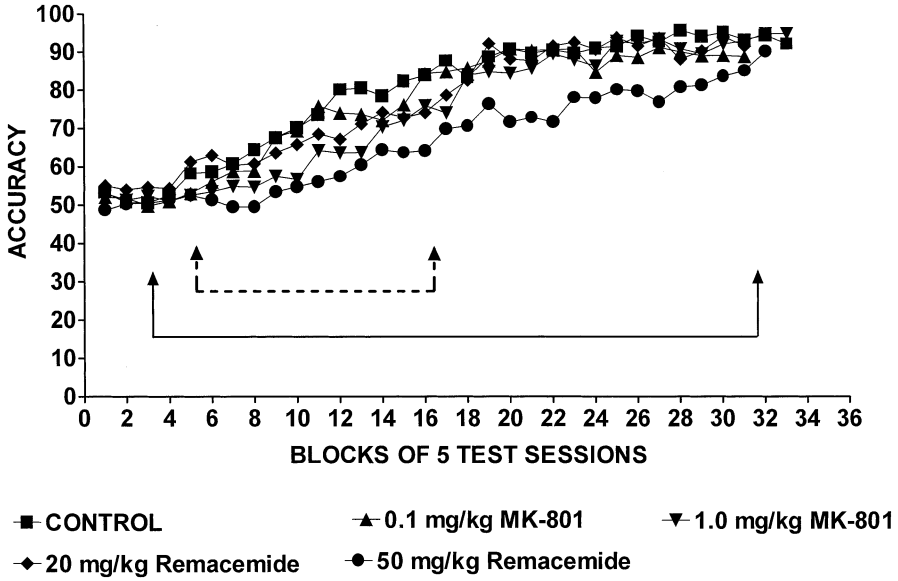

Fig. 1. Presents the effects of chronic treatment on CPR accuracy measured during the 18-month treatment phase. Each block of the five test sessions corresponds to 2 full weeks of drug treatment. Solid brackets encompass the points at which the 50-mg/kg remacemide group differed significantly from control. Dashed brackets encompass the points at which the 1.0-mg/kg MK-801 group differed significantly from control ($P < .05$).

(center press plate) and the presentation of the subsequent choices. Six possible delay durations could be defined. The presentation of these delays was pseudorandomized such that each delay could be presented no more than 20 times during a given test session. Initially, all delays were set at 0.01 s (i.e., possible delays included: 0.01, 0.01, 0.01, 0.01, 0.01, or 0.01 s). After three consecutive sessions during which subjects achieved $\geq 60\%$ accuracy and completed at least 50 trials, possible delay durations were changed to include delays of 1 s (i.e., possible delays included: 0.01, 0.01, 0.01, 1,0, 1.0, or 1.0 s). Under this delay schedule, subjects could be presented with a total of 60 trials with 0.01-s delay and 60 trials with delay set at 1 s. After three consecutive sessions during which subjects achieved $\geq 60\%$ accuracy and completed at least 50 trials, possible delay durations were changed to include delays of 2 s (0.01, 0.01, 1.0, 1.0, 2.0, or 2.0 s). When performance across this delay schedule achieved the same criterion, the difficulty of the delay schedule was changed to include 4 s (0.01, 1.0, 1.0, 2.0, 2.0, or 4.0 s), 8 s (0.01, 1.0, 1.0, 2.0, 4.0, or 8.0 s), 16 s (0.01, 1.0, 2.0, 4.0, 8.0, or 16.0 s), 32 s (0.01, 2.0, 4.0, 8.0, 16.0, or 32.0 s), 48 s (0.01, 4.0, 8.0, 16.0, 32.0, or 48.0 s) and finally 64 s (0.01, 8.0, 16.0, 32.0, 48.0, or 64.0 s). Subjects began progressive ratio training after they had earned 2750 reinforcers at DMTS training level 6. All behavioral assessments were conducted at the same time of the day (within 1 h), Monday through Friday, and lasted approximately 50 min/day. Subjects were rotated through the behavioral test chambers such that no subject was tested in the same chamber on consecutive test days. Performance of the CPR and DMTS tasks occurred every other day, Monday–Friday, and lasted a total of 50 min.

2.7. Behavioral endpoints

Accuracy, response rate, observing response latency, and choice response latency were monitored for each behavioral task. Accuracy was defined as the number of correct responses divided by the total number of choice responses made, times 100. Response rate was defined as the total number of responses made divided by the total running time (in seconds) for the task. Observing response latency was defined as the average time required for subjects to acknowledge (with a response) the colored stimulus (for CPR) or the geometric "sample" (DMTS). Choice response latency was defined as the average time required for subjects to make a choice response after the choices were presented. Timeout periods were not included in the calculation of response rates or observing response latencies.

2.8. Treatment of data and statistical analyses

Data collected during treatment were grouped into blocks of five sessions for each behavioral endpoint. An animal's block mean was only included in the group mean when the block contained all five sessions. Group means were only used in the analysis when four or more animals had data in that block of sessions. Regression equations were fitted to the mean of each group and comparisons between groups were made using Wald statistics based on a chi-square distribution [16]. To obtain an accurate fit to the regression model, accuracy data for both tasks were transformed prior to analyses using: $\log(p/(1-p))$.

Data collected during the withdrawal period were grouped by week (encompassing two to three test sessions per subject per week) and are expressed as a percent

