

## Assessing the potential toxicity of MK-801 and remacemide: Chronic exposure in juvenile rhesus monkeys

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Received 26 October 2001; received in revised form 21 January 2002; accepted 29 January 2002

### Abstract

The present experiment examined the effects of chronic exposure to either 0.1 or 1.0 mg/kg MK-801 [a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist] or 20.0 or 50.0 mg/kg remacemide (an NMDA receptor antagonist which also blocks fast sodium channels) in juvenile rhesus monkeys. Endpoints were monitored to provide a general index of subjects' health and included measures of clinical chemistry, hematology, ophthalmology, spontaneous home-cage behavior, and peak drug plasma levels. In general, both drugs were well tolerated and produced no treatment-related effects during 2 years of dosing and assessment. Periodic plasma drug level determinations provided limited evidence that both compounds may induce their own metabolism. The present results contrast sharply with previously reported effects of long-lasting impairments in the acquisition of incremental learning and in the development of color and position discrimination in these same subjects. These observations highlight the importance of collecting a broad range of toxicology data, including tests of cognitive function, to make comprehensive assessments of new drug safety. In the present case, the less obvious effects of these drugs on cognition defined the toxicologic response. © 2002 Elsevier Science Inc. All rights reserved.

**Keywords:** Remacemide; MK-801; *N*-Methyl-D-aspartate (NMDA); Fast sodium channels; Clinical chemistry; Hematology; Ophthalmology; Home-cage behavior; Rhesus monkeys

### 1. Introduction

*N*-Methyl-D-aspartate (NMDA) receptors and fast sodium channels represent important targets for pharmaceutical drug discovery. Sodium channel blockade provides the mechanism of action underlying the antiarrhythmic effects of lidocaine, quinidine, procainamide, and disopyramide [10,24]. Other sodium channel blocking agents such as phenytoin and its congeners mephenytoin, ethotoin, and phenacemide, have been proven useful in the treatment of

partial and generalized tonic-clonic seizures [23]. Drugs which exert their effects via NMDA receptors have received less clinical attention but nonclinical studies suggest that agents which block NMDA receptor activity may have utility in the treatment of epilepsy [16] and as neuroprotective therapy following hypoxic insult [1,6,9,28].

Despite the potential usefulness of these compounds, very little has been reported regarding their developmental/toxicologic effects. In particular, relatively little attention has been paid to the role of NMDA receptors and fast sodium channels outside of the central nervous system. Reports indicate the presence of NMDA receptor subunits in the heart, skeletal muscle, pancreas, and dermal-epidermal junction, yet little is known regarding NMDA receptor-mediated toxicity at these sites [4,12]. Other reports suggest that peripheral NMDA receptors are present in the lung and may mediate nitric oxide-induced toxicity [25].

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In the present experiment, we examined the effect of long-term developmental exposure to MK-801 (a prototypic agent which selectively and noncompetitively inhibits NMDA receptor activity) or remacemide (a relatively novel compound that blocks NMDA receptors and fast sodium channels concurrently) on peripherally—as well as centrally mediated toxicity in juvenile rhesus monkeys (*Macaca mulatta*). The rhesus monkey was deemed to be an appropriate model for study based on the known binding properties of MK-801 and remacemide [22,26] and on the significant homology of NMDA receptor subunits across mammalian species [12]. The decision to study the effects of these drugs in juveniles was based on the established importance of NMDA receptor function during development [14] and on speculation that the developing animal may be more responsive to agents that affect NMDA receptor function than are adults [5]. Because the potential for peripheral toxicity from these compounds is largely unknown, endpoints were selected to provide a broad index of subjects' health and included measures of clinical chemistry, hematology, and ophthalmology as well as home-cage behavioral observations and periodic determinations of drug plasma levels.

## 2. Methods

### 2.1. Subjects and housing

Subjects were 30 captive-bred female rhesus monkeys (*M. mulatta*) with a mean weight of 1.8 kg at the start of the experiment (weight range = 1.3–2.3 kg). In addition to serving as subjects in the present experiment, all animals also served in studies to assess the effects of MK-801 and remacemide on the acquisition of complex operant behaviors. The results of these behavioral assessments indicate that the doses of MK-801 and remacemide used presently can have profound deleterious effects on the acquisition of incremental learning and color and position discrimination in these same subjects [20,21]. 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 model the period from middle childhood to the onset of puberty [8]. Subjects were individually housed and 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). Food 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 [17–19]. Subjects were housed under a 12-h light/dark cycle (lights on at 6:00 a.m. CST) with temperature and relative humidity of  $25 \pm 2$  °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

The dosing period was comprised of an 18-month treatment phase which was followed by a 6-month, two-step dose-reduction phase. Drugs were administered at the same time of day 7 days/week. During the 18-month chronic treatment phase, doses of remacemide (20 or 50 mg/kg/day, calculated as free base, dosed as remacemide HCl) and MK-801 (0.1 or 1.0 mg/kg/day, calculated and doses as HCl salt) were prepared in untreated tap water and were administered via oral gavage. During the oral gavage procedure, subjects were confined to portable restraint chairs (Primate Products, Redwood City, CA) and the fore- and hindlimbs were manually restrained by several technicians. This method of restraint allows subjects free movement of fore and hind limbs, 360° rotational capacity, and the ability to rest naturally on their haunches. The additional manual restraint ensured that subjects' limbs were not free to interfere with the gavage procedure. The low dose of remacemide was chosen to produce plasma levels which would be comparable to those measured during human clinical trials. 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 [3]. The low and high doses of MK-801 were based on acute dose pilot studies in monkeys and represent a no-effect dose and the maximum tolerated dose, respectively. The maximum tolerated dose of MK-801 represents the highest dose that could be administered to subjects without rendering them incapacitated. Subjects in the control condition received equivalent volumes of untreated tap water alone administered via oral gavage. For the first 7 days of daily dosing, all remacemide-treated subjects received 20 mg/kg remacemide per 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 18-month treatment phase of the experiment. This “ramping” procedure allowed subjects to habituate to the transient emetic-producing effects that sometimes accompany high-dose oral remacemide treatment in nonhuman primates. Each subject's daily dose was administered as a 5.0-ml bolus which was immediately followed by a 5.0-ml flush with untreated tap water to ensure that no test compound remained in the oral gavage tube. Each plastic oral gavage tube was 48.8 cm long, had an internal diameter of 0.64 cm, was trimmed at an angle, and was seared with an open flame to remove sharp edges on the insertion end. 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 each set of gavage tubes was used for a single subject, exclusively.

### 2.2.2. "Dose-reduction" phase

The 18-month treatment phase was followed by a 6-month, two-stage dose-reduction phase. During the first 3 months of dose reduction, 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 dose-reduction phase, all subjects received water only. Thus, animals that had received the low doses of either compound were gavaged with water only for the entire 6 months of the dose-reduction phase, whereas the animals that had received the high dose of either compound were gavaged with the low dose for the first 3 months followed by water for the remaining 3 months. The purpose of the two-step dose-reduction procedure used in each of the high-dose groups was to minimize the potential for withdrawal-induced seizures which can accompany NMDA receptor up-regulation following chronic exposure [27].

### 2.3. Blood collection for clinical chemistry and hematology assessments

Blood samples for clinical chemistry and hematology assessments were collected prior to the start of dosing and again after 4, 12, 21, 47, and 90 weeks of dosing. At each sampling time, approximately 3 ml of blood was sampled from all subjects using a 3.0-cm<sup>3</sup> syringe. The portion of each sample designated for hematology analyses (approximately 1.5 ml) was transferred to a Monoject brand collection tube containing 0.05 ml 15% ethylenediamine tetraacetic acid and agitated gently to inhibit clotting. The remainder of the sample, designated for clinical chemistry analyses, was transferred to a Vacutainer brand collection tube containing SST gel and clot activator. After clotting had occurred (approximately 30 min), samples designated for clinical chemistry were placed into a bench-top centrifuge (Damon/IEC Division, Model IEC-HN-S, Needham Heights, MA, USA) and spun at approximately 1000 × *g* for 10 min. Serum was then drawn from the samples using Pasteur pipettes and the remaining sample was spun for an additional 10 min at approximately 1000 × *g*. After the second and final centrifugation, serum was again collected using Pasteur pipettes and transferred to micro centrifuge tubes for immediate analysis.

#### 2.3.1. Clinical chemistry analysis

For clinical chemistry analyses, a Cobas Mira Plus analyzer (Roche Diagnostic Systems, Somerville, NJ, USA) was used with Roche Diagnostic reagents and standards to measure cholesterol, triglycerides, alanine trans-

aminase, alkaline phosphatase, creatine kinase, blood urea nitrogen, albumin, total protein, glucose, total bilirubin, calcium, sodium, potassium, and chloride. Sigma reagents (Sigma, St. Louis, MO) were used for lactate, lipase, and amylase measurements. Instruments were calibrated according to manufacturers' instructions and two levels of assayed controls were included in the analyses as internal controls.

