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Differential effects of two NMDA receptor antagonists on cognitive–behavioral development in nonhuman primates I☆

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Abstract

The present experiment examined effects of chronic exposure to remacemide (an *N*-methyl-D-aspartate [NMDA] antagonist which also blocks fast sodium channels) or MK-801 (which blocks NMDA receptors, exclusively) on learning and motivation in young rhesus monkeys. Remacemide (20 or 50 mg/kg/day) or MK-801 (0.1 or 1.0 mg/kg/day) was administered every day to separate groups of animals via orogastric gavage for up to 2 years. Immediately prior to dosing, 5 days per week (M–F), throughout the 2-year dosing period, an incremental repeated acquisition (IRA) task was used to assess learning and a progressive ratio (PR) task was used to assess motivation. The results indicate an effect of 50 mg/kg/day remacemide to impair learning (IRA) which persisted even after drug treatment was discontinued. MK-801 had no effect on learning but transiently increased motivation. Because the effects of remacemide occurred independently of changes in motivation or response rates, they are likely due to specific cognitive impairments and are not due to an inability of subjects to fulfill the motoric requirements of the task. The fact that MK-801 did not alter learning suggests that NMDA antagonism alone may be insufficient to produce learning deficits in young monkeys and that such deficits may rely on the ancillary blockade of fast sodium channels.

Keywords: Remacemide; MK-801; *N*-methyl-D-aspartate (NMDA); Fast sodium channels; Operant behavior; Learning; Motivation

1. Introduction

Remacemide hydrochloride is a relatively new compound that exhibits promising neuroprotective and anticonvulsant properties. In animal models, remacemide can prevent seizures induced by the application of maximal [11] and subthreshold [23] electroshock as well as those induced by *N*-methyl-D-aspartate (NMDA) [23] and kainic acid [8]. In human clinical trials, remacemide has proven effective in reducing the frequency of seizures in subjects adjunctive to other antiepileptic drugs [9].

The mechanisms that underlie remacemide's neuroprotective and anticonvulsant properties are thought to involve noncompetitive antagonism of NMDA receptors and the blocking of fast sodium channels. 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 [5]. Long-term potentiation has been characterized as an increase in synaptic efficiency that can be induced by repeated tetanic stimulation [6,32]. It is generally believed that the mechanisms involved with the production and maintenance of long-term potentiation are also involved with learning and memory processes [17].

In addition to their purported role in learning and memory, the excitatory amino acids play an important role during development by regulating neuronal survival, axonal and dendritic structure, and synaptic genesis and plasticity [22]. Developmental observations in humans indicate marked differences in excitatory amino acid binding sites from the neonatal period through the 10th decade of life [7,10,20,31].

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Consistent 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 [10].

The purpose of the present experiment was to examine the developmental effects of chronic administration of remacemide, and of the classical NMDA receptor antagonist MK-801, on the acquisition of behavioral tasks which are designed to model learning and motivation in rhesus monkeys. Drugs were administered daily (7 days/week) to separate groups of juvenile rhesus monkeys (*Macaca mulatta*) via orogastric gavage and the ability of subjects to acquire complex operant behaviors was measured. On behavior testing days (Monday–Friday), dosing immediately followed the assessment of behavior. This dosing and testing procedure served to minimize the role that acute drug effects played in the results and enabled analyses to focus on the long-term effects of chronic drug exposure rather than on the acute effects of drug intoxication. Concurrent investigation of these two compounds enabled direct comparisons to be made between the cognitive–behavioral effects of a relatively novel NMDA receptor antagonist, remacemide, with those of the well-characterized NMDA receptor antagonist, MK-801. In addition to monitoring behavior during chronic drug treatment, behavior also was monitored during a two-step drug withdrawal procedure.

The operant behaviors monitored included: incremental repeated acquisition (IRA) to assess learning and progressive ratio (PR) to assess appetitive motivation. (Subjects also were assessed using a color and position discrimination and delayed-matching-to-sample task. The results of these assessments will be presented elsewhere.) The IRA task requires subjects to perform a specific sequence of lever presses to receive reinforcers. Because the specific lever sequence is different for each test session, the IRA task can provide an index of a subject's ability to learn novel sequences [4]. Performance on this task has been shown to be positively correlated with IQ in children [27]. The PR task requires an incrementally greater number of responses (on a single operant lever) for subsequent reinforcers. This task has been used extensively to assess aspects of appetitive motivation [14,15], the reinforcing effects of drugs [12,29], and the motivational effects of chronic marijuana exposure [26]. 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.

2. Methods

2.1. Subjects and housing

Subjects were 30 experimentally naive female rhesus monkeys (*M. 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. All subjects were born in captivity (vaginal births) at one of three facilities maintained by Labs of Virginia, Inc. (i.e., Hampton, SC; Morgan Island, SC; Yemmassee, SC). Subjects were weaned immediately prior to their arrival at NCTR and were individually housed from the time of arrival (i.e., 3 months prior to the start of treatment and testing) until the end of the experiment. Females were chosen in light of published reports which indicate that female rats are more sensitive to the behavioral and biochemical effects of dizocilpine [1,16,34] and remacemide (Astra-Zeneca, Personal Communication) than are males. Daily access to food (High Protein Monkey Diet, PMI Nutrition International, Brentwood, MO) was supplemented with fresh fruit (three times per week) 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 [24–26]. Subjects were housed under a 12-h light/dark cycle (lights on at 6:00 a.m. 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, subjects began an 18-month daily dosing regimen, which was followed by a 6-month, two-step washout phase (i.e., a total of 730 dosing days per subject). Drugs were administered 7 days/week, within 1 h after daily (Monday–Friday) behavioral test sessions, and at the same time of day on Saturday and Sunday. Administration of drugs after daily behavioral assessment (rather than before) served to minimize the impact of acute drug effects and allow analyses to focus on the long-term effects of chronic treatment. During the 18 month initial treatment phase, doses of remacemide (20 or 50 mg/kg/day, free base) and MK-801 (0.1 mg/kg/day 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 which 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. During the gavage procedure, subjects were