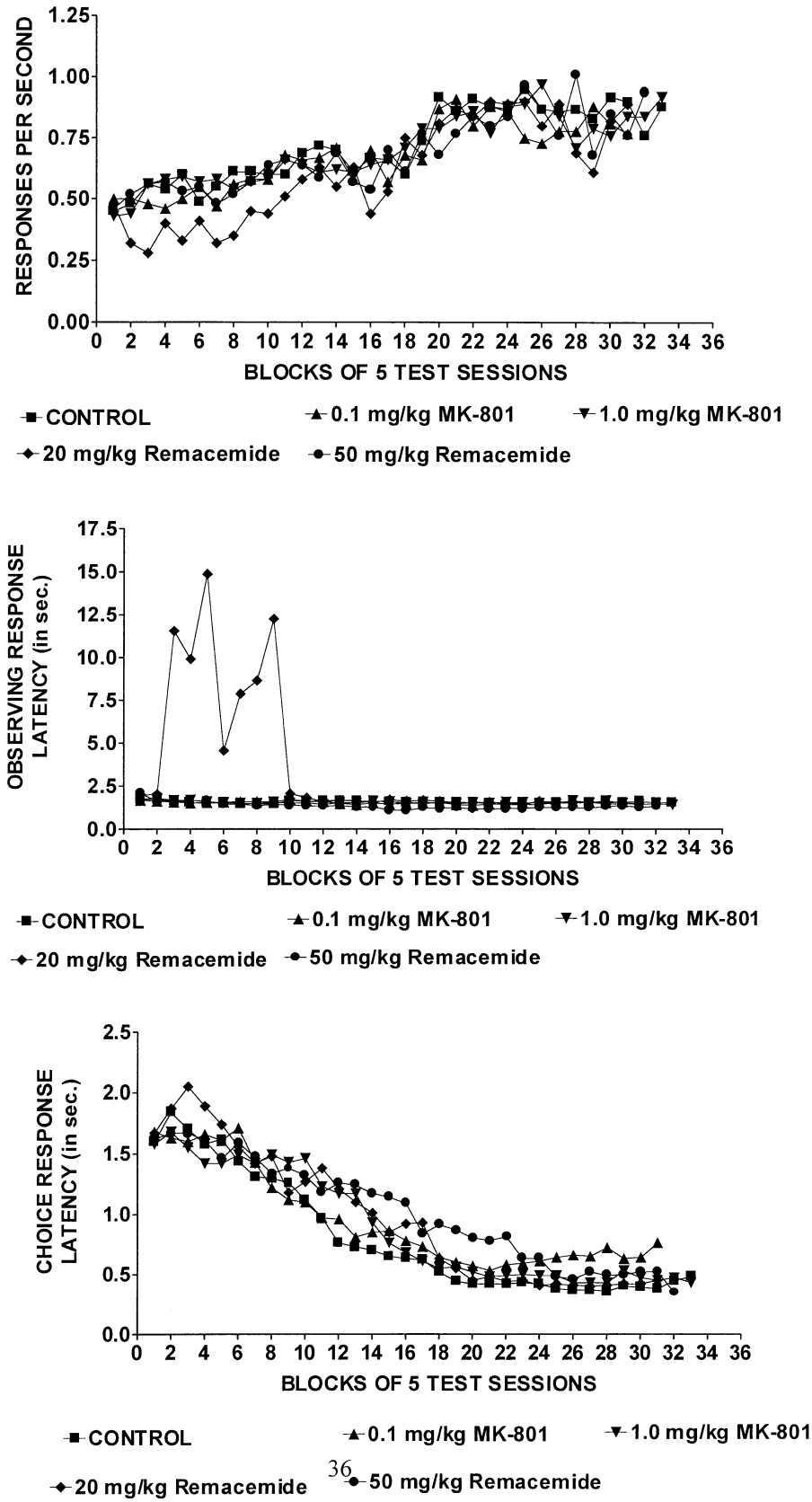

Fig. 2. Presents the effects of chronic treatment on CPR response rates (a) observing response latency (b) and choice response latency (c) measured during the 18-month treatment phase. Each block of the five test sessions corresponds to 2 full weeks of drug treatment.

Table 1
Number of sessions at each delay set for the DMTS task

Possible delay sets (0 denotes 0.01 s)	Control	0.1 mg/kg MK-801	1.0 mg/kg MK-801	20 mg/kg Remacemide	50 mg/kg Remacemide
0, 0, 0, 0, 0, or 0 s	72.0	61.7 (P=.73)	64.2 (P=1.0)	67.8 (P=.88)	71.7 (P=.99)
0, 0, 0, 1, 1, or 1 s	6.7	10.0 (P=.98)	34.3 (P=.55)	17.2 (P=.97)	41.2 (P=.14)
0, 0, 1, 1, 2, or 2 s	5.0	8.8 (P=.34)	15.0 (P=.42)	4.2 (P=1.0)	11.2 (P=.40)
0, 1, 1, 2, 2, or 4 s	5.7	4.7 (P=1.0)	4.8 (P=1.0)	5.6 (P=1.0)	12.0 (P=.08)
0, 1, 1, 2, 4, or 8 s	6.5	4.8 (P=.80)	8.3 (P=.95)	5.4 (P=.98)	11.3 (P=.51)
0, 1, 2, 4, 8, or 16 s	8.7	4.5 (P=.49)	4.8 (P=.73)	2.8 (P=.08)	10.0 (P=1.0)
0, 2, 4, 8, 16, or 32 s	12.3	5.0 (P=.59)	13.5 (P=.99)	8.2 (P=.95)	12.5 (P=.98)
0, 4, 8, 16, 32, or 48 s	9.7	14.6 (P=.65)	9.0 (P=1.0)	8.8 (P=1.0)	18.5 (P=.78)
0, 8, 16, 32, 48, or 64 s	86.0	94.4 (P=.86)	103.0 (P=.64)	107.0 (P=.64)	55.5 (P=.08)

Mean P value established using Dunnett's post hoc test to compare each treatment group to control.

change from baseline. For data collected during the first half of the withdrawal period (i.e., the first 3 months posttreatment), the baseline was defined using data collected during the last 4 weeks of treatment. For data collected during the second half of the withdrawal period (i.e., the second 3 months posttreatment), the baseline was defined using data collected during the last 4 weeks of the first withdrawal period. Data collected during withdrawal were analyzed as described above [16].

3. Results

3.1. Effects of chronic drug treatment on CPR

The effects of chronic drug treatment on CPR accuracy are presented in Fig. 1. The results indicate effects of high-dose remacemide and high-dose MK-801 to slow the acquisition of accurate CPR performance relative to controls (P<.05). The effects of remacemide emerged during the third block of five test sessions and persisted through most of the 18-month treatment phase. The effects of MK-801, on the other hand, emerged during the fifth block of five test sessions and persisted only through the seventeenth block of five test sessions.

The effects of chronic drug treatment on CPR response rate, observing response latency, and choice response latency are presented in Fig. 2a–c, respectively. There were no statistically significant effects of drug treatment on any of these three endpoints. The apparent effect of low-dose remacemide to reduce response rates and to increase latencies were due to extremely low rates of responding by a single subject, which initially skewed the response rate measures down and the latency measures up for this group.