#### 2.3.2. Hematology analysis

For hematology analyses, a Cobas Minos Vet analyzer (Roche Diagnostic Systems) was used to count cells (red cells, white cells, and platelets) and to measure hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Reticulocyte and differential white blood cell slides were prepared and stained with Sigma Diagnostic Acustain. Three dilutions of normal controls were tested prior to daily analyses to confirm proper linear calibration.

### 2.4. Ophthalmic assessment

Ophthalmic examinations were performed 7 weeks prior to the start of daily dosing, and during the 2nd, 5th, 11th, and 17th months of treatment. Ophthalmic examinations also were performed during the fifth month of the dose-reduction phase. Immediately prior to ophthalmic examination, subjects were sedated using Ketamine HCl (10.0 mg/kg im). Appropriate mydriasis was induced using 1.0% Tropicamide administered topically in the eye. Following sedation and induction of mydriasis, eyelids, bulbar conjunctiva, cornea, iris, anterior chambers, and lens were examined using focal illumination and slit lamp biomicroscopy. The vitreous body and ocular fundus were examined by ophthalmoscopy. Retinal photographs were obtained using a fundus camera.

### 2.5. Home-cage behavioral assessment

Home-cage behavioral assessments were conducted 2 h after dosing to correspond with expected peak plasma levels of remacemide and MK-801 [3,11,26]. Behaviors monitored included: locomotion, environmental exploration, self-motion play, stereotypy, huddle, self-sex, self-bite, self-groom, self-aggression, eating, drinking, self-directed behaviors not otherwise described, threat, fear grimace, vocalization, hostility toward tester, submissiveness toward tester, orientation toward tester, collar manipulation, ataxia, wet-dog shakes, and nystagmus. Each subject was observed individually for 5 min in accordance with procedures outlined by Ferguson et al. [7]. A total of nine assessments were conducted during the drug treatment period (during 1st, 2nd, 3rd, 4th, 6th, 8th, 12th, 15th, and 18th months) with an additional two assessments conducted during the 2nd and 5th months of the dose-reduction period (20.5 and 23.5 months after the start of dosing, respectively).

Table 1

Clinical chemistry endpoints measured during 18 months of chronic treatment (means  $\pm$  S.E.M.)

Endpoint	Time after first drug treatment	Vehicle	0.1 mg/kg MK-801	1.0 mg/kg MK-801	20 mg/kg remacemide	50 mg/kg remacemide
Glucose (mg/dl)	Pretreatment (5 weeks)	73.8 $\pm$ 5.03	62.8 $\pm$ 6.65	70.2 $\pm$ 6.98	61.5 $\pm$ 6.65	67.8 $\pm$ 6.65
	4 weeks	76.2 $\pm$ 6.11	69.3 $\pm$ 6.39	71.5 $\pm$ 6.39	70.8 $\pm$ 6.70	72.6 $\pm$ 6.70
	12 weeks	76.5 $\pm$ 5.81	71.2 $\pm$ 11.63	74.0 $\pm$ 11.19	71.2 $\pm$ 11.19	66.5 $\pm$ 11.19
	21 weeks	67.2 $\pm$ 4.24	67.8 $\pm$ 7.59	63.7 $\pm$ 7.59	70.7 $\pm$ 7.59	68.3 $\pm$ 7.59
	47 weeks	71.2 $\pm$ 6.01	61.2 $\pm$ 6.86	74.5 $\pm$ 6.54	70.5 $\pm$ 6.54	66.0 $\pm$ 6.54
	90 weeks	62.0 $\pm$ 6.6	59.8 $\pm$ 9.69	67.0 $\pm$ 9.24	67.8 $\pm$ 9.24	67.8 $\pm$ 9.69
Total protein (g/dl)	Pretreatment (5 weeks)	7.0 $\pm$ 0.17	7.0 $\pm$ 0.25	7.0 $\pm$ 0.26	7.0 $\pm$ 0.25	7.0 $\pm$ 0.25
	4 weeks	7.8 $\pm$ 0.30	8.4 $\pm$ 0.37	7.7 $\pm$ 0.39	7.8 $\pm$ 0.39	7.7 $\pm$ 0.39
	12 weeks	7.6 $\pm$ 0.41	7.5 $\pm$ 0.42	7.6 $\pm$ 0.40	7.5 $\pm$ 0.40	7.5 $\pm$ 0.40
	21 weeks	7.2 $\pm$ 0.07	7.4 $\pm$ 0.22	7.2 $\pm$ 0.22	7.5 $\pm$ 0.22	7.3 $\pm$ 0.22
	47 weeks	7.7 $\pm$ 0.23	7.7 $\pm$ 0.43	8.1 $\pm$ 0.41	7.8 $\pm$ 0.41	7.5 $\pm$ 0.41
	90 weeks	7.5 $\pm$ 0.29	7.8 $\pm$ 0.37	7.5 $\pm$ 0.35	7.3 $\pm$ 0.35	7.5 $\pm$ 0.37
Albumin (g/dl)	Pretreatment (5 weeks)	4.6 $\pm$ 0.13	4.3 $\pm$ 0.26	4.4 $\pm$ 0.28	4.4 $\pm$ 0.26	4.4 $\pm$ 0.26
	4 weeks	5.2 $\pm$ 0.10	5.2 $\pm$ 0.16	5.0 $\pm$ 0.17	5.1 $\pm$ 0.17	5.0 $\pm$ 0.17
	12 weeks	5.1 $\pm$ 0.09	4.9 $\pm$ 0.22	5.2 $\pm$ 0.21	4.9 $\pm$ 0.21	4.8 $\pm$ 0.21
	21 weeks	5.3 $\pm$ 0.15	5.0 $\pm$ 0.21	4.9 $\pm$ 0.21	5.3 $\pm$ 0.21	5.0 $\pm$ 0.21
	47 weeks	4.9 $\pm$ 0.21	4.6 $\pm$ 0.24	4.7 $\pm$ 0.23	4.8 $\pm$ 0.23	4.6 $\pm$ 0.23
	90 weeks	5.3 $\pm$ 0.20	5.0 $\pm$ 0.31	5.6 $\pm$ 0.30	5.0 $\pm$ 0.30	5.0 $\pm$ 0.31
Total bilirubin (mg/dl)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	0.2 $\pm$ 0.05	0.1 $\pm$ 0.04	0.1 $\pm$ 0.04	0.1 $\pm$ 0.04	0.2 $\pm$ 0.04
	12 weeks	0.1 $\pm$ 0.0	0.1 $\pm$ 0.04	0.1 $\pm$ 0.04	0.1 $\pm$ 0.04	0.1 $\pm$ 0.04
	21 weeks	0.2 $\pm$ 0.02	0.1 $\pm$ 0.04	0.2 $\pm$ 0.04	0.2 $\pm$ 0.04	0.2 $\pm$ 0.02
	47 weeks	0.1 $\pm$ 0.02	0.1 $\pm$ 0.05	0.2 $\pm$ 0.05	0.1 $\pm$ 0.05	0.2 $\pm$ 0.05
	90 weeks	0.2 $\pm$ 0.03	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05
Alanine transaminase (U/l)	Pretreatment (5 weeks)	32.5 $\pm$ 1.52	31.5 $\pm$ 4.38	29.2 $\pm$ 4.59	33.3 $\pm$ 4.38	32.3 $\pm$ 4.38
	4 weeks	33.6 $\pm$ 6.62	29.3 $\pm$ 12.80	29.0 $\pm$ 12.80	40.2 $\pm$ 13.37	44.8 $\pm$ 13.37
	12 weeks	41.8 $\pm$ 3.12	44.6 $\pm$ 7.01	37.8 $\pm$ 6.74	42.8 $\pm$ 6.74	36.2 $\pm$ 7.01
	21 weeks	39.5 $\pm$ 6.34	35.7 $\pm$ 35.5	37.0 $\pm$ 7.41	47.2 $\pm$ 7.41	33.2 $\pm$ 7.41
	47 weeks	98.2 $\pm$ 31.21	51.6 $\pm$ 23.22	54.7 $\pm$ 22.14	58.5 $\pm$ 22.14	52.2 $\pm$ 22.14
	90 weeks	91.3 $\pm$ 23.05	59.6 $\pm$ 19.78	41.2 $\pm$ 18.86*	51.0 $\pm$ 18.86	60.8 $\pm$ 19.78
Alkaline phosphatase (U/l)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	ND	ND	ND	ND	ND
	12 weeks	ND	ND	ND	ND	ND
	21 weeks	875.3 $\pm$ 91.77	741.3 $\pm$ 137.11	850.3 $\pm$ 137.11	663.7 $\pm$ 137.11	951.2 $\pm$ 137.11
	47 weeks	682.5 $\pm$ 48.88	607.6 $\pm$ 93.11	694.5 $\pm$ 88.77	560.2 $\pm$ 88.77	664.3 $\pm$ 88.77
	90 weeks	524.3 $\pm$ 53.84	521.8 $\pm$ 102.01	618.0 $\pm$ 97.26	499.8 $\pm$ 97.26	605.8 $\pm$ 102.01
Amylase ( $\mu$ l)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	471.0 $\pm$ 121.23	247.7 $\pm$ 85.79	293.5 $\pm$ 85.79	261.4 $\pm$ 89.98	285.8 $\pm$ 89.98
	12 weeks	309.3 $\pm$ 58.05	253.0 $\pm$ 49.73	293.5 $\pm$ 47.85	259.8 $\pm$ 47.85	268.2 $\pm$ 49.73
	21 weeks	360.7 $\pm$ 42.33	321.3 $\pm$ 57.63	317.2 $\pm$ 57.63	350.0 $\pm$ 57.63	327.5 $\pm$ 57.63
	47 weeks	285.7 $\pm$ 45.17	285.4 $\pm$ 49.45	290.8 $\pm$ 47.15	279.8 $\pm$ 47.15	269.8 $\pm$ 47.15
	90 weeks	364.7 $\pm$ 38.01	348.8 $\pm$ 53.55	369.8 $\pm$ 51.06	319.8 $\pm$ 51.06	287.2 $\pm$ 53.55
Chloride (mmol/l)	Pretreatment (5 weeks)	108.2 $\pm$ 0.60	108.3 $\pm$ 1.42	108.0 $\pm$ 1.49	108.3 $\pm$ 1.42	109.0 $\pm$ 1.42
	4 weeks	110.7 $\pm$ 1.15	109.8 $\pm$ 1.61	108.8 $\pm$ 1.69	110.4 $\pm$ 1.69	108.6 $\pm$ 1.69
	12 weeks	115.0 $\pm$ 0.91	112.6 $\pm$ 2.11	111.0 $\pm$ 2.03	113.3 $\pm$ 2.03	113.8 $\pm$ 2.03
	21 weeks	112.5 $\pm$ 1.61	110.0 $\pm$ 2.00	109.5 $\pm$ 2.00	112.2 $\pm$ 2.00	111.3 $\pm$ 2.00
	47 weeks	114.2 $\pm$ 0.60	110.8 $\pm$ 1.56	112.5 $\pm$ 1.39	113.5 $\pm$ 1.39	112.5 $\pm$ 1.39
	90 weeks	113.5 $\pm$ 0.99	106.8 $\pm$ 2.81	110.5 $\pm$ 2.51	110.8 $\pm$ 2.51	111.4 $\pm$ 2.63
Lactic acid (mg/dl)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	59.4 $\pm$ 19.57	64.1 $\pm$ 17.37	63.9 $\pm$ 18.14	63.9 $\pm$ 18.14	77.5 $\pm$ 18.14
	12 weeks	72.0 $\pm$ 5.09	69.6 $\pm$ 11.68	59.7 $\pm$ 11.24	57.4 $\pm$ 11.24	52.9 $\pm$ 11.24
	21 weeks	58.3 $\pm$ 11.51	61.7 $\pm$ 10.89	53.2 $\pm$ 10.89	61.6 $\pm$ 10.89	57.8 $\pm$ 10.89
	47 weeks	89.3 $\pm$ 94.4	92.2 $\pm$ 17.56	67.4 $\pm$ 16.67	82.3 $\pm$ 16.67	86.5 $\pm$ 17.48
	90 weeks	51.0 $\pm$ 7.49	31.8 $\pm$ 10.51	57.4 $\pm$ 9.86	47.1 $\pm$ 9.40	39.5 $\pm$ 9.86
Lipase ( $\mu$ l)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	43.8 $\pm$ 16.54	54.8 $\pm$ 34.25	51.7 $\pm$ 35.77	27.5 $\pm$ 35.77	59.2 $\pm$ 35.77
	12 weeks	91.5 $\pm$ 17.53	55.7 $\pm$ 28.66	47.1 $\pm$ 27.58	29.7 $\pm$ 27.58	50.2 $\pm$ 27.58
	21 weeks	71.4 $\pm$ 17.91	62.2 $\pm$ 28.85	51.4 $\pm$ 28.85	43.7 $\pm$ 28.85	61.6 $\pm$ 28.85
	47 weeks	80.2 $\pm$ 19.48	50.8 $\pm$ 7.62	46.2 $\pm$ 18.76	43.1 $\pm$ 18.76	50.2 $\pm$ 19.68
	90 weeks	69.1 $\pm$ 18.36	253.8 $\pm$ 88.59	55.4 $\pm$ 84.70	69.7 $\pm$ 84.47	54.9 $\pm$ 88.59

