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Functional alteration by NMDA antagonist: Effects of L-Dopa, neuroleptics drug and postnatal administration

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Summary. Antiakinsic effects of the uncompetitive NMDA antagonists, memantine, amantadine and MK-801, and competitive antagonists, CGP 40116, alone or in co-administration with acute subthreshold dose of L-Dopa (5 mg/kg) in MPTP-treated mice, functional alterations induced by acute MK-801 in combinations with neuroleptic compounds or behavioural deficits following postnatal administration of MK-801 were investigated. Memantine and amantadine injected 60 min before the subthreshold dose of L-Dopa (5 mg/kg), induced antiakinesic actions in hypokinesic MPTP-treated mice. Concurrently, higher doses of memantine and MK-801 caused dyskinesic changes, reducing further rearing (10 and 30 mg/kg) and locomotor (30 mg/kg) behaviour of the MPTP mice; MK-801 elevated locomotion (0.1 mg/kg) but reduced rearing (0.3 mg/kg). In control, saline-treated mice, memantine (3, 10 and 30 mg/kg) and MK-801 (0.1 and 0.3 mg/kg) increased locomotor behaviour but decreased rearing behaviour. In rats, MK-801 induced marked increases in locomotor activity and disruptions of circular swim maze acquisition that were to greater or lesser extents blocked or potentiated by neuroleptic compounds: SCH 23390 (0.005 and 0.05 mg/kg) and clozapine (5.0 and 10.0 mg/kg) dose-dependently antagonised MK-801 (0.3 mg/kg) induced locomotor activity whereas raclopride (0.1 mg/kg) and haloperidol (0.1 mg/kg) attenuated it dose-specifically. Amperozide (0.5 mg/kg) attenuated the MK-801 effect but potentiated it at the 2.0 mg/kg dose. In the circular swim maze, raclopride (0.01 mg/kg) and SCH 23390 (0.05 mg/kg) improved the acquisitive performance of rats administered MK-801 (0.03 mg/kg) acutely whereas clozapine (10.0 mg/kg) and amperozide (2.0 mg/kg) deteriorated the performance of MK-801-treated rats. Postnatal administration of MK-801 (0.05 mg/kg, day 11 after birth) induced severe functional alterations in adult mice. At 70 days of age, MK-801 mice showed an initial hypoactivity followed by marked hyperactivity in the motor activity test chambers. These mice showed deficits in habituation, a nonassociative form of learning. Their hyperactivity in the test chambers was reversed by a low dose of d-amphetamine (0.25 mg/kg). Taken together, these findings display a wide range of acute/long-term functional alterations induced by NMDA antagonists, particularly MK-801, associated with animal models of brain disorders.

Keywords: MPTP – Hypokinesia – Dyskinesia – Locomotion – Rearing – Memantine – Amantadine – MK-801 – L-Dopa – 5 mg/kg – Co-administration – Motor fluctuations – C57 BL/6 mice – Activity test cages – Hypoactivity – Hyperactivity – Wistar rats – Circular swim maze – Acquisition performance – Clozapine – Haloperidol – Amperozide – SCH 23390 – Raclopride – Antagonism – Potentiation – Postnatal administration – Habituation – Neonatal – Adult – Rats – Mice

Introduction

The functional alterations induced acutely by N-methyl-D-aspartate (NMDA) receptor antagonists have been shown to range over a multitude of spontaneous and learned behaviours (e.g. Bubser et al., 1992; Hoffman, 1992; Krystal et al., 1994; Liljequist et al., 1991; Schmidt, 1994; Wozniak et al., 1990). Compounds like MK-801, memantine and phencyclidine invariably elevate locomotor activity (Clineschmidt et al., 1982; Kretschmer et al., 1992; McCann et al., 1989), particularly in the case of MK-801 that has been investigated exhaustively (Ford et al., 1989; Lehmann-Masten and Geyer, 1991; Liljequist, 1991; Maj et al., 1991; Murase et al., 1993). Other studies have examined the effects of MK-801 in a variety of instrumental and spatial learning tasks (Butelman, 1989; Danysz et al., 1988; Parada-Turska and Turski, 1990; Ward et al., 1990; Wishaw and Auer, 1989). The functional and putative therapeutic profiles of NMDA receptors are reviewed comprehensively (Danysz and Parsons, 1998).

MPTP induces parkinsonism in human and nonhuman primates (Langston, 1985), inducing the loss of substantia nigra cells in the pars compacta of adult animals. It was previously shown that injections

of MPTP (2 × 40 mg/kg) in C57 BL/6 mice induced L-Dopa reversible hypoactivity (Fredriksson et al., 1990; Sundström et al., 1990), a less rigorous dose treatment, e.g. 2 × 20, 25 or 30 mg/kg of MPTP has been found not to reduce motility in the C57 black mice although DA concentrations may indicate upto 50–80% reduction (Heikkila et al., 1989; Sonsalla and Heikkila, 1986; Weimuller et al., 1989). The parameters of MPTP treatment in this mouse strain are long-lasting (up to and above 52 weeks after treatment) with a good correlation between the functional defect, hypokinesia, the neurochemical concomitant, a severe depletion of DA, and a dose- and time-dependent recovery of several parameters of motor behaviour after treatment with L-Dopa (cf. Fredriksson and Archer, 1994; Fredriksson et al., 1990). Earlier studies demonstrated that the acute co-administration of a subthreshold dose of L-Dopa (5 mg/kg) with low doses of either the noncompetitive NMDA antagonist, MK-801, or the competitive antagonist, CGP 40116 produced a synergistic elevation of motor activity in MPTP-treated mice (Archer et al., 1996; Fredriksson et al., 1994a,b). Interestingly, also in this model an phenomenon analogous to "wearing off" following chronic L-Dopa treatment has been observed recently (Archer and Fredriksson, 1999; Fredriksson et al., 1999).