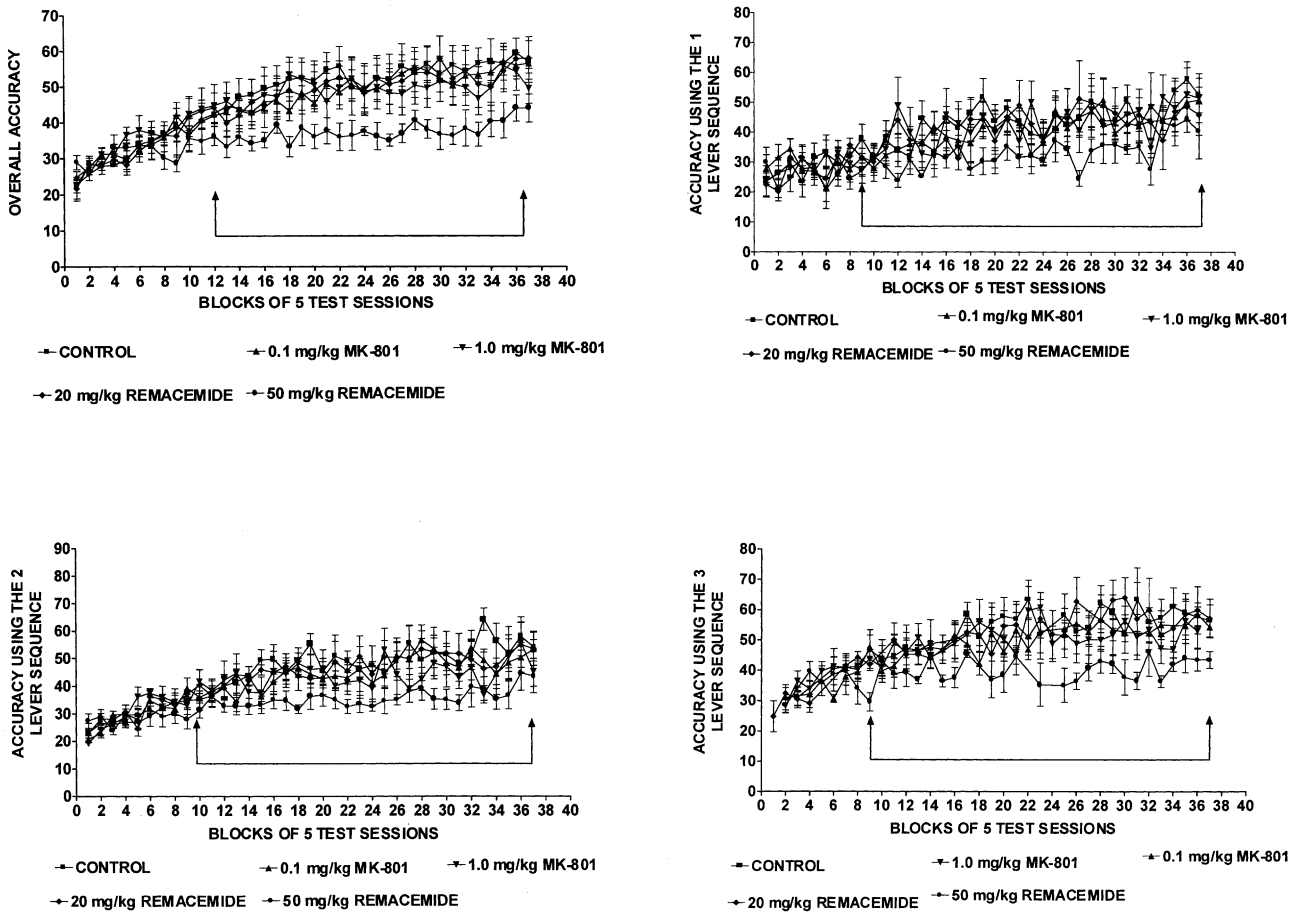


Fig. 1. Effects of chronic treatment on IRA accuracy measured during the 18-month treatment phase (means ± S.E.M.). Each block of five test sessions represents 2 weeks of daily drug exposure. (a) Overall accuracy. (b–d) Accuracy for each of IRA levels 1–3, respectively. Brackets encompass the points at which the 50 mg/kg remacemide group differed significantly from control ($P < .05$).

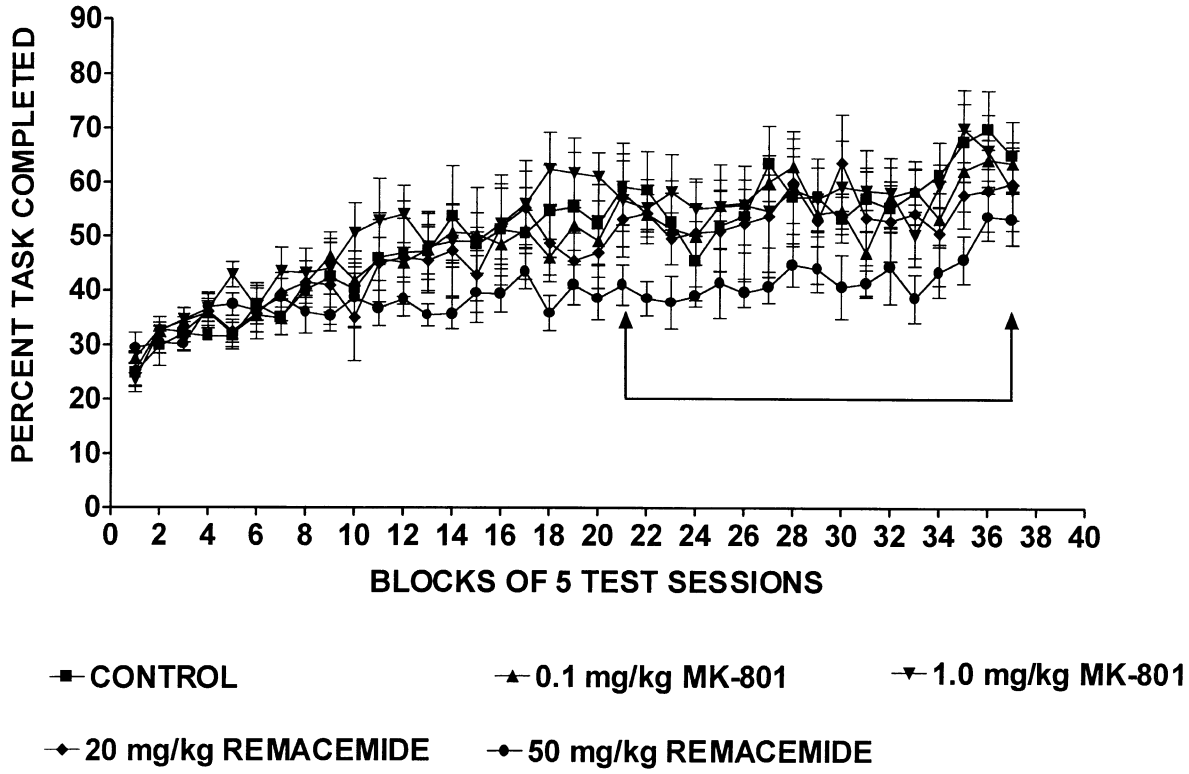
confined to portable restraint chairs (Primate Products, Redwood City, CA) and were held in place by a restraint collar and several technicians. This method of restraint allows subjects free movement of fore and hind limbs, 360° rotation capacity and the ability to rest naturally on their haunches. During the actual gavage, subjects were additionally restrained by several technicians. 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 43.8 cm orogastric gavage tube was cut from a length of plastic intravenous tubing (internal gauge = 0.63 cm). One end of the 43.8-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. "Washout" phase

During the first 3 months of the 6-month washout phase, 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. During the second 3 months of the 6-month washout phase, all subjects received water only. Thus, all low-dose animals were gavaged with water only for the entire 6 months of washout, whereas the high dose animals were gavaged with the low dose for the first 3 months of washout followed by water for the remaining 3 months.

2.3. Behavioral testing apparatus

Prior to operant behavior testing, subjects were placed into portable restraint chairs as described above. In addition to affording the subject free movement of fore and hind limbs, the portable restraint chair positions the subject's head in a manner optimal for viewing stimuli presented during behavioral testing. After placement in the restraint