(continued on next page)

Table 1 (continued)

Endpoint	Time after first drug treatment	Vehicle	0.1 mg/kg MK-801	1.0 mg/kg MK-801	20 mg/kg remacemide	50 mg/kg remacemide
Blood urea nitrogen (mg/dl)	Pretreatment (5 weeks)	23.9 ± 1.20	21.0 ± 2.66	20.2 ± 2.79	21.5 ± 2.66	21.2 ± 2.66
	4 weeks	31.0 ± 2.46	25.8 ± 2.79	24.9 ± 2.92	25.8 ± 2.92	26.6 ± 2.92
	12 weeks	29.1 ± 3.66	29.5 ± 3.87	27.7 ± 3.72	26.8 ± 3.72	30.7 ± 3.72
	21 weeks	32.2 ± 6.37	23.8 ± 4.69	21.6 ± 4.69	20.8 ± 4.69	22.7 ± 4.69
	47 weeks	31.2 ± 2.25	34.2 ± 5.69	33.8 ± 5.42	33.1 ± 5.42	36.7 ± 5.42
	90 weeks	25.3 ± 0.93	28.0 ± 2.06	22.7 ± 1.97	24.5 ± 1.97	24.8 ± 2.06
Creatinine (mg/dl)	Pretreatment (5 weeks)	0.8 ± 0.06	0.6 ± 0.07	0.7 ± 0.07	0.7 ± 0.07	0.7 ± 0.07
	4 weeks	0.6 ± 0.1	0.7 ± 0.08	0.6 ± 0.08	0.7 ± 0.08	0.7 ± 0.08
	12 weeks	0.9 ± 0.04	0.9 ± 0.06	0.8 ± 0.06	0.9 ± 0.06	0.8 ± 0.06
	21 weeks	0.7 ± 0.06	0.6 ± 0.05	0.6 ± 0.05	0.7 ± 0.05	0.7 ± 0.05
	47 weeks	0.8 ± 0.04	0.8 ± 0.09	0.7 ± 0.09	0.7 ± 0.09	0.8 ± 0.09
	90 weeks	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04
Total cholesterol (mg/dl)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	151.2 ± 7.62	180.2 ± 13.67	149.2 ± 14.34	177.2 ± 14.34	175.0 ± 14.34
	12 weeks	158.3 ± 16.29	154.6 ± 23.58	176.8 ± 22.69	162.0 ± 22.69	161.7 ± 22.69
	21 weeks	151.0 ± 9.75	176.3 ± 17.23	177.5 ± 17.23	175.8 ± 17.23	172.5 ± 17.23
	47 weeks	143.8 ± 9.69	168.4 ± 18.11	166.4 ± 17.27	155.3 ± 17.27	150.2 ± 17.27
	90 weeks	164.0 ± 6.82	183.0 ± 23.00	184.3 ± 21.93	173.0 ± 21.93	173.8 ± 23.00
Calcium (mg/dl)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	11.2 ± 0.37	10.9 ± 1.26	10.7 ± 1.31	11.8 ± 1.38	12.2 ± 1.31
	12 weeks	10.9 ± 0.34	11.6 ± 0.56	11.5 ± 0.54	11.2 ± 0.56	10.8 ± 0.56
	21 weeks	10.3 ± 0.15	10.5 ± 0.41	10.3 ± 0.41	10.7 ± 0.41	10.0 ± 0.41
	47 weeks	11.0 ± 0.23	11.2 ± 0.50	11.3 ± 0.47	11.3 ± 0.47	10.6 ± 0.47
	90 weeks	10.6 ± 0.2	10.8 ± 0.27	10.5 ± 0.26	10.8 ± 0.26	10.7 ± 0.27
Sodium (mmol/l)	Pretreatment (5 weeks)	151.0 ± 0.52	150.8 ± 1.14	150.0 ± 1.20	151.3 ± 1.14	151.5 ± 1.14
	4 weeks	159.0 ± 1.65	157.3 ± 2.70	154.3 ± 2.70	156.0 ± 2.83	157.2 ± 2.83
	12 weeks	159.5 ± 0.65	157.2 ± 3.06	154.7 ± 2.95	156.2 ± 2.95	157.0 ± 2.95
	21 weeks	156.0 ± 2.37	154.5 ± 2.19	153.5 ± 2.19	157.3 ± 2.19	154.8 ± 2.19
	47 weeks	155.8 ± 0.48	156.0 ± 1.70	155.3 ± 1.52	155.3 ± 1.52	154.8 ± 1.52
	90 weeks	157.8 ± 1.28	153.5 ± 3.76	157.2 ± 3.36	157.5 ± 3.36	157.8 ± 3.52
Potassium (mmol/l)	Pretreatment (5 weeks)	4.0 ± 0.29	3.8 ± 0.26	3.8 ± 0.27	3.9 ± 3.8	3.7 ± 0.26
	4 weeks	6.1 ± 0.26	6.1 ± 0.57	6.0 ± 0.60	6.1 ± 0.60	5.8 ± 0.60
	12 weeks	6.2 ± 0.17	5.7 ± 0.59	6.1 ± 0.56	5.4 ± 0.56	5.0 ± 0.59
	21 weeks	5.6 ± 0.16	5.6 ± 0.35	5.2 ± 0.35	5.5 ± 0.35	5.7 ± 0.37
	47 weeks	5.4 ± 0.16	5.4 ± 0.27	5.3 ± 0.24	5.6 ± 0.24	5.4 ± 0.24
	90 weeks	5.1 ± 0.31	4.6 ± 0.29	4.7 ± 0.29	4.5 ± 0.28	4.5 ± 0.29
Aspartate aminotransferase (U/l)	Pretreatment (5 weeks)	38.8 ± 3.07	45.5 ± 4.74	35.0 ± 4.98	39.8 ± 4.74	43.7 ± 4.74
	4 weeks	57.8 ± 8.98	48.7 ± 12.65	48.5 ± 12.65	52.6 ± 13.27	73.8 ± 13.27
	12 weeks	55.8 ± 8.56	55.2 ± 15.6	63.0 ± 15.02	56.5 ± 15.02	50.8 ± 15.6
	21 weeks	63.0 ± 8.27	70.7 ± 17.39	50.2 ± 17.39	60.7 ± 17.39	72.0 ± 17.39
	47 weeks	63.7 ± 5.86	74.6 ± 9.8	53.8 ± 9.34	61.3 ± 9.34	67.5 ± 9.34
	90 weeks	90.8 ± 25.94	83.8 ± 23.87	51.8 ± 22.76	55.0 ± 22.76	56.4 ± 23.87
Creatine kinase (U/l)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	561.8 ± 403.65	336.5 ± 328.72	350.0 ± 328.72	214.8 ± 344.70	597.0 ± 344.77
	12 weeks	263.5 ± 61.22	231.4 ± 341.43	750.8 ± 328.54	129.0 ± 328.54	153.6 ± 341.43
	21 weeks	568.2 ± 215.44	534.2 ± 279.24	74.7 ± 279.24	391.0 ± 279.24	165.3 ± 279.24
	47 weeks	171.3 ± 79.58	258.5 ± 88.94	64.7 ± 79.55	160.2 ± 83.44	224.8 ± 83.44
	90 weeks	171.8 ± 67.65	147.4 ± 199.71	337.5 ± 191.21	436.0 ± 191.21	245.0 ± 199.71