3.2. Effects of chronic drug treatment on DMTS

Subjects in the present experiment failed to gain proficiency in performing the DMTS task using longer delays, regardless of treatment group. Thus, data for only the 0.01-s delay condition are presented. Under these conditions, the DMTS task is very similar to the CPR task except that: (1)

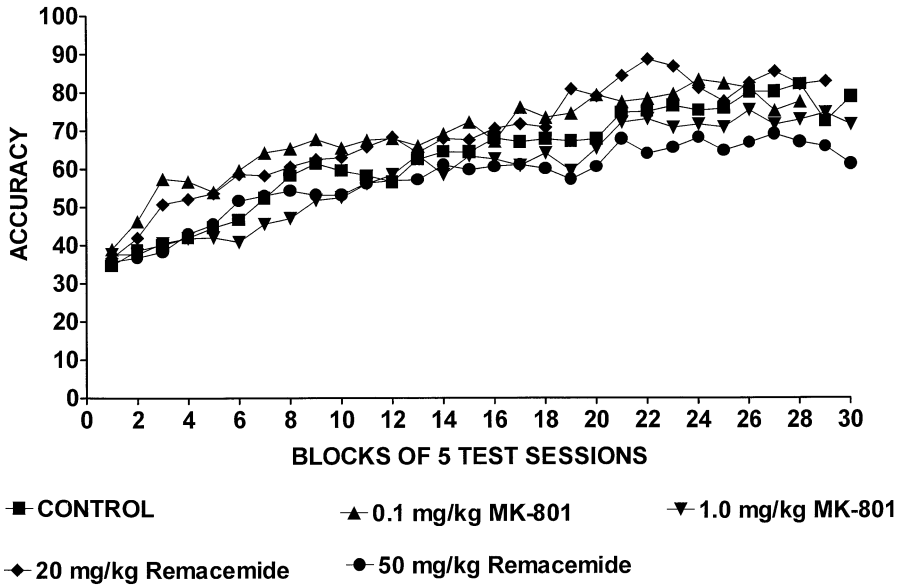

Fig. 3. Presents the effects of chronic treatment on DMTS accuracy measured during the 18-month treatment phase. Each block of the five test sessions corresponds to 2 full weeks of drug treatment.

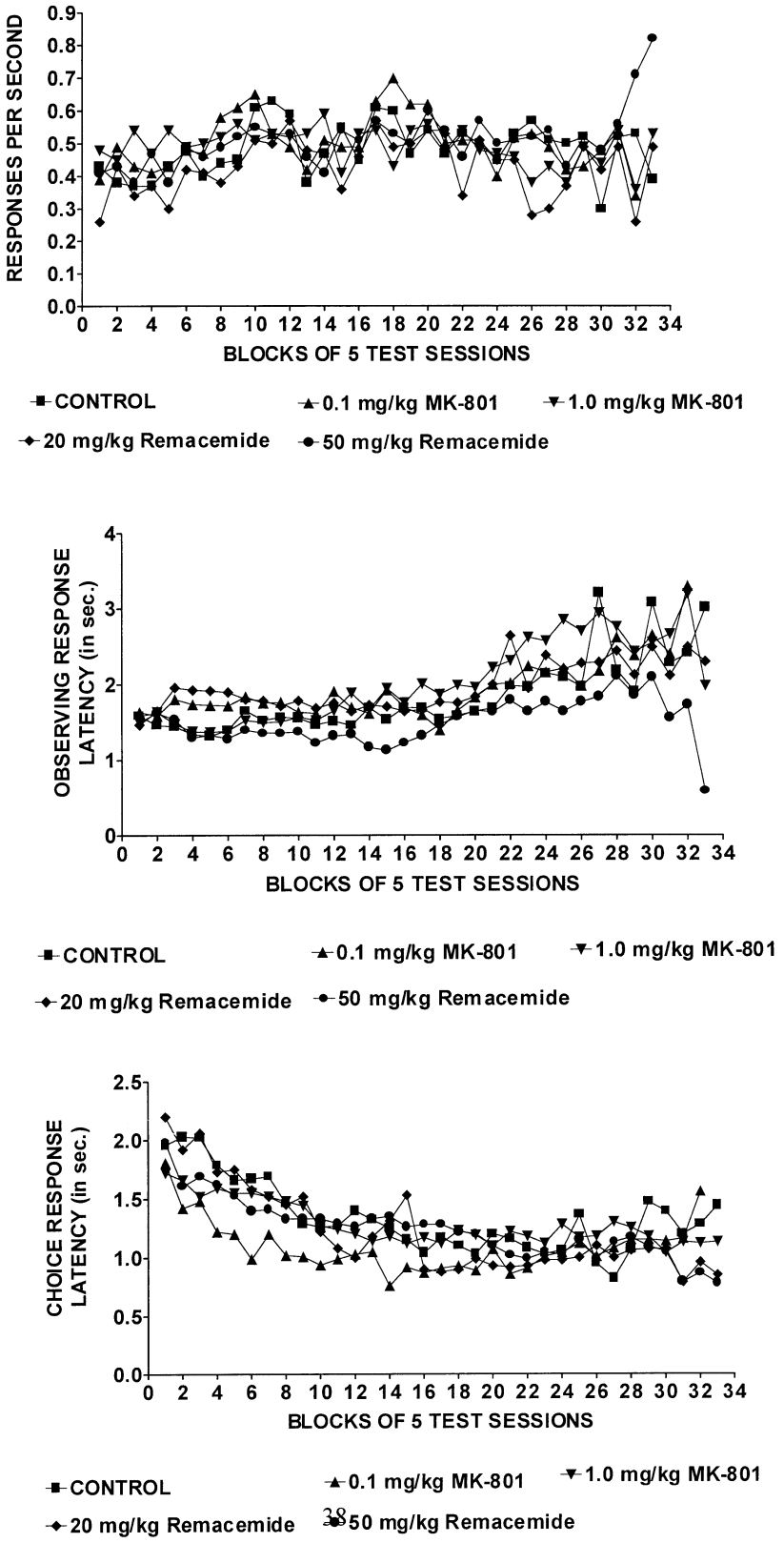

Fig. 4. Presents the effects of chronic treatment on DMTS response rates (a) observing response latency (b) and choice response latency (c) measured during the 18-month treatment phase. Each block of the five test sessions corresponds to 2 full weeks of drug treatment.

subjects must choose from among three choices rather than two; (2) subjects must discriminate symbols rather than colors; and (3) subjects must respond at random positions rather than set positions. Although there were no significant

treatment-related effects on the rate of progression through the various delay criteria, subjects in the high-dose remacemide group occasionally took longer to complete the delay "sets" than did the control group. These data are presented in Table 1.