PERCENT TASK COMPLETED
0
10
20
30
40
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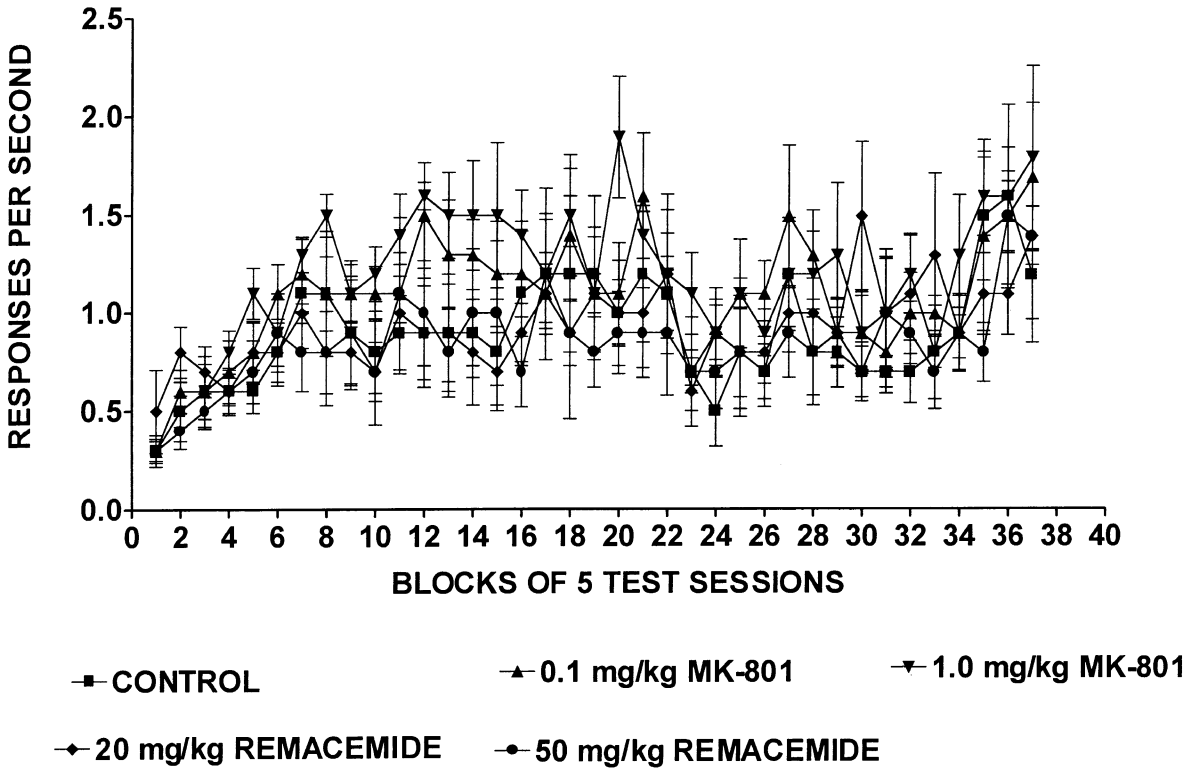
60
70
80
90
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
BLOCKS OF 5 TEST SESSIONS
CONTROL
0.1 mg/kg MK-801
1.0 mg/kg MK-801
20 mg/kg REMACEMIDE
50 mg/kg REMACEMIDE


RESPONSES PER SECOND
0.0
0.5
1.0
1.5
2.0
2.5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
BLOCKS OF 5 TEST SESSIONS
CONTROL
0.1 mg/kg MK-801
1.0 mg/kg MK-801
20 mg/kg REMACEMIDE
50 mg/kg REMACEMIDE

chairs, subjects were placed into 1 of 10 sound-attenuating operant test chambers (Model PCP-001, BRS/LVE, Beltsville, MD). Each test chamber measured 111.8 × 68.6 × 127 cm and was equipped with a house light, a ventilating fan, and a food trough. The test chambers were additionally equipped with four model RRL-001 retractable levers, which were used during the IRA and PR tasks, as well as three model PPC-012 projection press plates (BRS/LVE), six serial position lights, correct and incorrect response indicator lights, and a speaker through which ambient white noise was delivered. A diagram of the operant test panel is provided elsewhere [30]. Each operant test panel was controlled by a computerized input/output controller (developed at the National Center for Toxicological Research, Jefferson, AR) that administered the behavioral tasks and recorded behavioral responses. The software controlling the computers and response panels was written at the National Center for Toxicological Research.

2.4. Training and testing procedure

Prior to the start of the experiment, all subjects underwent a 2-week (30 min/day) procedure designed to acclimate them to the portable restraint chairs (see above) and to familiarize them with the banana-flavored food pellets that were used as reinforcers during subsequent behavioral testing. During this procedure, which all subjects completed concurrently, subjects were confined to the restraint chairs and were offered exactly 20 banana-flavored food pellets per day by an experimenter. By the end of this 2-week procedure, all subjects consumed all of the proffered pellets within each 30-min acclimation period. At the end of this familiarization process, subjects were assigned to one of five treatment groups that were balanced with respect to age, place of birth, and body weight. For the first 7 days of daily dosing all remacemide-treated subjects received 20 mg/kg/day remacemide per day. After 7 days of treatment, half of these subjects began treatment with 50 mg/kg/day remacemide and continued to receive this dose for the remainder of the treatment phase. This "ramping" procedure was designed to allow subjects to habituate to the transient emetic effects that sometimes accompany the initial exposure to oral remacemide. Indeed, by the end of the 2-year experiment, only 23 episodes of emesis were documented out of a total of 4380 high-dose treatments.

On the third day of drug treatment (i.e., the same day for all monkeys), subjects began interacting with the behavioral test apparatus while performing the IRA task as described below. Because there was no pretreatment period of operant training, data collected during the training of this, and each of the other behavioral tasks, constituted the primary endpoint(s) of the study. All subjects completed the same number of IRA test sessions regardless of treatment group or task performance. Behavioral testing was conducted at the same time of day, Monday through Friday, and lasted approximately 50 min per day. Subjects were rotated through the 10 behavioral test chambers such that no subject was tested in the same chamber on consecutive test days. The purpose of this procedure was to ensure that any subtle variation which may have resulted from undetectable differences in equipment function would be equally distributed across all subjects and across all treatment groups. Performance of the IRA and PR tasks occurred every other day, Monday–Friday and lasted a total of 50 min (40 min of IRA followed by 10 min of PR). On days when subjects were not assessed for performance of IRA and PR, they were assessed for performance on a conditioned position responding task and a delayed-matching-to-sample task. The data collected using these other behavioral tasks will be presented elsewhere [28].

2.5. Incremental repeated acquisition

During IRA training (40-min sessions), subjects progressed through four performance levels. At Level 1, a single press on any one of the four extended response levers resulted in the delivery of a food pellet reinforcer. The purpose of training level 1 was to familiarize subjects with the operant levers and with the procedure for receiving reinforcers. After 40 reinforcers had been earned, training progressed to Level 2 in which one of the four levers was randomly deactivated. Thus, a single response on any of the three active levers produced a reinforcer, while responses on the deactivated lever had no programmed consequences. After 40 reinforcers had been earned at Level 2, training progressed to Level 3, in which another of the active levers was randomly deactivated, leaving only two active levers. After 40 reinforcers were earned at Level 3, training proceeded to Level 4, which actually represented subjects' first exposure to the full IRA task. At this level, only one of the four levers remained active and each press on that lever resulted in reinforcer delivery. If subjects did not complete training for IRA in one session, they continued during the next test session at the level at which they stopped on the previous test day. Thus, if 38 pellets were earned at Level 2 on one test day, subjects were allowed to obtain two more reinforcers at Level 2 during the next test session prior to moving to Level 3, and so on. After completing IRA training, subjects began performance of the full IRA task during the next scheduled test session. During performance of the full IRA task, subjects were required to perform a specific sequence of lever presses to obtain reinforcers. At

Fig. 2. Effects of chronic treatment on IRA percent task completed (2a) and response rate (2b) measured during the 18-month treatment phase (means ± S.E.M.). Each block of five test sessions represents 2 weeks of daily drug exposure. Brackets encompass the points at which the 50 mg/kg remacemide group differed significantly from control ($P < .05$).

the beginning of each session, subjects could respond on any of the four retractable levers, but only responses to one of these resulted in reinforcer delivery. After 20 reinforcers were earned, a 1-min timeout was presented, during which all levers were inactivated and all panel lights were extinguished. Immediately after this timeout period, an "incremented" two-lever sequence was presented. At this level, a response on an additional lever was required before a response on the initial correct lever produced food. After the 20th errorless two-lever sequence, a 1-min timeout was followed by an incremented three-lever sequence. At this level, an additional lever press was required prior to the previously learned two-lever sequence (i.e., a total of three specific lever presses were required) to produce a reinforcer. After completing 20 errorless three-lever sequences, a four-lever sequence was initiated, followed by a five-, and finally, a six-lever sequence, depending on the subject's performance. The sequence of lever presses differed for each subject and for each test session. All sequences were determined randomly with the following exceptions: (1) Sequences were never presented in order either from left to right or from right to left; (2) Sequences were never presented in which the same lever was used consecutively; (3) With the exception of the single lever sequences, sequences were never presented in which fewer than two levers were utilized. A maximum of 120 errorless sequences (20 reinforcers per sequence × 6 sequences = 120) could be performed during each 40-min IRA test session. Immediately after the completion of the IRA test session, all levers were retracted for 60 s and all panel lights were extinguished. At the end of this 1-min "quiet period," the far right retractable lever was extended, signaling the start of the 10-min PR test.