Blank cells indicate missing data.

ND = no data.

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

## 2.6. Blood collection for drug plasma level determinations

For the control group, the low-dose remacemide group, and both of the two MK-801 groups, blood was sampled for determination of plasma drug concentrations on the first day of drug dosing, and after 1 week, 1 month, 3 months, 6 months, 12 months, and 18 months of daily treatment. Blood was also sampled from each group during the first month of the dose-reduction phase (Month 19) and from the

50-mg/kg remacemide and 1.0-mg/kg MK-801 groups during the third and fourth months of the dose-reduction phase (Months 21 and 21.5, respectively). For the 50-mg/kg remacemide group, the first blood sample was collected on the first day of treatment with 50 mg/kg, which was actually the seventh day of drug treatment (see Section 2.2). Subsequent blood samples were collected from these subjects as described above. At each of the sampling time points, 2–3 ml of blood was sampled from two monkeys

Table 2

Hematology endpoints measured during 18 months of chronic treatment (means  $\pm$  S.E.M.)

Endpoint	Time after first drug treatment	Vehicle	0.1 mg/kg MK-801	1.0 mg/kg MK-801	20 mg/kg Remacemide	50 mg/kg Remacemide
Red blood cells ( $10^6/\text{mm}^3$ )	Pretreatment (5 weeks)	5.4 $\pm$ 0.15	5.1 $\pm$ 0.23	5.8 $\pm$ 0.24	5.4 $\pm$ 0.23	5.3 $\pm$ 0.23
	4 weeks	5.7 $\pm$ 0.14	6.0 $\pm$ 0.25	6.2 $\pm$ 0.25	5.6 $\pm$ 0.26	5.7 $\pm$ 0.27
	12 weeks	5.5 $\pm$ 0.17	5.5 $\pm$ 0.27	6.0 $\pm$ 0.27	5.4 $\pm$ 0.26	5.3 $\pm$ 0.26
	21 weeks	5.2 $\pm$ 0.15	5.3 $\pm$ 0.23	5.8 $\pm$ 0.22	5.3 $\pm$ 0.22	5.0 $\pm$ 0.25
	47 weeks	5.2 $\pm$ 0.07	5.3 $\pm$ 0.2	5.8 $\pm$ 0.19*	5.4 $\pm$ 0.19	5.3 $\pm$ 0.2
	90 weeks	5.0 $\pm$ 0.10	5.3 $\pm$ 0.29	5.5 $\pm$ 0.28	5.4 $\pm$ 0.28	4.9 $\pm$ 0.29
White blood cells ( $10^3/\text{mm}^3$ )	Pretreatment (5 weeks)	9.9 $\pm$ 1.57	8.9 $\pm$ 2.33	8.5 $\pm$ 2.44	8.8 $\pm$ 2.33	7.6 $\pm$ 2.33
	4 weeks	11.2 $\pm$ 1.78	9.1 $\pm$ 1.81	9.5 $\pm$ 1.81	7.0 $\pm$ 1.89	9.2 $\pm$ 2.02
	12 weeks	10.1 $\pm$ 1.82	9.9 $\pm$ 1.89	8.0 $\pm$ 1.89	7.2 $\pm$ 1.82	10.5 $\pm$ 1.82
	21 weeks	9.3 $\pm$ 1.69	10.7 $\pm$ 1.75	9.4 $\pm$ 1.67	7.1 $\pm$ 1.67	7.4 $\pm$ 1.67
	47 weeks	11.4 $\pm$ 1.89	9.9 $\pm$ 2.29	12.0 $\pm$ 2.19	10.3 $\pm$ 2.19	9.9 $\pm$ 2.29
	90 weeks	9.4 $\pm$ 1.02	11.0 $\pm$ 1.70	9.2 $\pm$ 1.62	8.6 $\pm$ 1.62	9.2 $\pm$ 1.70
Hemoglobin (g/dl)	Pretreatment (5 weeks)	12.1 $\pm$ 0.26	11.4 $\pm$ 0.55	12.7 $\pm$ 0.57	11.9 $\pm$ 0.55	12.3 $\pm$ 0.55
	4 weeks	13.6 $\pm$ 0.36	14.1 $\pm$ 0.50	14.2 $\pm$ 0.50	13.5 $\pm$ 0.52	13.2 $\pm$ 0.56
	12 weeks	13.2 $\pm$ 0.37	13.5 $\pm$ 0.56	14.3 $\pm$ 0.56	13.3 $\pm$ 0.54	13.0 $\pm$ 0.54
	21 weeks	13.3 $\pm$ 0.13	13.5 $\pm$ 0.53	13.9 $\pm$ 0.50	13.5 $\pm$ 0.50	13.5 $\pm$ 0.50
	47 weeks	14.0 $\pm$ 0.36	14.1 $\pm$ 0.55	15.3 $\pm$ 0.52	14.5 $\pm$ 0.52	14.1 $\pm$ 0.55
	90 weeks	12.3 $\pm$ 0.28	12.8 $\pm$ 0.62	13.0 $\pm$ 0.59	13.0 $\pm$ 0.59	12.1 $\pm$ 0.62
Reticulocyte count (%)	Pretreatment (5 weeks)	ND	ND	ND	ND	ND
	4 weeks	0.9 $\pm$ 0.04	1.0 $\pm$ 0.10	0.8 $\pm$ 0.10	0.8 $\pm$ 0.10	0.8 $\pm$ 0.11
	12 weeks	1.1 $\pm$ 0.09	0.8 $\pm$ 0.10	0.8 $\pm$ 0.10	0.9 $\pm$ 0.10	0.8 $\pm$ 0.10
	21 weeks	1.0 $\pm$ 0.07	1.0 $\pm$ 0.09	0.8 $\pm$ 0.09	0.9 $\pm$ 0.09	0.8 $\pm$ 0.09
	47 weeks	1.0 $\pm$ 0.09	1.0 $\pm$ 0.08	1.0 $\pm$ 0.08	1.0 $\pm$ 0.08	1.0 $\pm$ 0.08
	90 weeks	1.0 $\pm$ 0.04	1.1 $\pm$ 0.06	1.0 $\pm$ 0.06	1.1 $\pm$ 0.06	1.1 $\pm$ 0.06
Platelets ( $10^3/\text{mm}^3$ )	Pretreatment (5 weeks)	407.3 $\pm$ 32.92	425.5 $\pm$ 55.00	364.6 $\pm$ 57.69	436.7 $\pm$ 55.00	390.0 $\pm$ 55.00
	4 weeks	358.8 $\pm$ 59.98	386.8 $\pm$ 65.20	390.7 $\pm$ 65.20	483.6 $\pm$ 68.38	342.8 $\pm$ 72.89
	12 weeks	292.3 $\pm$ 37.55	298.2 $\pm$ 57.43	222.8 $\pm$ 57.43	286.2 $\pm$ 55.26	334.2 $\pm$ 55.26