Effects of chronic drug treatment on DMTS accuracy (0.01-s delay) are presented in Fig. 3. Although accuracies for both the low-dose MK-801 and the low-dose remacemide groups appear higher than those for all other groups, none of these data differ significantly from control. There also were no effects of treatment on any of the measures of DMTS response rates or latencies (Fig. 4a–c).

3.3. Effects on CPR measured during withdrawal

Fig. 5 presents the effects of decreasing or eliminating drug treatment on CPR accuracy measured during each of the 3-month withdrawal periods. During the first period of withdrawal, when the high doses of each drug were reduced to the low doses and the low doses were reduced to vehicle, there was a transient effect of prior high-dose remacemide treatment to increase CPR accuracy, which abated by the second week of the withdrawal ($P<.05$). During the second period of withdrawal, when all subjects received vehicle only, there was a significant effect of drug withdrawal in the high-dose remacemide group to impair CPR accuracy relative to control ($P<.05$). This effect was evident during Weeks 15–20 and again during Weeks 24–26. There were no effects of withdrawal on CPR accuracy in any of the other treatment groups.

Figs. 6a–c present effects of decreasing or eliminating drug treatment on CPR response rate, observing response latency, and choice response latency, respectively. As was the case for CPR accuracy, there were no consistent effects on response rate when the doses of either drug were reduced during the first period of withdrawal. During the second period of withdrawal, however, there was a significant relative decrease in CPR response rates among subjects that had received the high dose of remacemide during treatment ($P<.05$). This effect persisted from Week 15 through Week 21 of withdrawal. As was the case for CPR response rates, there was no effect on response latencies when the doses of either drug were reduced during the first period of withdrawal. During the second period of withdrawal, however, there was a significant relative increase in CPR response latencies among subjects that had received the high dose of remacemide during treatment. For observing response latency, this effect was evident during Weeks 19–21. For choice response latency, this effect emerged during Week 15 and continued throughout the remainder of the withdrawal period ($P<.05$).

3.4. Effects on DMTS measured during withdrawal

Fig. 7 presents the effects of drug withdrawal or reduction on DMTS accuracy (0.01-s delay) during each of the two withdrawal periods. During the first period of withdrawal, reducing the daily dose of remacemide from 50 to 20 mg/kg/day resulted in a significant increase in DMTS accuracy but only for Weeks 9 through 13 ($P<.05$). There were no further increases in accuracy observed when the doses were removed altogether during the second withdrawal period.

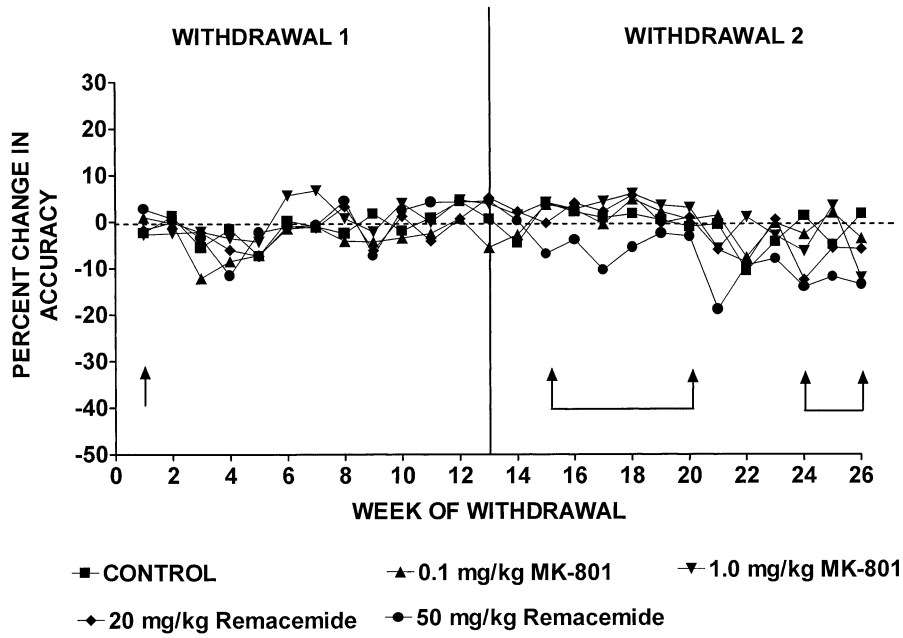

Fig. 5. Presents the effects of chronic treatment on CPR accuracy measured during the 6-month washout phase. Solid brackets and arrows denote points at which subjects in the 50-mg/kg remacemide group differed significantly from control ($P<.05$).