2.6. Progressive ratio

PR training began after specific performance criteria had been met using the delayed-matching-to-sample procedure that was presented on alternate test days (data not shown). The number of dosing days that preceded the commencement of PR training ranged from 162 to 380 (age range = 412–712 days), but did not differ between treatment groups (P=.63).

PR training began 1 min after the end of the IRA session and lasted for 10 min. For level one of PR training, only one of the retractable operant levers was extended and every response made on that lever resulted in reinforcement. After subjects had earned 100 reinforcers under the training schedule, subjects were presented with the full PR schedule during the next test session. During full PR, the first pellet earned during a given test session required a single lever press and an incrementally greater number of responses was required for each subsequent reinforcer. Thus, the first reinforcer earned during each session required a single response on the operant lever. The second reinforcer required two responses, the third reinforcer required three responses, etc. The PR task continued for a total task time of 10 min.

2.7. Behavioral endpoints

For the IRA task, accuracy, response rate, and percent task completed were monitored. Accuracy was defined as the total number of correct responses divided by the total number of responses made, times 100. A response was considered "correct" as long as it corresponded to the next required lever in that sequence. Incorrect responses made within a sequence neither reset the sequence nor required subjects to start again at the beginning. Overall accuracy (i.e., collapsed across all sequence levels) was monitored as was accuracy during performance of each of the first three sequence levels. All subjects occasionally reached sequence levels 4–6, but this did not happen with enough regularity to permit meaningful independent analysis. Response rate was defined as the total number of lever responses divided by the total running time (in seconds) for a given test session. Percent task completed was defined as the number of errorless sequences completed, divided by the total number of errorless sequences possible (here, 120), times 100. The percent task completed measure provides a metric of the length of IRA sequence learned by subjects during a given test session. Thus, percent task completed values between 0 and 16.7% indicate completion of only one lever sequences, percent task completed values between 16.7% and 33.3% indicate that subjects completed at least a two-lever sequence, but had not yet completed any three-lever sequences, etc.

For the PR task, response rate and last ratio completed were monitored. As was the case for the IRA task, response rate was defined as the total number of responses divided by the total running time (in seconds) for a given test session. The last ratio completed was defined as the total number of responses made for the last reinforcer earned in a given test session.

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. Subjects were excluded from analyses when it appeared that they failed to make any responses during a given test session and when there was independent confirmation (from daily logs maintained in the animal testing room) that this resulted from equipment malfunction. Using this criteria, 17 sessions of individual animal data were eliminated during the course of the study. Regression equations were fit to the mean of each group and comparisons between groups were made using the Wald statistic. The Wald statistic, calculated as the square of the ratio of an

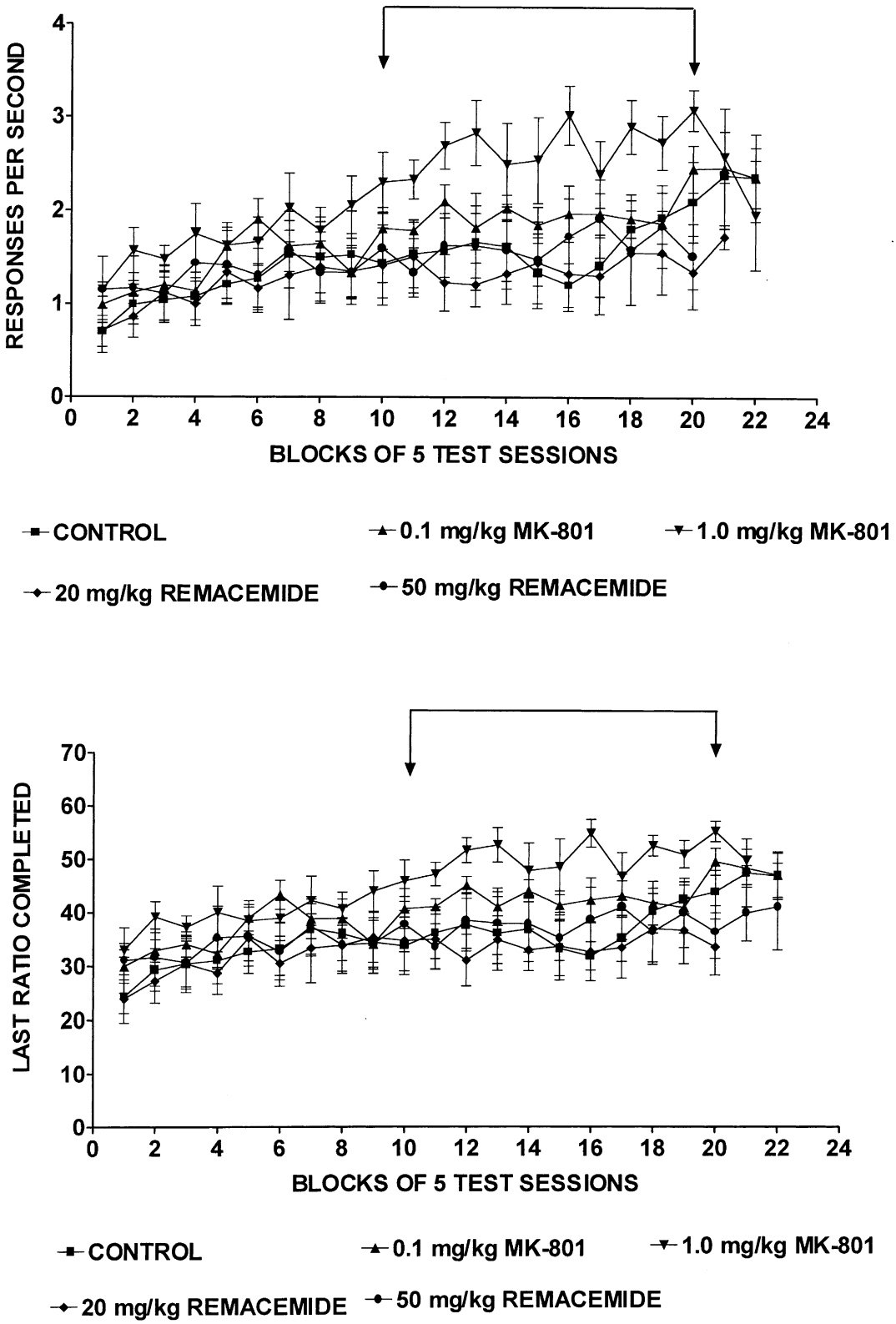


Fig. 3. Effects of chronic treatment on PR response rate (a) and last ratio completed (b) measured during the 18-month treatment phase (means ± S.E.M.). Each block of five test sessions represents 2 weeks of daily drug exposure. Brackets encompass the points at which the 1.0 mg/kg MK-801 group differed significantly from control ($P < .05$).

estimate and its standard error, follows a chi-square distribution and, if not squared, is identical to the normally distributed t (or z) statistic [21]. To obtain an accurate fit to the regression model, data for IRA accuracy and IRA percent task completed were transformed prior to analysis using: $T = \log(p/(1-p))$ where p indicates percent (for percent task completed) or percent accuracy (for accuracy). The logistic transformation produces an elongated S-shaped curve, and was chosen to closely approximate a typical learning function.