	21 weeks	295.8 $\pm$ 43.36	325.6 $\pm$ 55.84	315.2 $\pm$ 56.11	313.8 $\pm$ 56.11	333.2 $\pm$ 56.11
	47 weeks	305.8 $\pm$ 28.54	236.4 $\pm$ 47.74	235.2 $\pm$ 45.71	272.8 $\pm$ 45.71	257.0 $\pm$ 47.74
	90 weeks	246.6 $\pm$ 50.65	385.0 $\pm$ 64.49	343.4 $\pm$ 64.49	379.5 $\pm$ 61.74	363.5 $\pm$ 68.40
Hematocrit (%)	Pretreatment (5 weeks)	37.7 $\pm$ 0.58	36.5 $\pm$ 1.17	39.2 $\pm$ 1.23	38.0 $\pm$ 1.17	37.9 $\pm$ 1.17
	4 weeks	41.8 $\pm$ 1.32	42.7 $\pm$ 1.40	43.1 $\pm$ 1.40	41.0 $\pm$ 1.47	41.2 $\pm$ 1.56
	12 weeks	39.5 $\pm$ 1.10	40.1 $\pm$ 1.58	42.3 $\pm$ 1.58	39.9 $\pm$ 1.52	38.9 $\pm$ 1.52
	21 weeks	38.0 $\pm$ 1.10	38.5 $\pm$ 1.44	39.3 $\pm$ 1.37	38.2 $\pm$ 1.37	38.5 $\pm$ 1.37
	47 weeks	37.7 $\pm$ 0.78	38.1 $\pm$ 1.27	40.8 $\pm$ 1.22	39.6 $\pm$ 1.22	38.6 $\pm$ 1.27
	90 weeks	39.3 $\pm$ 0.76	40.5 $\pm$ 2.35	39.3 $\pm$ 2.24	41.5 $\pm$ 2.24	38.9 $\pm$ 2.35
Mean corpuscular hemoglobin (pg)	Pretreatment (5 weeks)	22.7 $\pm$ 0.61	22.2 $\pm$ 0.93	21.9 $\pm$ 0.97	22.1 $\pm$ 0.93	23.3 $\pm$ 0.93
	4 weeks	23.8 $\pm$ 0.49	23.7 $\pm$ 0.63	23.1 $\pm$ 0.63	24.2 $\pm$ 0.66	23.2 $\pm$ 0.70
	12 weeks	24.2 $\pm$ 0.47	24.4 $\pm$ 0.70	23.9 $\pm$ 0.70	24.4 $\pm$ 0.67	24.7 $\pm$ 0.67
	21 weeks	25.7 $\pm$ 0.49	26.0 $\pm$ 0.65	25.0 $\pm$ 0.62	26.1 $\pm$ 0.62	26.0 $\pm$ 0.62
	47 weeks	26.7 $\pm$ 0.48	26.4 $\pm$ 0.75	26.2 $\pm$ 0.72	26.8 $\pm$ 0.72	26.6 $\pm$ 0.75
	90 weeks	24.3 $\pm$ 0.44	24.3 $\pm$ 0.57	23.6 $\pm$ 0.54	24.4 $\pm$ 0.54	24.6 $\pm$ 0.57
Mean corpuscular hemoglobin concentration (g/dl)	pretreatment (5 weeks)	24.6 $\pm$ 0.49	23.9 $\pm$ 0.66	23.7 $\pm$ 0.63	24.1 $\pm$ 0.63	24.5 $\pm$ 0.66
	4 weeks	32.6 $\pm$ 0.30	33.1 $\pm$ 0.43	32.9 $\pm$ 0.43	32.7 $\pm$ 0.45	31.9 $\pm$ 0.48
	12 weeks	33.5 $\pm$ 0.35	33.6 $\pm$ 0.65	33.7 $\pm$ 0.65	33.1 $\pm$ 0.62	33.5 $\pm$ 0.62
	21 weeks	35.0 $\pm$ 0.35	35.5 $\pm$ 0.39	35.4 $\pm$ 0.37	35.2 $\pm$ 0.37	35.1 $\pm$ 0.37
	47 weeks	37.1 $\pm$ 0.39	37.0 $\pm$ 0.36	37.5 $\pm$ 0.35	36.7 $\pm$ 0.35	36.4 $\pm$ 0.36
	90 weeks	32.0 $\pm$ 0.30	31.7 $\pm$ 0.37	32.2 $\pm$ 0.35	31.6 $\pm$ 0.35	31.6 $\pm$ 0.37
Mean corpuscular volume (fl)	Pretreatment (5 weeks)	70.7 $\pm$ 1.58	71.0 $\pm$ 1.97	68.0 $\pm$ 2.07	70.8 $\pm$ 1.97	71.7 $\pm$ 1.97
	4 weeks	72.8 $\pm$ 1.19	71.8 $\pm$ 1.72	70.0 $\pm$ 1.72	74.0 $\pm$ 1.80	72.8 $\pm$ 1.92
	12 weeks	72.3 $\pm$ 0.75	72.8 $\pm$ 1.77	70.8 $\pm$ 1.77	73.7 $\pm$ 1.71	73.7 $\pm$ 1.71
	21 weeks	73.5 $\pm$ 1.12	74.2 $\pm$ 1.76	70.5 $\pm$ 1.68	74.5 $\pm$ 1.68	74.0 $\pm$ 1.68
	47 weeks	71.8 $\pm$ 0.80	71.4 $\pm$ 1.92	69.8 $\pm$ 1.84	73.3 $\pm$ 1.84	73.0 $\pm$ 1.92
	90 weeks	73.3 $\pm$ 1.26	73.0 $\pm$ 1.82	70.7 $\pm$ 1.73	73.5 $\pm$ 1.73	74.6 $\pm$ 1.82
Lymphocytes (%)	Pretreatment (5 weeks)	53.5 $\pm$ 3.92	50.8 $\pm$ 4.64	55.4 $\pm$ 4.86	55.5 $\pm$ 4.64	57.8 $\pm$ 4.64
	4 weeks	53.2 $\pm$ 2.83	44.2 $\pm$ 5.21	50.2 $\pm$ 5.21	52.6 $\pm$ 5.46	55.3 $\pm$ 5.82
	12 weeks	51.3 $\pm$ 1.49	46.4 $\pm$ 3.75	50.6 $\pm$ 3.75	48.8 $\pm$ 3.61	47.2 $\pm$ 3.61
	21 weeks	51.3 $\pm$ 1.36	43.0 $\pm$ 2.84*	47.8 $\pm$ 2.71	49.3 $\pm$ 2.71	48.7 $\pm$ 2.71
	47 weeks	75.0 $\pm$ 3.32	63.4 $\pm$ 9.98	62.5 $\pm$ 9.56	57.8 $\pm$ 9.56	56.4 $\pm$ 9.98
	90 weeks	61.3 $\pm$ 3.56	55.0 $\pm$ 4.72	61.7 $\pm$ 4.50	58.7 $\pm$ 4.50	66.2 $\pm$ 4.72

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Table 2 (continued)

Endpoint	Time after first drug treatment	Vehicle	0.1 mg/kg MK-801	1.0 mg/kg MK-801	20 mg/kg Remacemide	50 mg/kg Remacemide
Segmented neutrophils (%)	Pretreatment (5 weeks)	45.3 ± 3.73	47.7 ± 4.58	43.4 ± 4.81	44.2 ± 4.58	41.5 ± 4.58
	4 weeks	46.0 ± 3.01	53.8 ± 5.24	48.8 ± 5.24	47.0 ± 5.49	43.5 ± 5.86
	12 weeks	48.3 ± 1.25	52.8 ± 3.63	48.8 ± 3.63	51.2 ± 3.49	52.7 ± 3.49
	21 weeks	48.3 ± 1.28	56.4 ± 2.98*	51.7 ± 2.84	51.7 ± 2.84	51.0 ± 2.84
	47 weeks	24.0 ± 3.65	35.8 ± 10.33	36.7 ± 9.89	41.3 ± 9.89	43.4 ± 10.33
	90 weeks	35.5 ± 3.59	41.4 ± 6.14	36.5 ± 5.85	39.5 ± 5.85	31.4 ± 6.14

ND = no data.