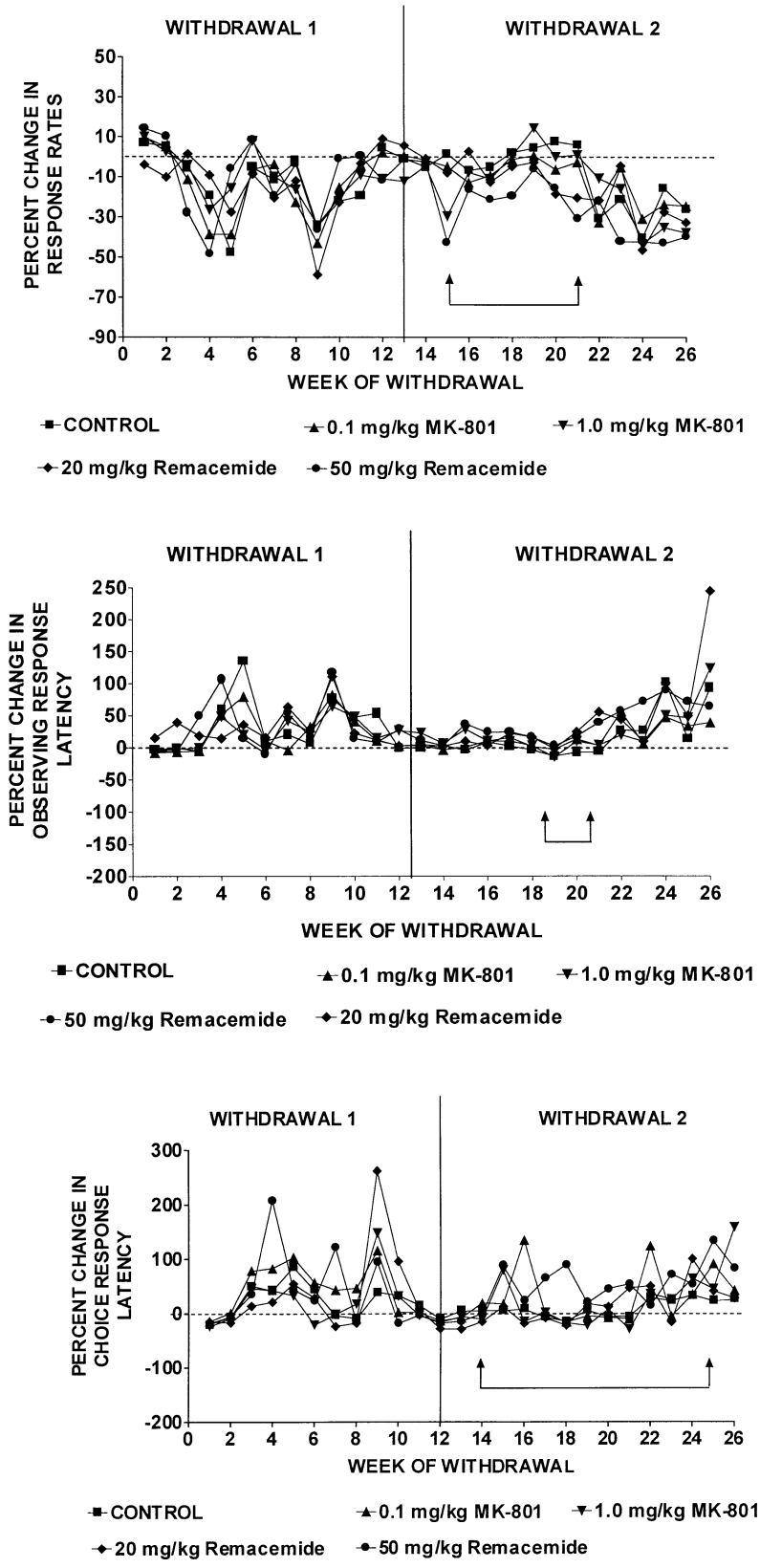

Fig. 6. Presents the effects of chronic treatment on CPR response rate (a) observing response latency (b) and choice response latency (c) measured during the 6-month washout phase. Brackets encompass the points at which the 50-mg/kg remacemide group differed significantly from control ($P < .05$).

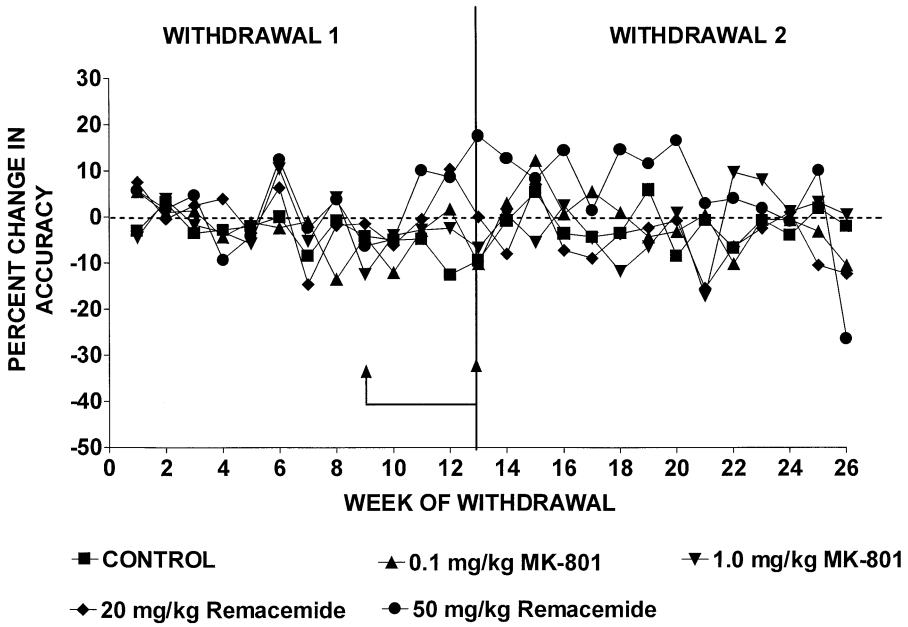

Fig. 7. Presents the effects of chronic treatment on DMTS accuracy measured during the 6-month washout phase. Brackets denote points at which subjects in the 50-mg/kg remacemide group differed significantly from control ($P < .05$).

Fig. 8a–c present effects of reducing or eliminating treatment on DMTS response rate, observing response latency, and choice response latency, respectively. During the first withdrawal phase, the low-dose MK-801 group exhibited transient, but significant increases in relative response rate (Weeks 6–11) and observing response latency (Weeks 1 and 2). The effect on observing response latency not only disappeared but also reversed during Weeks 19–22 (second withdrawal phase), in that relative changes in observing response latencies for this group became significantly less than those noted for controls. There were no significant effects observed for choice response latency at any point during withdrawal.

4. Discussion

The present experiment examined the effects of chronic remacemide and MK-801 treatment on the acquisition of behavioral tasks designed to model color and position discrimination and short-term memory in juvenile rhesus monkeys. When administered at high doses, both drugs impaired subjects' abilities to acquire and perform color and position discrimination but neither drug altered their ability to acquire or perform the short-term memory task. The effects of MK-801 on color and position discrimination abated after the 17th block of five test sessions, whereas the effects of remacemide persisted to the end of the 18-month dosing period. In neither case were the effects on accuracy associated with significant changes in response rate or in the time required by subjects to make a choice response.