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 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. The purpose of normalizing withdrawal data to its own baseline was to allow analyses to examine within-groups effects (i.e., does drug withdrawal alter a group's performance relative to itself?) as well as between-groups effects (i.e., does drug withdrawal alter a group's performance relative to other groups?) during each stage of withdrawal. From an experimental design standpoint, the withdrawal phase was treated as an independent experiment with "treatment" being replaced by "withdrawal of treatment" as the independent variable. Each withdrawal period commenced during the same calendar week for all subjects. Data collected during withdrawal were analyzed as previously described.

3. Results

3.1. Effects of chronic drug treatment on IRA

The effects of chronic drug treatment on IRA accuracy are presented in Fig. 1a–d. There was a significant impairment in overall accuracy (Fig. 1a) produced by 50 mg/kg/day

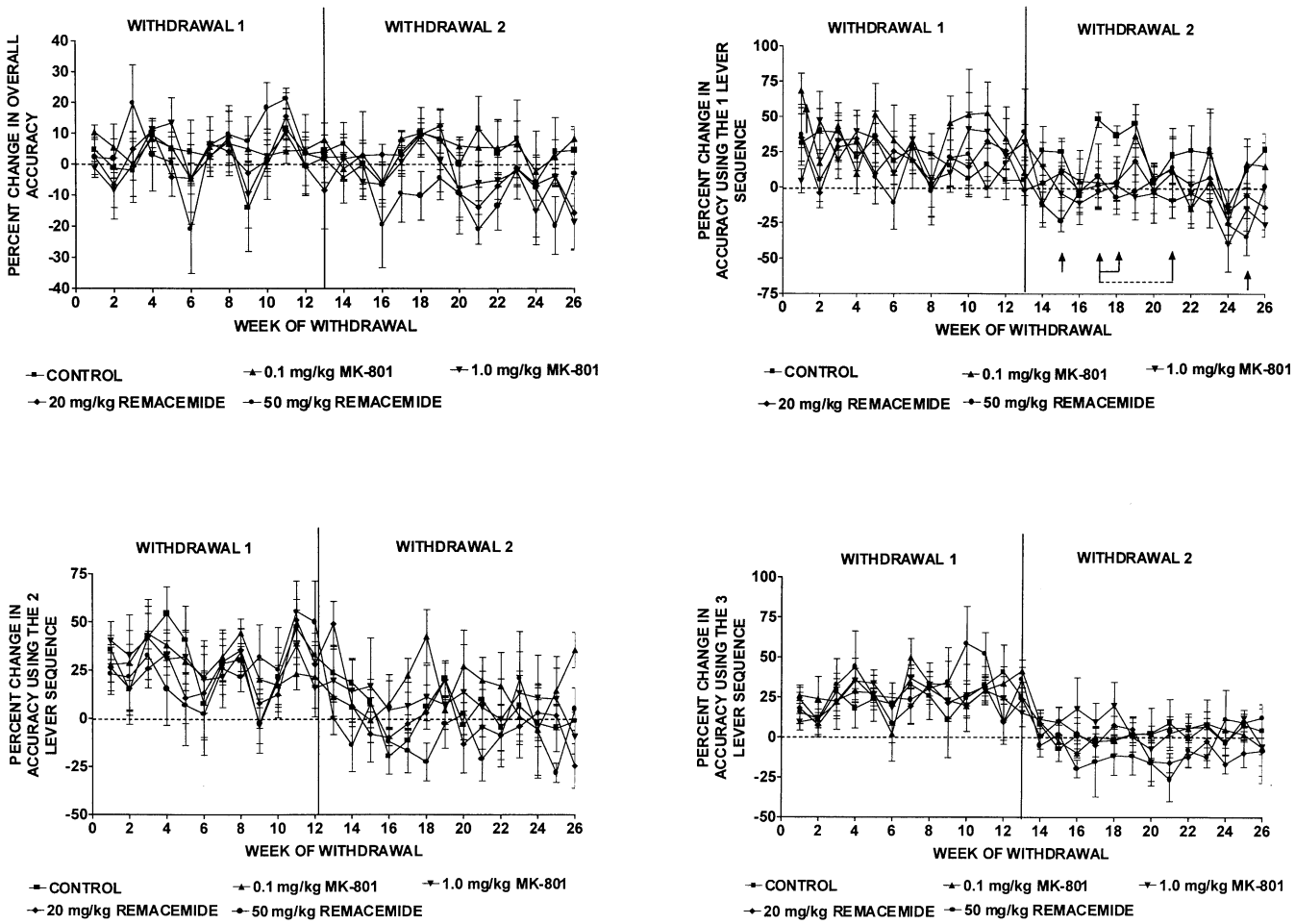


Fig. 4. Effects of chronic treatment on IRA accuracy measured during the 6-month washout phase (means ± S.E.M.). (a) Overall accuracy. (b–d) Accuracy for each of IRA levels 1–3, respectively. Brackets and individual arrows denote the points at which the 50 mg/kg remacemide group differed significantly from control ($P < .05$). Dashed line and arrows denote the points at which the 1.0 mg/kg MK-801 group differed significantly from control ($P < .05$).

remacemide which emerged during Block 12 and persisted for the duration of the 18-month dosing period ($P < .05$ at each time point). A similar pattern of results emerged when each of the first three IRA levels were considered alone with the deleterious effect of 50 mg/kg/day remacemide emerging during Block 9 for IRA level 1 (Fig. 1b), and during Block 10 for IRA levels 2 and 3 (Fig. 1c and d, respectively). Interestingly, there were no significant effects of either dose of MK-801 to impair accuracy overall, or when each of the first three sequence levels were considered separately. For the percent task completed measure (Fig. 2a), the deleterious effects of 50 mg/kg remacemide did not emerge as statistically significant until Block 21 but, like the effects of remacemide on accuracy, these effects persisted throughout the remainder of the 18-month dosing period. Again, there were no significant effects of MK-801 on percent task completed at either dose tested. Similarly, there were no effects of either drug on IRA response rates (Fig. 2b), indicating that the effects of chronic remacemide treatment on IRA accuracy were not associated with an inability to meet the motoric requirements of this task.

3.2. Effects of chronic drug treatment on PR

The effects of chronic drug treatment on PR response rate and on the last PR ratio completed are presented in Fig. 3a and b, respectively. There was a significant effect of 1.0 mg/kg/day MK-801 to increase PR response rate and last ratio completed during Blocks 10–20. There were no such effects of remacemide at either dose tested.