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

from each treatment group using 3.0 cm<sup>3</sup> heparinized syringes with 20-gauge needles. Samples were drawn immediately prior to daily dosing, and again 1, 2, 4, 10, 17, and 24 h after daily dosing. After collection, samples were immediately transferred to 12 × 75 mm glass tubes and centrifuged at 3000 × *g* for 10 min. Plasma was then collected using Pasteur pipettes and was stored at −70 °C for later analysis.

#### 2.6.1. Measurement of remacemide levels in plasma

Plasma levels of remacemide and of its primary desglycyl metabolite were determined using high-performance liquid chromatography (HPLC) according to procedures developed by AstraZeneca. Briefly, samples were thawed and centrifuged at 800 × *g* for 10 min. One hundred to two hundred microliters of each sample were then transferred to glass tubes containing 1.5 ml 0.01M phosphate buffer (pH 6.0). The plasma-buffer solution was then transferred to 1 of 10 wells of an Advanced Automated Sample Processor (AASP; Varian, Birchwood Science Park, Warrington, Cheshire, UK) and eluted onto an AASP CBA cartridge (AI 12222-9011; Varian) under nitrogen pressure (10–50 psi). The CBA cartridge was then removed from the AASP prep-station and loaded onto an AASP autosampler (Varian) for HPLC analysis.

Samples were washed from the cartridges with mobile phase (22:78 v/v MeCN: 0.1% trifluoroacetic acid) onto a Symmetry C18 HPLC column (4.6 × 250 mm) equipped with a C18 Symmetry Sentry guard column (Waters Associates, Milford, MA, USA). A Waters 510 HPLC pump was used to maintain a flow rate of 1.5 ml/min and a Fiatron CH-30 column heater was used to maintain the column at a temperature of 40 °C. A Waters 486 tunable absorbance detector and a Perkin-Elmer LCI-100 integrator were used to collect and store the data.

#### 2.6.2. Measurement of MK-801 levels in plasma

Plasma levels of MK-801 were determined using HPLC according to procedures developed at the National Center for Toxicological Research. Briefly, 500–1000 µl of each sample + 100 µl 1.0 N KOH was added to 100 × 13 mm screw-topped glass tubes and vortexed. Four milliliters of methylene chloride (MeCl<sub>2</sub>) was then added to each tube and the tubes were agitated gently using a platform rocker. After 10 min of agitation, the tubes were rotated 180° (to

wet all internal surfaces) and rocked for an additional 10 min. After rocking, the samples were centrifuged for 10 min (800 × *g*) and the organic phase was transferred to a set of 8 ml tubes containing 800 µl of melonic acid solution (2.0 mg/ml in acetone). Three milliliters of MeCl<sub>2</sub> was then added to each sample and extraction, centrifugation, and transfer procedures were repeated. After the second transfer, samples were evaporated to dryness under a nitrogen stream and resuspended in 500 µl 10/90 acetonitrile/water. Samples were then transferred to individual 600 µl glass inserts suspended by Waters coil springs inside Waters 4-ml vials. The vials were sealed with Teflon caps and stored at approximately 4 °C for later HPLC analysis.

HPLC was performed using a model 600 solvent delivery system which pumped a mobile phase of 70/30 acetonitrile/water: 0.01 N KH<sub>2</sub>PO<sub>4</sub> (pH 4.4) at 1.0 ml/min and at a pressure of 660 psi through a Superflo 5 µm × 4.6 × 250 mm diphenyl column. A Phenomenex 4 × 3.0 mm C18 guard column also was used. All injections were 100 µl in volume using a Waters 717+ autoinjector. The detector was a Waters model 474 fluorescent detector set at Ex = 229 nm and Em = 291 nm. The retention time of MK-801 was 7.8 min.

#### 2.7. Statistical analyses

Data were analyzed using between-subjects analyses of variance at each time point. Dunnett's *a posteriori* comparisons were used to compare values for each drug treatment group to those of control. A probability of .05 or less was used to determine statistical significance.

### 3. Results

#### 3.1. Clinical chemistry monitoring

The results of clinical chemistry analyses revealed only occasional and sporadic differences among treatment groups (Table 1). In fact, the only significant result noted was lower alanine transaminase levels among subjects treated with 1.0 mg/kg/day MK-801 at 90 weeks of treatment than among controls ( $P < .05$ ). Due to the large number of pairwise comparisons made, it is likely that this effect reflects an artifact owing to Type-I error rather than true drug effects.

Table 3  
Home-cage behavioral observations (mean number of observations per treatment group)

Group	Date											
	2 weeks	1 month	1.5 months	2.5 months	3.5 months	5.5 months	7.5 months	11.5 months	14.5 months	17.5 months	20.5 months	23.5 months
<i>Locomotion</i>												
Vehicle	7.0	7.3	6.7	6.8	9.2	8.2	10.7	9.2	7.8	10.2	7.0	5.0
0.1 mg/kg MK-801	6.7	4.5	7.3	5.0	6.5	11.0	8.7	11.4	7.4	5.8	7.5	5.8
1.0 mg/kg MK-801	7.5	5.7	11.0	8.7	8.5	9.8	8.0	7.5	7.0	9.0	8.5	6.5
20 mg/kg remacemide	8.3	6.7	7.7	8.5	6.8	8.0	7.2	8.2	9.3	8.3	6.0	7.0
50 mg/kg remacemide	7.8	2.5	11.3	7.0	10.0	8.7	9.7	9.3	8.7	7.4	4.8	7.8
<i>Foraging</i>												
Vehicle	0.0	0.5	0.3	0.8	0.7	2.0	1.7	1.5	0.3	1.8	2.0	1.3
0.1 mg/kg MK-801	0.8	1.5	0.2	0.8	2.3	1.5	1.0	0.4	2.6	1.0	1.0	1.2
1.0 mg/kg MK-801	1.0	1.7	0.0	1.3	2.3	1.2	0.0	0.5	0.5	0.8	1.3	1.5
20 mg/kg remacemide	1.3	1.3	1.7	2.2	2.3	2.7	1.2	1.8	2.5	1.3	1.0	3.2
50 mg/kg remacemide	0.0	1.0	0.7	0.7	1.2	2.7	1.0	1.3	1.7	3.2	1.5	3.5
<i>Environmental exploration (other)</i>												
Vehicle	1.8	0.5	0.2	1.5	1.2	1.0	0.7	1.2	0.3	0.5	0.0	1.2
0.1 mg/kg MK-801	1.2	0.3	1.0	1.5	0.3	1.3	0.3	0.8	1.6	1.4	0.2	0.5
1.0 mg/kg MK-801	0.8	1.0	1.5	2.0	0.2	0.3	0.0	0.3	0.5	0.3	0.5	0.3
20 mg/kg remacemide	0.3	0.7	0.5	0.8	0.3	0.8	0.2	1.0	0.3	0.3	0.5	0.5
50 mg/kg remacemide	1.2	0.7	1.3	2.0	0.2	1.0	0.2	1.0	1.0	0.2	0.0	0.0
<i>Self-motion play</i>												
Vehicle	1.2	0.5	0.2	1.3	2.3	1.3	1.8	0.7	0.5	1.0	.05	0.3
0.1 mg/kg MK-801	1.5	0.2	0.8	0.7	1.0	1.2	1.3	0.4	1.0	0.4	1.8	0.3
1.0 mg/kg MK-801	2.8	1.3	2.2	1.8	0.8	2.5	1.8	2.5	2.5	0.8	0.7	2.0
20 mg/kg remacemide	2.2	0.7	1.0	2.5	1.2	1.2	1.2	2.3	1.5	2.0	1.2	1.2
50 mg/kg remacemide	1.5	0.2	1.3	1.3	0.8	0.8	1.8	0.8	1.2	0.0	0.5	1.8
<i>Inactivity</i>												
Vehicle	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.3	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Stereotypy</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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Table 3 (continued)

Group	Date											
	2 weeks	1 month	1.5 months	2.5 months	3.5 months	5.5 months	7.5 months	11.5 months	14.5 months	17.5 months	20.5 months	23.5 months
<i>Stereotype</i>												
1.0 mg/kg MK-801	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Huddle</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.7	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Self-sex</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Self-bite</i>												
Vehicle	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.2	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Self-groom</i>												
Vehicle	1.0	0.8	0.2	0.5	0.7	0.3	0.3	0.7	0.5	0.3	0.5	1.0
0.1 mg/kg MK-801	1.3	0.8	1.0	1.5	0.3	1.2	1.0	1.8	0.6	1.0	0.8	0.5
1.0 mg/kg MK-801	0.7	0.2	0.3	0.3	0.5	1.5	0.2	0.3	0.3	0.8	0.5	0.2
20 mg/kg remacemide	1.8	0.5	0.7	1.2	0.5	1.2	0.5	0.5	0.7	1.7	0.8	1.2
50 mg/kg remacemide	0.7	0.7	0.5	1.0	0.3	0.5	0.0	0.5	0.8	0.6	0.2	0.8
<i>Self-aggression</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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Table 3 (continued)