During the first period of withdrawal, when the high dose of each drug was reduced to the low dose and the low dose was reduced to vehicle, there was a transient increase in CPR accuracy among the high-dose remacemide group, which abated almost immediately. A similarly transient effect was seen for the short-term memory task. During the second period of withdrawal, when all subjects received vehicle only, the effects of prior remacemide treatment on CPR were generally consistent with reductions in accuracy and response rate with concomitant increases in observing and choice response latencies. The effects of prior remacemide treatment on short-term memory were somewhat less consistent, with only a transient increase in observing response latency. Taken together, these results suggest that chronic treatment with relatively high doses of remacemide or MK-801 can delay the acquisition of complex operant behaviors but that these effects can be overcome with reduced drug exposure or additional behavioral experience. This pattern of results is consistent with previously reported effects of MK-801 in rats (see Ref. [8] for review) and provides important new information regarding the effects of remacemide.

One of the most notable results of the present experiment is the finding that chronic treatment with both remacemide and MK-801 delayed the acquisition of color and position discrimination behavior but that the effects of remacemide were considerably more persistent than were those of MK-801. The effects of MK-801 appeared during the 10th week of testing (fifth block of five test sessions) and abated by the 34th week of testing (17th block of five test sessions), a period corresponding to only 6 months of chronic treatment. The effects of remacemide, on the other hand, emerged during the 6th week of testing (third block of five test

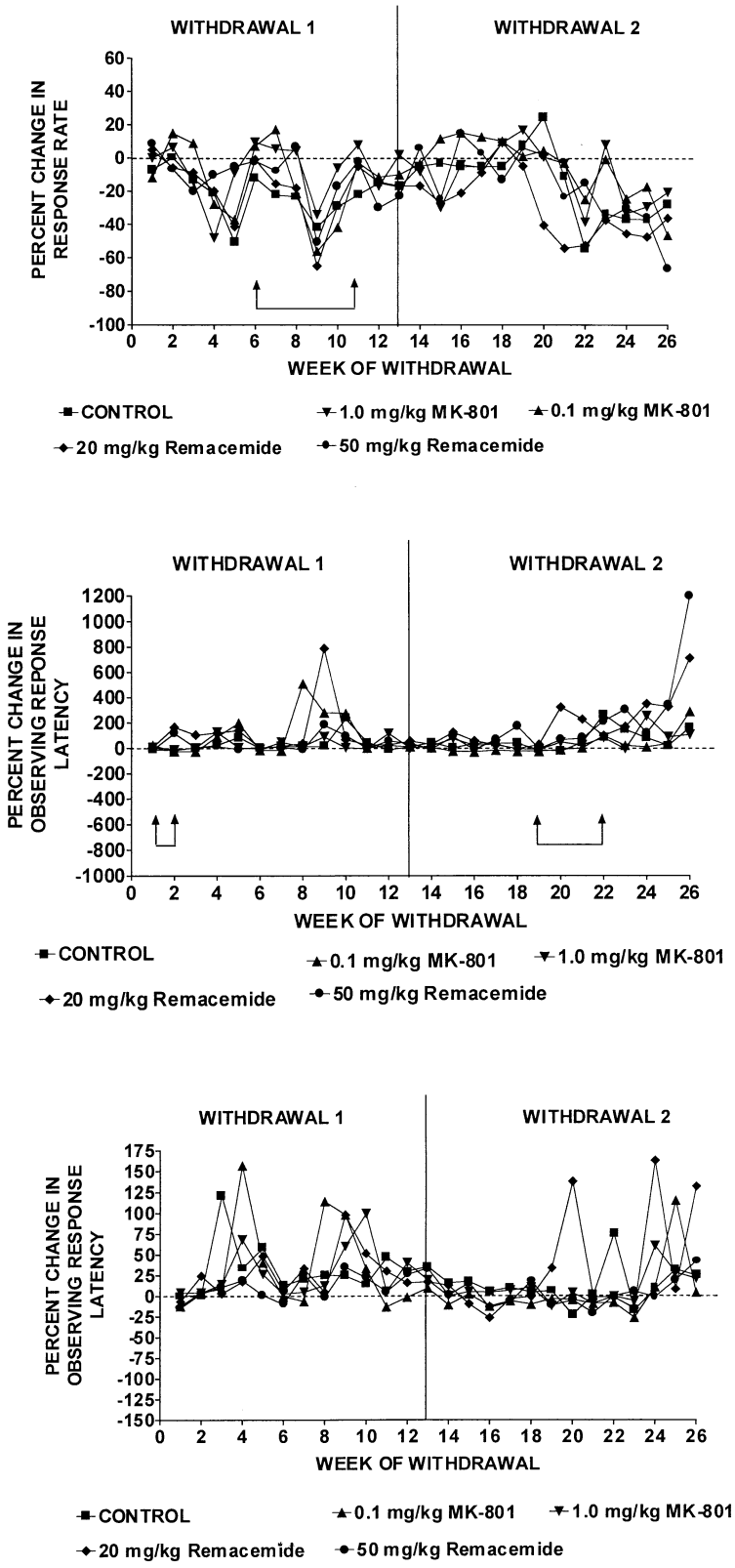

Fig. 8. Presents the effects of chronic treatment on DMTS response rate (a) observing response latency (b) and choice response latency (c) measured during the 6-month washout phase. Brackets encompass the points at which the 50-mg/kg remacemide group differed significantly from control ($P < .05$).

sessions) and persisted almost to the end of the 18-month treatment phase. Because the high dose of MK-801 represented the highest dose that could be administered without rendering subjects unconscious (unpublished pilot study), it

seems unlikely that the differential effects of remacemide and MK-801 resulted from the MK-801 dose being too low. Rather, this pattern of results suggests that subjects that were treated with MK-801 were able to overcome its deleterious effects, either through subsequent behavioral practice or through tolerance to the effects of the drug, whereas the subjects that had been treated with remacemide were not. Because there were no significant differences in response rates between the subjects treated with MK-801 relative to those that had been treated with remacemide, and likewise, no significant differences in the number of behavioral trials completed, it seems unlikely that differences in behavioral practice underlie differences in the rate of ''recovery'' between these two groups. Rather, it seems more likely that differences in tolerance or developmental disruption may have influenced the differences in the effects of these two drugs. Huang and Stevens [12] reported that acute injections of MK-801 resulted in impaired passive avoidance performance in rats but that these effects disappeared after 14 days of chronic treatment. In the present experiment, MK-801 produced significant impairments in CPR performance which disappeared by block 17. In these same subjects, MK-801 has been shown to increase aspects of motivation (measured using a progressive ratio schedule of reinforcement) with effects emerging during the 10 blocks of treatment disappearing by block 20 [24]. The suggestion that differences in tolerance may underlie differences in the effects of these drugs is further supported by findings that remacemide neither induces nor inhibits its own metabolism following chronic exposure (unpublished observations), an observation that is consistent with the apparent lack of tolerance observed presently. Taken together, these results suggest that tolerance can develop to the behavioral effects of MK-801 (or that developmental processes can overcome these effects) and that these phenomena may account for the differences in the effects of MK-801 and remacemide seen presently.