3.3. Effects of treatment on IRA measured during withdrawal

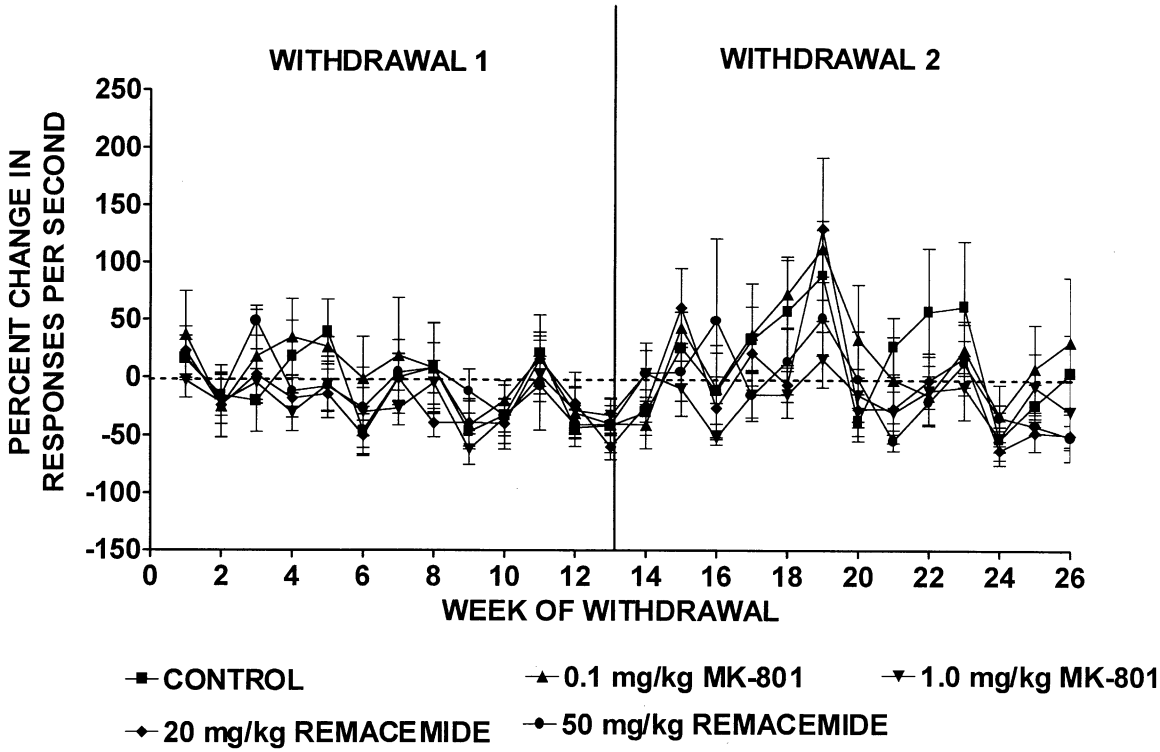
The effects of drug withdrawal on IRA accuracy are presented in Fig. 4a–d. There were no significant effects observed during the first period of drug withdrawal for overall accuracy, or for accuracy when each of the first three IRA levels were considered separately. Summary data for IRA accuracy measured at the end of each experimental phase supports the conclusion that the effects that emerged during treatment persisted throughout withdrawal (see Table 1).

During the second period of withdrawal, the 50 mg/kg/day remacemide and 1.0 mg/kg MK-801 groups showed transient impairments in accuracy at IRA level 1 (Fig. 4b). For the 50 mg/kg/day remacemide group, this effect was statistically significant during Weeks 15, 17, 18, and 26. For the 1.0 mg/kg MK-801 group, this effect was statistically significant during Weeks 17–21. There also were transient effects of withdrawal on IRA response rates with the 50 mg/kg/day group showing slower response rates during the second period of withdrawal than did controls (Fig. 5a). However, this effect was significant only during Week 25. There were no effects of MK-801 on response rate during

Table 1
Group mean accuracy scores for each treatment group measured at the end of each experimental phase (mean ± S.E.M. averaged over the last 4 weeks of each phase)

Treatment group	18-month treatment phase	Withdrawal 1	Withdrawal 2
Overall accuracy			
Control	57.9 ± 5.21	61.3 ± 4.94	60.0 ± 3.45
0.1 mg/kg MK-801	57.2 ± 1.83	58.7 ± 1.71	60.5 ± 1.12
1.0 mg/kg MK-801	56.3 ± 4.93	58.5 ± 5.09	54.3 ± 3.59
20 mg/kg remacemide	56.6 ± 5.94	58.7 ± 5.07	54.3 ± 5.69
50 mg/kg remacemide	41.9 ± 3.73 *	46.7 ± 4.02 *	43.1 ± 5.40 *
Level 1 accuracy			
Control	52.9 ± 5.10	43.2 ± 3.59	42.7 ± 4.21
0.1 mg/kg MK-801	54.1 ± 4.25	50.6 ± 4.56	49.7 ± 4.65
1.0 mg/kg MK-801	50.3 ± 5.51	52.5 ± 5.28	34.9 ± 3.50
20 mg/kg remacemide	46.5 ± 6.14	45.6 ± 4.50	41.8 ± 2.49
50 mg/kg remacemide	31.8 ± 4.59 *	36.1 ± 5.60	31.0 ± 5.10
Level 2 accuracy			
Control	54.7 ± 4.83	56.9 ± 5.24	53.0 ± 1.32
0.1 mg/kg MK-801	52.7 ± 4.56	49.6 ± 4.93	47.0 ± 5.58
1.0 mg/kg MK-801	53.0 ± 6.36	50.2 ± 5.70	48.6 ± 3.58
20 mg/kg remacemide	51.1 ± 6.23	57.6 ± 6.29	51.7 ± 5.31
50 mg/kg remacemide	42.0 ± 4.71	43.7 ± 5.35	37.5 ± 5.89 *
Level 3 accuracy			
Control	57.6 ± 4.15	62.6 ± 4.35	64.0 ± 4.96
0.1 mg/kg MK-801	56.3 ± 2.08	59.9 ± 2.90	62.8 ± 3.37
1.0 mg/kg MK-801	55.7 ± 5.57	57.1 ± 5.70	58.9 ± 4.30
20 mg/kg remacemide	56.3 ± 5.71	59.5 ± 5.21	53.3 ± 3.98
50 mg/kg remacemide	43.1 ± 2.55 *	50.0 ± 2.34	50.3 ± 3.18 *

* Indicates significant difference from control ($P < .05$).