Group	Date											
	2 weeks	1 month	1.5 months	2.5 months	3.5 months	5.5 months	7.5 months	11.5 months	14.5 months	17.5 months	20.5 months	23.5 months
<i>Self-aggression</i>												
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Eating</i>												
Vehicle	1.5	1.7	2.0	1.7	1.7	2.0	1.3	0.8	1.5	1.7	2.5	1.3
0.1 mg/kg MK-801	0.8	0.8	1.0	0.2	1.5	0.7	1.2	0.4	2.2	1.2	2.5	2.2
1.0 mg/kg MK-801	0.5	0.8	0.5	0.2	1.0	0.0	1.5	1.0	0.3	0.8	0.3	1.3
20 mg/kg remacemide	0.8	1.3	0.7	1.0	2.5	2.2	1.3	1.5	1.8	0.7	4.5	1.5
50 mg/kg remacemide	0.3	1.2	1.7	1.7	1.5	1.2	3.3	1.5	1.3	1.6	5.5	2.5
<i>Drinking</i>												
Vehicle	0.9	0.8	0.7	0.8	1.0	0.9	1.0	0.9	0.7	0.9	0.0	1.3
0.1 mg/kg MK-801	1.0	0.6	0.7	0.8	0.9	1.1	0.8	0.9	0.9	0.6	0.7	1.2
1.0 mg/kg MK-801	1.0	0.7	1.0	0.9	0.8	1.0	0.7	0.8	0.7	0.8	0.2	1.2
20 mg/kg remacemide	1.1	0.8	0.8	1.1	0.9	1.0	0.7	0.9	1.0	0.9	0.5	2.0
50 mg/kg remacemide	0.9	0.5	1.0	0.9	1.0	0.9	1.0	0.9	0.9	0.8	1.8	1.2
<i>Self-directed behavior (other)</i>												
Vehicle	0.2	0.0	0.0	0.0	0.0	0.0	0.8	0.5	0.5	0.0	0.0	0.7
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.2
1.0 mg/kg MK-801	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
<i>Threat</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Fear grimace</i>												
Vehicle	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.3	0.2	0.7	0.0	0.5	0.3	0.3	0.3	0.0	1.3	0.0
20 mg/kg remacemide	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	1.3	1.0	0.0	0.2	0.0	0.2	0.5	0.0	0.0	0.4	0.0	0.0

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Table 3 (continued)

Group	Date											
	2 weeks	1 month	1.5 months	2.5 months	3.5 months	5.5 months	7.5 months	11.5 months	14.5 months	17.5 months	20.5 months	23.5 months
<i>Vocalization</i>												
Vehicle	3.2	2.5	1.0	1.0	0.5	1.7	1.0	0.3	1.7	1.0	1.5	1.7
0.1 mg/kg MK-801	2.3	0.8	5.2	0.0	0.0	2.5	0.5	2.8	1.2	2.2	0.3	0.3
1.0 mg/kg MK-801	0.2	1.8	2.0	2.8	1.2	0.2	0.3	0.2	0.0	0.8	1.5	1.5
20 mg/kg remacemide	3.0	1.5	0.0	2.7	4.3	1.0	1.3	2.0	0.5	0.8	0.2	0.0
50 mg/kg remacemide	3.3	3.8	5.0	3.0	0.8	0.8	1.2	2.3	2.3	0.6	0.2	2.5
<i>Hostility toward tester</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0
0.1 mg/kg MK-801	0.2	0.3	0.2	0.3	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.2
1.0 mg/kg MK-801	0.0	0.0	0.5	0.0	0.2	0.0	0.0	0.0	0.0	0.7	0.2	0.0
20 mg/kg remacemide	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Submissive toward tester</i>												
Vehicle	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.5	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.3	0.2	0.0	0.5	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.7	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
50 mg/kg remacemide	1.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Orientation toward tester</i>												
Vehicle	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.5	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.3	0.2	0.0	0.5	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.7	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	1.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Collar manipulation</i>												
Vehicle	1.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
0.1 mg/kg MK-801	0.3	0.0	0.3	0.5	0.5	0.3	0.2	0.0	1.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.5	0.5	0.2	0.0	0.3	0.7	0.2	0.5	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.2	0.3	0.3	0.2	0.0	0.3	0.3	0.2	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.5	0.5	0.2	0.3	0.0	0.7	0.0	0.2	0.7	0.0	0.0	0.0
<i>Ataxia</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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Table 3 (continued)

Group	Date											
	2 weeks	1 month	1.5 months	2.5 months	3.5 months	5.5 months	7.5 months	11.5 months	14.5 months	17.5 months	20.5 months	23.5 months
<i>Ataxia</i>												
1.0 mg/kg MK-801	0.0	0.2	0.2	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nystagmus</i>												
Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0 mg/kg MK-801	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50 mg/kg remacemide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Wet-dog shakes</i>												
Vehicle	0.0	0.0	0.2	0.0	0.3	0.0	0.2	0.2	0.3	0.0	0.0	0.2
0.1 mg/kg MK-801	0.0	0.2	0.2	0.3	0.5	0.2	0.3	0.0	0.2	0.0	0.2	0.0
1.0 mg/kg MK-801	0.3	0.3	0.7	0.2	0.7	0.2	0.5	0.3	0.0	0.0	0.0	0.2
20 mg/kg remacemide	0.0	0.3	0.3	0.2	0.2	0.5	0.3	0.0	0.5	0.0	0.0	0.2
50 mg/kg remacemide	0.5	0.3	0.3	0.5	0.7	0.3	0.7	0.8	0.2	0.0	0.2	0.2

### 3.2. Hematology monitoring

As was the case for the clinical chemistry assessments, the results of the hematology analyses revealed only occasional and sporadic effects between treatment groups (see Table 2). Specifically, the 1.0-mg/kg/day MK-801 group had higher red blood cell counts than did controls during the 47th week and the 0.1-mg/kg/day MK-801 group showed lower lymphocyte and higher segmented neutrophil levels (%) during the 21st week than did controls ( $P < .05$ ). There were no significant differences between remacemide-treated subjects and control subjects at any time during the study.

### 3.3. Ophthalmic examinations

Routine ophthalmologic examinations conducted throughout the study did not detect any ocular abnormalities (data not shown).

### 3.4. Home-cage behavioral observation

The results of the home-cage observations are presented in Table 3. Most of the behaviors monitored occurred at such a low frequency as to be uninformative. For those behaviors

that were observed often enough to be of interest, there were no statistically significant treatment-related effects.

### 3.5. Plasma level monitoring

Peak plasma levels of remacemide and its desglycyl metabolite are presented in Table 4a. During the chronic treatment phase, peak plasma remacemide levels ranged from 183 to 1265 ng/ml 1–4 h after the 20-mg/kg doses and from about 198 to 3504 ng/ml 1–4 h after the 50-mg/kg doses. Mean peak plasma levels for the metabolite ranged from about 25 to 613 ng/ml and from approximately 70 to 1861 ng/ml 2–4 h after the 20- and 50-mg/kg doses, respectively. The wide variability in the plasma levels achieved in individual animals is generally consistent with the route of administration and with clinical observations in humans [3]. It is of note that one subject that was initially assigned to the high-dose remacemide group was moved to the low-dose remacemide group on the third day of the study to replace a subject that would not tolerate the orogastric gavage procedure. In accordance with the “ramping” procedure followed for all high-dose remacemide subjects, this subject was treated with 20 mg/kg remacemide for 7 days of dosing prior to collection of the first blood sample. As a result, the averaged plasma level data pre-

Table 4

a. Peak levels of remacemide and of its desglycinyll metabolite in plasma (ng/ml). Data represent  $C_{\max}$  measured in two subjects per treatment group ( $T_{\max}$  presented in parentheses). For the 20-mg/kg/day group, data presented for the 19-month sample reflect administration of water only. For the 50-mg/kg/day group, data presented for the 19- and 21-month samples reflect administration of 20 mg/kg and data presented for the 21.5-month sample reflect administration of water only. <MDL indicates levels below the minimum detectable limits of the assay (i.e., 10.0 ng/ml). ND = no data. Data presented for Subject AK3K (first day) were collected following 7 days of treatment with 20 mg/kg remacemide.