In addition to the tolerance that may develop to effects of MK-801, it is also possible that differences in the effects of MK-801 and remacemide reflect differences in their respective mechanisms of action. More specifically, it is likely that the more persistent effects of remacemide on color and position discrimination reflect its activity at fast sodium channels (or its capacity to block NMDA receptors and fast sodium channels concurrently), whereas the less persistent effects of MK-801 reflect its effects to block NMDA receptors more selectively. Previous experiments that have examined effects of sodium channel antagonists on behavior have revealed effects that are qualitatively similar to those seen presently for remacemide. Tetrodotoxin, for example, has been shown to disrupt behavioral acquisition in a variety of paradigms involving taste aversion conditioning [14], inhibitory avoidance, and spatial memory [1]. Also notable is the fact that, in each of these previous experiments, the effects of tetrodotoxin were evident even when the drug was administered after testing, results that are consistent with the dosing regimen used presently and with the suggestion that sodium channel blockade can have disruptive effects on behavioral performance.

Another notable result of the present experiment is the fact that both MK-801 and remacemide impaired color and position discrimination but that neither drug impaired performance of the short-term memory task. This is particularly striking given the fact that, when presented without delays (or with delays of only 0.01 s), the short-term memory task is fundamentally similar to the color and position discrimination task. Differences in the effects of drugs on these behaviors may reflect procedural differences in the way the tasks are presented rather than differences in the effects of these drugs on the specific cognitive functions being modeled. The CPR task, for example, requires accurate color discrimination whereas the DMTS task does not. Although regularly scheduled opthalmic examinations failed to reveal any evidence of compound-related ocular disease in the present subjects, the possibility that drug treatment may have impaired subjects' ability to accurately discriminate color, and therefore accurately perform the color and position discrimination task, cannot be ruled out. Alternatively, the difference in the effects of treatment on these two tasks may simply reflect the fact that the matching-to-sample task, which requires subjects to select from three random choices, may be inherently more difficult than the CPR task, which requires subjects to select from only two preset choices. This suggestion is supported by the fact that baseline (control) performance on the short-term memory task was significantly poorer than was baseline performance on the color and position discrimination task (see Figs. 1 and 3, respectively). Therefore, the possibility that difference in baseline task performance may have helped to mask any drug-induced performance deficits cannot be ruled out.

Finally, it is important to comment on the possible implications of the present results for our current understanding of learning and memory and of its proposed relationship to LTP. LTP is a neural phenomenon whereby synaptic efficiency is increased through repeated tetanic stimulation [3,28]. Although the physiologic relevance of this phenomenon is still a matter of some debate, it is generally believed that the mechanisms involved with the production and maintenance of LTP are intimately related to those that underlie learning and memory [12]. Further, it has often been argued that the mechanisms that underlie LTP are intimately tied to the function of NMDA receptors [4]. Drugs that block NMDA receptors (such as MK-801) have been shown to block the development of LTP and also to block the formation of certain types of memory [12]. In the present experiment, blocking NMDA receptors alone (with MK-801) had less persistent effects on behavioral performance than did the presumed disruption of NMDA receptors and fast sodium channels concurrently (with remacemide). Although definitive conclusions regarding the respective roles of NMDA receptors and LTP as mediators of learning cannot be drawn from the present data, the results suggest

that the mechanisms that underlie the acquisition of complex operant behavior in developing primates may be significantly more complicated than suggested by the model of NMDA receptor-mediated induction of LTP.

The present experiment examined the effects of remacemide (an NMDA antagonist, which also blocks fast sodium channels) or MK-801 (which blocks NMDA receptors selectively) on the acquisition of color and position discrimination and short-term memory performance in juvenile rhesus monkeys. Chronic exposure to high doses of either drug impaired the acquisition of accurate color and position discrimination but neither drug altered performance of the short-term memory task. In the case of MK-801, these effects abated within 6 months of the start of treatment. In the case of remacemide, the effects persisted for most of the 18-month dosing period. The fact that the effects of remacemide on behavioral performance were more persistent than those seen for MK-801 suggests that (a) tolerance may develop to the behavioral effects of MK-801, which does not develop to the effects of remacemide or (b) that the concurrent antagonism of NMDA receptors and fast sodium channels may have more profound consequences for behavior than does the more selective antagonism of NMDA receptors alone. Future experiments are necessary to determine whether the results reported here reflect a specific effect of these NMDA receptor antagonists during development or whether a similar pattern of results would emerge in adult animals that were chronically exposed in this way.

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References

[1] F. Bermudez-Rattoni, I.B. Introni-Collison, J.L. McGaugh, Reversible interaction of the insular cortex by tetrodotoxin produces retrograde and antereograde amnesia for inhibitory avoidance and spatial learning, Proc. Natl. Acad. Sci. 88 (12) (1991) 5379–5382.

[2] G.L. Collingridge, S.J. Kehl, H. McLennan, Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus, J. Phys. 334 (1983) 33–46.

[3] G.L. Collingridge, R.A.J. Lester, Excitatory amino acid receptors in the vertebrate central nervous system, Pharm. Rev. 40 (1989) 143–210.

[4] J.A. Court, E.K. Perry, M. Johnson, M.A. Piggott, J.A. Kerwin, R.H. Ince, P.G. Ince, Regional patterns of cholinergic and glutamate activity in the developing and aging human brain, Dev. Brain Res. 71 (1993) 73–82.

[5] C.L. Cramer, M.L. Stagnitto, M.A. Knowles, G.C. Palmer, Kanic acid and 4-aminopyridine seizure models in mice: evaluation of efficacy of anti-epileptic agents and calcium antagonists, Life Sci. 54 (1994) PL271–PL274.

[6] P. Crawford, A. Richens, G. Mawer, P. Cooper, J.B. Hutchinson, A double-blind placebo-controlled crossover study of remacemide hydrochloride on adjunct therapy in patients with refractory epilepsy, Epilepsy 1 (1992) 7–17.