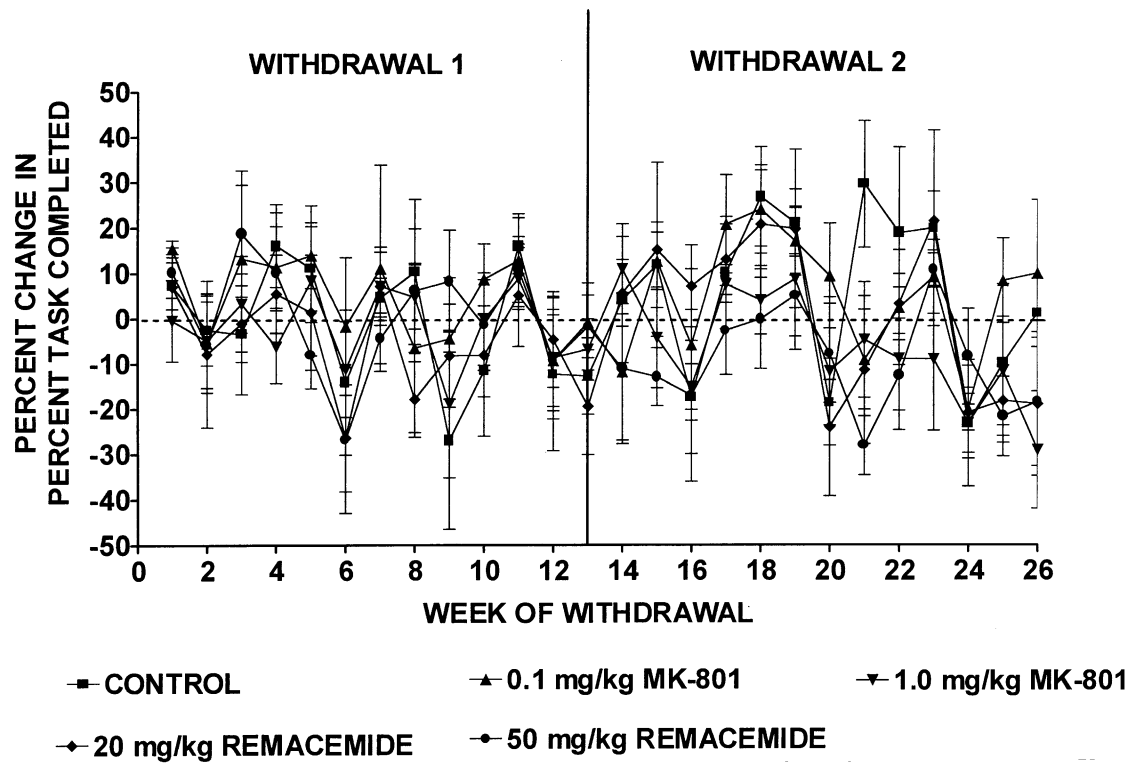
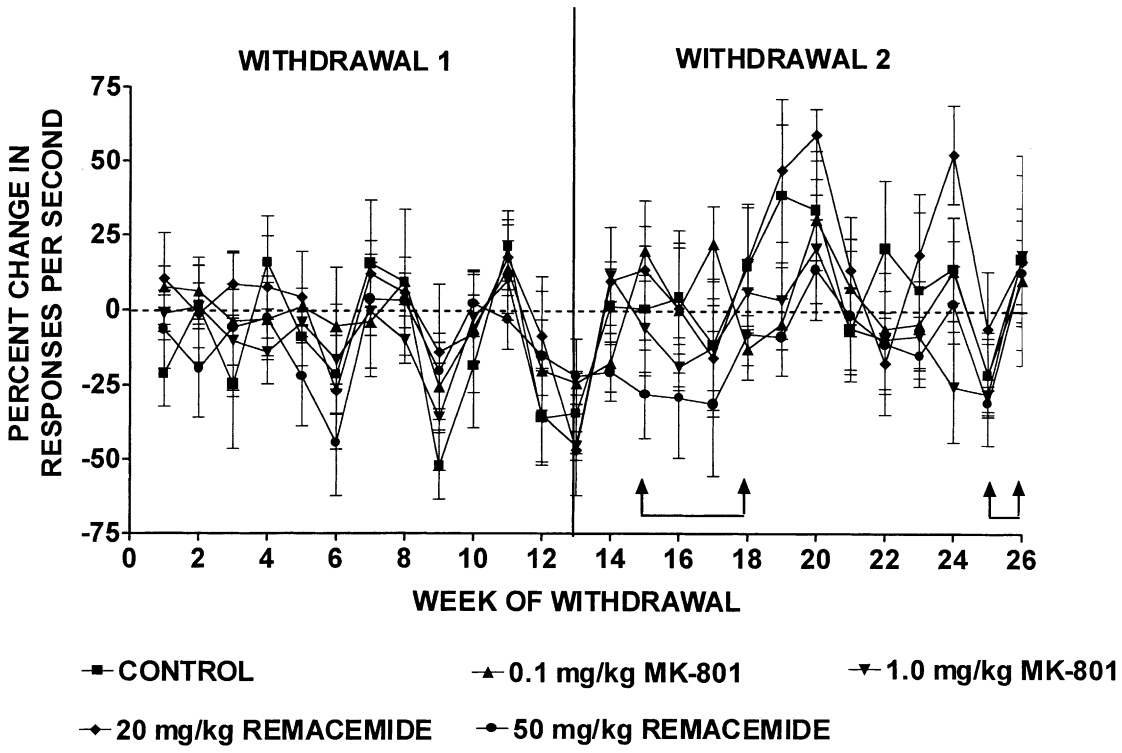


Fig. 5. Effects of chronic treatment on IRA percent task completed (a) and response rate (b) measured during the 6-month washout phase (means ± S.E.M.).



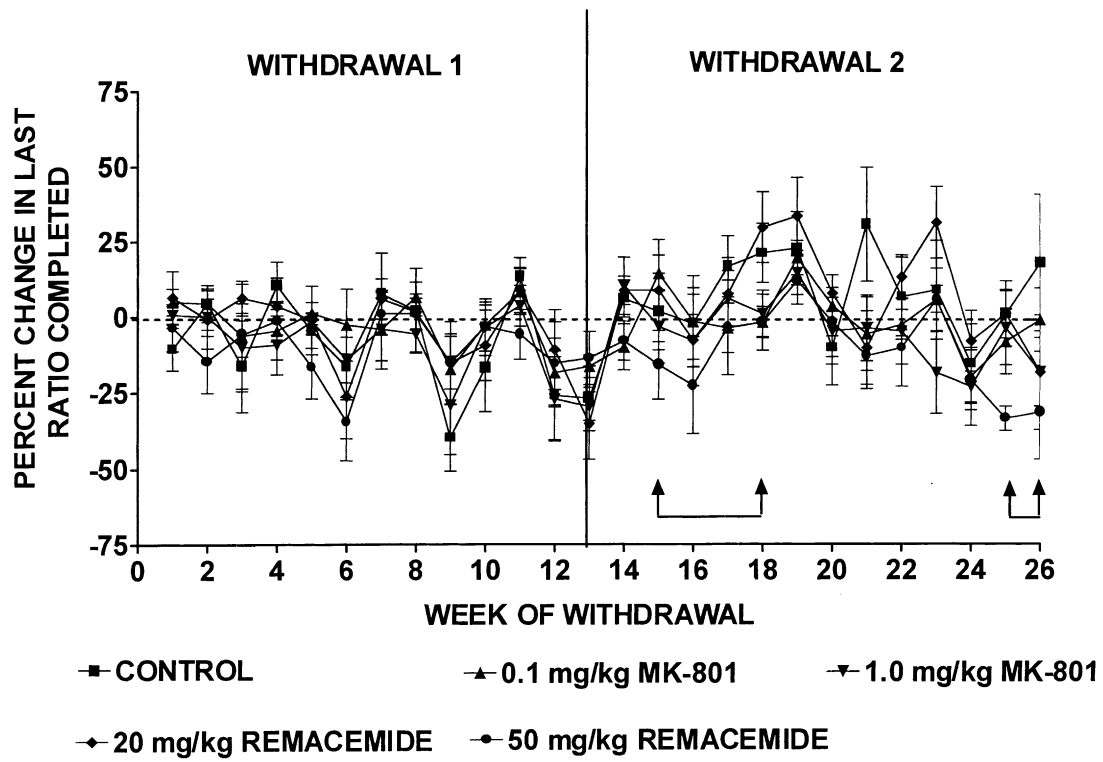


Fig. 6. Effects of chronic treatment on PR response rate (a) and last ratio completed (b) measured during the 6-month washout phase (means ± S.E.M.). Brackets encompass the points at which the 1.0 mg/kg MK-801 group differed significantly from control ($P < .05$).

either period of withdrawal nor were there any effect of either drug on percent task completed during withdrawal (Fig. 5a and b, respectively)

3.4. Effects of treatment on PR measured during withdrawal

Although there were no effects of chronic treatment on PR response rate during the first period of withdrawal, an inconsistent effect emerged in the 50 mg/kg/day remacemide group during the second period of withdrawal. This effect was evident during Weeks 15–18 and again during Weeks 25–26 (Fig. 6a). A similar effect was evident for the last ratio completed (Fig. 6b) with the 50 mg/kg/day remacemide group differing from controls during Weeks 15–18 and 25–26 of the second withdrawal period. There were no effects observed in the MK-801-treated groups during either period of withdrawal.