Time after first drug treatment	Plasma constituent	20 mg/kg Remacemide (ng/ml)		50 mg/kg Remacemide (ng/ml)	
		Subject AK3K	Subject AK22	Subject AH3W	Subject JEC
First day	Remacemide	1265 (2 h)	464 (2 h)	2487 (4 h)	2351 (1 h)
	Desglycinyll metabolite	613 (1 h)	106 (4 h)	1334 (4 h)	768 (4 h)
1 month	Remacemide	324 (1 h)	751 (2 h)	1394 (2 h)	2350 (4 h)
	Desglycinyll metabolite	29 (4 h)	174 (2 h)	804 (2 h)	1861 (4 h)
3 months	Remacemide	ND	183 (4 h)	3001 (4 h)	440 (2 h)
	Desglycinyll metabolite	ND	25 (10 h)	1253 (4 h)	70 (2 h)
6 months	Remacemide	638 (2 h)	598 (1 h)	3053 (2 h)	593 (4 h)
	Desglycinyll metabolite	147 (4 h)	90 (4 h)	1301 (4 h)	129 (4 h)
12 months	Remacemide	224 (4 h)	289 (1 h)	2269 (4 h)	198 (10 h)
	Desglycinyll metabolite	26 (4 h)	35 (24 h)	1043 (1 h)	167 (10 h)
18 months	Remacemide	1255 (2 h)	211 (1 h)	3504 (4 h)	938 (1 h)
	Desglycinyll metabolite	81 (baseline)	43 (4 h)	984 (4 h)	696 (2 h)
19 months	Remacemide	54 (4 h)	<MDL	1529 (2 h)	317 (4 h)
	Desglycinyll metabolite	<MDL	<MDL	268 (2 h)	24 (4 h)
21 months	Remacemide	ND	ND	1755 (2 h)	395 (2 h)
	Desglycinyll metabolite	ND	ND	496 (2 h)	130 (4 h)
21.5 months	Remacemide	ND	ND	<MDL	<MDL
	Desglycinyll metabolite	ND	ND	<MDL	<MDL

b. Peak plasma levels of MK-801 in plasma (ng/ml). Data represent  $C_{\max}$  measured in two subjects per treatment group ( $T_{\max}$  presented in parentheses). “<MDL” indicates that peak plasma level values were below the minimum detectable limits of the assay (i.e., < 1.0 ng/ml). ND = no data.

Time after first drug treatment	0.1 mg/kg MK-801 (ng/ml)		1.0 mg/kg MK-801 (ng/ml)	
	Subject AK74	Subject AH8W	Subject JBX	Subject JEX
First day	ND	ND	8.7 (1 h)	5.6 (1 h)
1 month	ND	ND	3.4 (1 h)	8.1 (1 h)
3 months	1.0 (1 h)	1.4 (2 h)	5.8 (1 h)	5.4 (2 h)
6 months	<MDL	1.6 (1 h)	4.4 (1 h)	4.1 (1 h)
12 months	<MDL	1.7 (1 h)	6.8 (2 h)	4.4 (1 h)
18 months	ND	ND	ND	ND
19 months	ND	ND	ND	ND
21 months	ND	ND	ND	ND
21.5 months	ND	ND	ND	ND

sented in Table 4a include data that were collected after 7 days of daily dosing and may not be representative of the plasma levels that would have been present on the very first day of dosing.

Peak plasma levels of MK-801 are presented in Table 4b. On the first day of treatment, administration of 1.0 mg/kg MK-801 resulted in peak plasma levels of 8.7 ng/ml measured 1 h after oral dosing. Subsequent administration produced somewhat lower peak plasma levels of only 4.1–8.1 ng/ml. Although the use of only two subjects per treatment group precluded definitive statistical analyses, this pattern of results may suggest an effect of chronic MK-801 treatment to alter its own metabolism. Alternatively, this pattern of results may simply reflect natural individual animal variation that can result from oral exposure to drugs. Among subjects treated with 0.1 mg/kg/day MK-801, peak plasma levels were extremely low (i.e., < 1.0 ng/ml) and were generally below the minimum detectable limits of the assay.

#### 4. Discussion

The present experiment examined effects of long-term developmental exposure to MK-801 (a prototypic agent which selectively inhibits NMDA receptor activity) or remacemide (a relatively novel compound which blocks NMDA receptors and fast sodium channels concurrently) in juvenile rhesus monkeys (*M. mulatta*). Endpoints were selected to provide a broad index of subjects' health and included clinical chemistry, hematology, ophthalmic measurements, home-cage behavioral observations, and periodic plasma drug level assessments.

In general, the results indicate that both MK-801 and remacemide were well tolerated and produced no consistent alterations in clinical chemistry, hematology, ophthalmic morphology, or spontaneous home-cage behavior. This pattern of results is consistent with previous clinical reports in which laboratory variables were unaffected by chronic

(4–6 weeks) remacemide treatment [2,3]. Where statistically significant effects did emerge in the present study (i.e., lymphocytes and segmented neutrophils at 21 weeks; red blood cells at 47 weeks; alanine transaminase at 90 weeks), they were inconsistent and of limited magnitude. The fact that all values, even those that were determined to be different from control, fell within the range of normal clinical values [13,15], suggests that the pattern of results reported here reflects chance variability rather than compound-related toxicity. Alternatively, it is conceivable that the inclusion of only six subjects per treatment group limited statistical power to such an extent that subtle, yet meaningful, toxicologic effects were not detected.

The results of the home-cage behavioral assessments revealed no significant treatment-related effects. Early during the course of treatment, subjects that had received the high dose MK-801 appeared to be more overtly sedated and ataxic than were subjects in all other treatment groups. On the days scheduled for home-cage observations, however, sedation/ataxia were not significant findings. It is possible that the presence of the observer sufficiently aroused subjects such that they did not exhibit the sedative/ataxic effects of MK-801 treatment.

Among the notable effects that did emerge during the course of treatment were the apparent changes in peak plasma levels of the desglycyl remacemide metabolite produced by chronic treatment with 20 mg/kg/day and in peak plasma levels of MK-801 produced by chronic treatment with 1.0 mg/kg/day MK-801. Although the inclusion of only two subjects per treatment group precludes definitive analysis, this pattern of results could be taken to suggest that chronic administration of either of these compounds may have altered their own absorption, distribution, metabolism, or excretion. It is important to note, however, that the changes in peak plasma levels were not related to the duration of treatment (i.e., they emerged within the first month and remained constant thereafter) and, in the case of remacemide, were not positively related to the dose of the compound administered. Further, it is relevant to note that the plasma levels measured in the high-dose remacemide group (50 mg/kg/day) during the first phase of dose reduction (after their daily dose had been reduced to 20 mg/kg/day) were very similar to those obtained from the low-dose group during the first 18 months of treatment. This would not have been expected if the metabolism of remacemide had been altered by prior exposure. There also may be age-related changes in the metabolism of MK-801 and remacemide which are entirely independent of either drug's capacity to induce its own metabolism. In the absence of more definitive data, therefore, no firm conclusions can be drawn regarding the potential of these compounds to affect their own absorption, distribution, metabolism, or excretion in this species.

It is interesting to note that although there were no significant effects of treatment on home-cage behavior, there were rather profound effects of treatment on the complex

operant behaviors monitored concurrently in these same subjects [20,21]. Specifically, remacemide (50 mg/kg/day), and to a lesser extent MK-801, had significant impairing effects on the acquisition of behaviors thought to model incremental learning and color and position discrimination. In the present study, neither the standard measures of toxicity (clinical chemistry, hematology, etc.) nor the home-cage behavioral observations were in any way indicative of these profound cognitive impairments. This contrast highlights the importance of using multiple types of measurement, including complex cognitive assessments, in making comprehensive assessments of drug safety.

Finally, it is relevant to comment on the fact that because animals in the present experiment were simultaneously assessed using food-reinforced operant behaviors, it was necessary to maintain strict control over their dietary intake. Specifically, daily access to food, supplemented with fresh fruit and chewable multivitamins, was rationed such that subjects gained between 0.05 and 1.0 kg body weight/month throughout the study. This rate of weight gain was similar across treatment groups and is consistent with previous studies conducted in our laboratory [17–19]. The fact that values for all clinically relevant endpoints were unremarkable and fell within expected normal ranges highlights the safety and effectiveness of this type of careful dietary control in rearing juvenile rhesus monkeys. However, because none of the animals in the present study were fed *ad libitum*, it is impossible to say unequivocally that the present feeding regimen did not influence the results.

In summary, the present experiment examined the effects of chronic exposure to remacemide or MK-801 on the general health and comportment of juvenile rhesus monkeys. Although both of these drugs were well tolerated and produced few consistent alterations in any of the endpoints monitored, it is important to note that a maximum tolerated dose of remacemide was not administered in the present study. Thus, it is impossible to say with certainty that remacemide would not have had effects if the doses were increased to a level outside of the therapeutic range. Plasma levels of both drugs peaked roughly 2 h after oral administration and fell within the range of expected values [3,8]. The fact that there were no effects on the home-cage behavior monitored presently is in sharp contrast to previously reported effects of these drugs on complex operant behaviors [20,21] in the same animals. This contrast highlights not only the advisability of using multiple endpoints, but also the importance and sensitivity of complex cognitive tasks in the evaluation of drug safety.

## Acknowledgments

The authors wish to thank Mr. Michael Gillam, The Bionetics Corporation, and Lionel Rubin, DVM for their important contributions to the present work.

This work was supported by AstraZeneca via a Cooperative Research and Development with the National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration. All procedures involving nonhuman subjects were reviewed by the NCTR Animal Care and Use Committee.

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