[7] W.W. Cumming, R. Berryman, The complex discriminated operant: Studies of matching-to-sample and related problems, in: D.I. Mostofsky (Ed.), Stimulus Generalization, Stanford Univ. Press, Stanford, CA, 1965, pp. 284–330.

[8] S.I. Deutsch, J. Mastropaolo, R.B. Rossem, Neurodevelopmental consequences of early exposure to phencyclidine and related drugs, Clin. Neuropharmacol. 21 (6) (1998) 320–332.

[9] S.W. D'Souza, S.E. McConnell, P. Slater, A.J. Barson, *N*-methyl-D-aspartate binding sites in neonatal and adult brain, Lancet 339 (8803) (1992) 1240.

[10] G.E. Garske, G.C. Palmer, J.J. Napier, R.C. Griffith, L.R. Freedman, E.W. Harris, R. Ray, S.A. McCreedy, J.C. Blosser, J.H. Woodhead, H.S. White, E.A. Swinyard, Preclinical profile of the anticonvulsant remacemide and its enantomers in the rat, Epilepsy Res. 9 (3) (1991) 161–174.

[11] M.S. Golub, M.E. Gershwin, Standardized neonatal assessment in the rhesus monkey, in: P.W. Nathaniel, J.T. Parer (Eds.), Research in Perinatal Medicine, Perinatology Press, New York, 1984, pp. 55–81.

[12] E.P. Huang, C.F. Stevens, The matter of mind: Molecular control of memory, Essays Biochem. 33 (1998) 165–178.

[13] H.B. Hucker, J.E. Hutt, S.D. White, B.H. Arison, A.G. Zacchei, Disposition and metabolism of (+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*] cyclohepten-5,10-imine in rats, dogs and monkeys, Drug Metab. Dispos. 11 (1) (1983) 54–58.

[14] S.F. Ivanova, J. Bures, Acquisition of conditioned taste aversion in rats is prevented by tetrodotoxin blockade of a small midbrain region centered around the parabranchial nuclei, Physiol. Behav. 48 (4) (1990) 543–549.

[15] M. Johnson, E.K. Perry, P.G. Ince, P.J. Shaw, R.H. Perry, Autoradiographic comparison of the distribution of [3H]MK801 and [3H]CNQX in the human cerebellum during development and aging, Brain Res. 615 (1993) 259–266.

[16] R.C. Little, G.A. Milliken, W.W. Stroup, R.D. Wolfinger, SAS® System for Mixed Models, SAS Institute, Cary, NC, 1996.

[17] J.W. McDonald, M.V. Johnston, Excitatory amino acid neurotoxicity in the developing brain, NIDA Res. Monog. 133 (1993) 185–205.

[18] D.T. Monaghan, H. Larsen, NR1 and NR2 subunit contributions to *N*-methyl-D-aspartate receptor channel blocker pharmacology, J. Pharmacol. Exp. Ther. 280 (2) (1997) 614–620.

[19] S.K. Norris, A.E. King, The stereo-isomers of the anticonvulsant, ARL-12495AA limit sustained repetitive firing and modify action potential properties of rat hippocampal neurons in vitro, J. Pharmacol. Exp. Ther. 281 (3) (1997) 1191–1198.

[20] G.C. Palmer, R.J. Murray, T.C.M. Wilson, M.S. Eisman, R.K. Ray, R.C. Griffith, J.J. Napier, M. Fedorchuk, M.L. Stagnitto, G.E. Garsky, Biological profile of the metabolites and potential metabolites of the anticonvulsant remacemide, Epilepsy Res. 12 (1992) 9–20.

[21] M.G. Paule, R.R. Allen, J.R. Bailey, A.C. Scallet, S.F. Ali, R.M. Slikker, W. Slikker, Chronic marijuana smoke exposure in the rhesus monkey: II. Effects on progressive ratio and conditioned position responding, J. Pharmacol. Exp. Ther. 260 (1) (1992) 210–222.

[22] M.G. Paule, P.J. Bushnell, J.P. Maurissen, G.R. Wenger, J.J. Buccafusco, J.J. Chelonis, R. Elliott, Symposium overview: The use of delayed matching-to-sample procedures in studies of short-term memory in animals and humans, Neurotoxicol. Teratol. 20 (5) (1998) 493–502.

[23] M.G. Paule, J.J. Chelonis, E.A. Buffalo, D.J. Blake, P.H. Casey, Operant test battery performance in children: Correlation with IQ, Neurotoxicol. Teratol. 21 (3) (1999) 223–230.

[24] E.J. Popke, R.R. Allen, E. Pearson, T. Hammond, M.G. Paule, Differential effects of two NMDA receptor antagonists on cognitive–behavioral development in non-human primates, Neurotoxicol. Teratol. (in preparation).

[25] G.E. Schulze, D.E. McMillan, J.R. Bailey, A. Scallett, S.F. Ali, W. Slikker, M.G. Paule, Acute effects of delta-9-tetrahydrocannabinol in rhesus monkeys as measured by performance in a battery of complex operant tests, J. Pharmacol. Exp. Ther. 245 (1) (1988) 178–186.
[26] G.E. Schulze, D.E. McMillan, J.R. Bailey, A. Scallett, S.F. Ali, W. Slikker, M.G. Paule, Effects of marijuana smoke on complex operant behavior in rhesus monkeys, Life Sci. 45 (1989) 465–475.
[27] P. Slater, S.E. McConnell, S.W. D'Souza, A.J. Barson, Postnatal changes in *N*-methyl-D-aspartate receptor binding and stimulation by glutamate and glycine of [3H]-MK-801 binding in human temporal cortex, Br. J. Pharmacol. 108 (4) (1993) 1143–1149.
[28] J. Tomita, Y. Shibata, T. Sakurai, Y. Okada, Involvement of a protein kinase C-dependent process in long-term potentiation formation in guinea pig superior colliculus slices, Brain Res. 536 (1990) 146–152.
[29] M. Tsuda, N. Shimizu, Y. Yajima, T. Suzuki, M. Misawa, Hypersusceptibility to DMCM-induced seizures during diazepam withdrawal in mice: Evidence for upregulation of NMDA receptors, Naunyn-Schmiedeberg's Arch. Pharmacol. 357 (3) (1998) 309–315.