4. Discussion

The present experiment examined the effects of remacemide and MK-801 on tasks designed to model learning and motivation in juvenile rhesus monkeys. Chronic treatment with remacemide (50 mg/kg/day) impaired subjects' performance on the learning (IRA) task whereas chronic treatment with MK-801 did not. Neither compound significantly altered IRA response rates, suggesting that the effects of remacemide observed during treatment resulted from a specific cognitive impairment and not from an inability of subjects to fulfill the motoric requirements of the task.

For the PR (motivation) task, chronic treatment with a high dose of MK-801 (1.0 mg/kg/day) increased response rates and increased the number of responses made for the last reinforcer earned (i.e., last ratio completed). There were no effects of chronic remacemide treatment on any aspect of PR performance. The fact that the PR task always followed IRA in the present experiment leaves open the possibility that PR performance may have been influenced by pellets earned during IRA. Although present data do not address this possibility directly, the observed pattern of results suggests that performance of PR is unrelated to performance of IRA. MK-801 increased PR response rates (and presumably motivation) despite the fact that IRA performance remained unchanged. Similarly, remacemide reduced IRA performance without producing an effect on PR performance. Although this pattern of results suggests that the performance of PR is largely unrelated to prior IRA performance, future experiments are required before this conclusion can be drawn unequivocally.

During the first period of withdrawal, reducing the dose of remacemide from 50 to 20 mg/kg/day (or reducing the dose of MK-801 from 1.0 to 0.1 mg/kg/day) did not alter the effect of prior high-dose treatment on either task. Similarly, there was no effect of abrupt cessation of treatment in subjects that had been treated with 20 mg/kg/day remacemide or 0.1 mg/kg/day MK-801. This pattern of results indicates that subjects (or treatment groups) that were performing at a high level of proficiency during the 18-month treatment period continued to perform at a high level during each of the two withdrawal periods. Similarly (and perhaps more importantly), subjects that had been performing at a low level of proficiency during the 18-month treatment period (such as the 50 mg/kg/day remacemide subjects) continued to perform at a low level during each of the two withdrawal periods. The fact that the pattern of poor behavior established during chronic exposure to 50 mg/kg remacemide persisted even after the daily dose of remacemide was reduced provides evidence that the effects of high-dose remacemide exposure on learning that were observed during chronic treatment may reflect a lasting cognitive impairment and are not dependent on continued high-dose administration of the drug. This interpretation is further supported by the raw summary data collected at the end of each experimental phase (Table 1).

During the second period of withdrawal there was a transient effect of eliminating drug exposure on IRA accuracy. Because the effects of withdrawal were apparent at only a few time points and often corresponded with non-significant increases in the performance of control subjects, interpretation of these effects should be made cautiously. However, this pattern of results may indicate that complete cessation of treatment following prolonged exposure can precipitate a moderate withdrawal syndrome which can manifest as impairments in learning task performance.

Also of note is the apparent decrease in performance on sequence levels 1–3 over the course of withdrawal without a concomitant decrease in overall accuracy over the same time period. Although there were not enough data to perform meaningful statistical analyses on sequence levels 4–6 individually, performance of these sequence levels nonetheless contributed to the overall accuracy scores and may have helped to maintain overall accuracy despite modest reductions in the performance of Levels 1–3. Finally, the fact that subjects in the 50 mg/kg/day group continued to perform as poorly during the second period of withdrawal (when the dose of drug was zero) as they had during each of the two previous phases provides further evidence of a persistent effect of prior high-dose remacemide exposure that is not dependent on continued administration of the drug.

One of the most striking results of the present experiment is the fact that chronic treatment with remacemide, an antagonist of NMDA receptors that also blocks fast sodium channels, impaired subjects' ability to perform the learning task, whereas chronic treatment with MK-801, an NMDA receptor antagonist that does not also block fast sodium channels, did not. Given the differential effects of these drugs and their somewhat different mechanisms of action, it is likely that the effects of remacemide on learning resulted either from its ancillary activity at fast sodium channels or from its effects to block NMDA receptors and sodium

channels concurrently. Drugs such as tetrodotoxin, which block sodium channels but have negligible activity at NMDA receptors, can produce disruptions in the performance of nonoperant learning procedures that are qualitatively similar to those produced by remacemide presently [2,19]. 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, a result that is consistent with the dosing regimen used presently.

While a role for sodium channel blockade in the effects of remacemide seems likely, it is also possible that the effects of remacemide (relative to MK-801) resulted in part from the way in which the drug was disposed in the body. Shortly after oral administration, remacemide is desglycinated to an active metabolite that has an even greater affinity for the NMDA receptor than does the parent compound. Thus, although the time course of MK-801 and remacemide disposition are similar (i.e., $\leq$ 17 h after high-dose administration) [18,33], the persistence of the active remacemide metabolite (up to 24 h after high-dose administration) may result in a somewhat longer inactivation of NMDA receptors following remacemide treatment than following treatment with MK-801. This, in turn, may have resulted in a greater functional exposure to NMDA receptor blockade (and a more prolonged blocking of the laying down of memory) after remacemide treatment than after MK-801 treatment.

Although the effects of remacemide presented here are noteworthy, so is the fact that chronic treatment with MK-801 had no such effects. Previous experiments, including those conducted in our own laboratory, indicate that acute treatment with MK-801 can have pronounced effects on the performance of operant behavioral tasks in adult monkeys [3,24]. Yet, in the present experiment, chronic administration of MK-801 during development was largely without effect. There is a substantial research literature which suggests that the excitatory amino acids, and NMDA receptors in particular, play an important role during development by regulating neuronal survival, axonal and dendritic structure, and synaptic genesis and plasticity [22]. 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 [7,10,20,31]. This finding has led some authors to speculate that the infant brain may be differentially sensitive to agents that affect NMDA receptor function relative to the adult brain [10]. If this were true, then it may help to explain why the juvenile subjects examined in the present experiment appeared to be insensitive to the effects of MK-801 relative to the adult subjects examined previously. It is important to emphasize, however, that the present experiment did not include subjects that began treatment/testing as adults. As a result, it is impossible to discern 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.

An alternative explanation for the relative absence of effects of chronic MK-801 treatment is that, over time, subjects became tolerant to its cognitive–behavioral effects. Hasselink et al. [13] 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 increases in response rates on the motivation (PR) task that emerged during Block 10 but subsequently disappeared by Block 20. Taken together, these results and observations suggest a tolerance-inducing effect of MK-801, which may help to account for the inconsistent nature of MK-801 effects seen presently.

In summary, the present results suggest that chronic developmental exposure to high doses of remacemide can have pronounced effects on learning whereas chronic developmental exposure to MK-801 does not. These effects occurred in the absence of reductions in motivation or response rate, suggesting that the effects of remacemide reflect specific cognitive impairments and are not due to changes in motivation or to an inability of subjects to fulfill the motoric requirements of the task. Furthermore, the effects of remacemide on learning persisted throughout a 6-month drug washout phase, suggesting an enduring effect of blocking NMDA receptors and fast sodium channels which persists long after the drugs are removed. Although the mechanisms that underlie the effects of remacemide are unknown, they are likely to involve the concurrent antagonism of NMDA receptors and fast sodium channels. The fact that chronic developmental exposure to MK-801 had no effect on subjects' ability to gain proficiency on a learning task highlights the need for additional research into the basic mechanisms of learning and memory during development.

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