6-Hydroxydopamine (6-OHDA) has been applied usefully in both adult (Breese and Traylor, 1972) and neonate (Creese and Iversen, 1973; Shaywitz et al., 1976; Smith et al., 1973) rats to study the behavioural effects of loss of mesencephalic dopamine (DA) neurons. Destruction of these neurons in newborn rats through intracerebral, i.e. intracisternal (ic) or intracerebroventricular (icv), administration of 6-OHDA induces behavioural changes characterised consistently by hyperactivity in tests of spontaneous motor activity and/or exploratory behaviour, that may or may not persist into adulthood but generally have been found to do so (Archer, 1989; Archer et al., 1988; Erinoff et al., 1979; Fobes and Olds, 1981; Miller et al., 1981). Intracisternal administration of 6-OHDA (50 μg in a volume of 10 on Day 1 after birth) 30 min after systemic injection of the noradrenaline reuptake inhibitor, desipramine (DMI, 25 mg/kg), to neonatal rat pups causes selective DA depletions in several brain regions (e.g. striatum, septum and nucleus accumbens) but generally does not affect motor activity in the adult animal unless administered at higher doses causing locomotor hyperactivity (Archer and Fredriksson, 1992; Luthman et al., 1989a,b, 1991, 1997).

MK-801 is considered the most selective compound of this type (Wong et al., 1986). Memantine shows good selectivity and an uncompetitive open-channel blockade (Chen and Lipton, 1997), whereas amantadine is poorly selective (Kornhuber et al., 1994; Parsons et al., 1993, 1996). Both memantine and amantadine have been classified as low-affinity open-channel blockers whereas MK-801 as a high-affinity open-channel blocker (Parsons et al., 1998). Postnatal administration of MK-801 on Day 7 after birth markedly increased the rate of apoptosis in rats (Ikonomidou et al., 2000). Here, mice were administered MK-801 on postnatal day 11 in order to assess the functional consequences of this accelerated apoptosis.

Methods and materials

Animals. In the experiments described, six month old male C57 BL/6 mice [I. Acute Administration of subthreshold L-Dopa] (ALAB, Sollentuna, Sweden), weighing 22–25 g, or four-month-old Wistar rats [III: MK-801-induced alterations: Effects of Neuroleptic compounds] (Möllegård, Denmark) weighing 345–390 g at the start of behavioural testing were used. Following arrival at the laboratory, the mice/rats were allowed to acclimatize for two weeks in a room with controlled temperature (21 ± 1°C), and a constant light-dark schedule (12 hr on/12 hr off, lights on between 06.00 and 18.00 hrs). Free access to food and water was maintained throughout. In the neonatal 6-OHDA experiments, four-to-five-month-old male Sprague-Dawley rats [Effects of NMDA antagonists in neonatal 6-OHDA rats] (ALAB, Sollentuna, Sweden), weighing 330–360 g at the start of the experiments, light-dark schedule (as above), were used. In the postnatal MK-801 experiment [Effects of postnatal MK-801 administration] C57/Bl6 mice, aged 70 days at testing were used. Pregnant Sprague-Dawley rats (Study II), as well as C57/BL6 mice (Study IV), were purchased from B&K, Sollentuna, Sweden. Each litter, adjusted within 48 h to 4–5 rats or 8–10 mice, respectively, and to contain offspring of either sex in about equal number, was kept together with its respective mother in a plastic cage in a room at temperature of 22 ± 1°C and a 12/12 hours constant light/dark cycle (lights on between 06.00 and 18.00 hrs). The male offspring only were used in the present studies. At the age of 4 weeks the rats/mice were weaned and the males were placed and raised in groups of 4 to 6 animals in a room maintained for male rats/mice only. Following arrival at the laboratory, pregnant rat/mouse dams were allowed to acclimatise for two-three weeks in a room with controlled temperature (21 ± 1°C), and a constant They were housed in groups of 4/10 animals, respectively. All testing of the offspring occurred only during the hours of light (08.00–15.00 hrs). All testing was performed in a normally lighted room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e. activity test cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and had a dimmed lighting.

Behavioural measurements and apparatus

ADEA test chambers for mice. An automated device, consisting of macrolon rodent test cages (40 × 25 × 15 cm) each placed within

two series of infra-red beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous and/or drug-induced motor activity of MPTP and control mice (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). The distance between the infra-red beams was as follows: the low level beams were 73 mm apart lengthwise and 58 mm apart breadthwise in relation to the test chamber; the high level beams, placed only along each longside of the test chamber were 28 mm apart. According to the procedures described previously (Archer et al., 1986, 1996), the following parameters were measured: LOCOMOTION was measured by the low grid of infra-red beams. Counts were registered only when the mouse in the horizontal plane, ambulating around the test-cage. REARING was registered throughout the time when at least one high level beam was interrupted, i.e. the number of counts registered was proportional to the amount of time spent rearing. TOTAL ACTIVITY was measured by a sensor (a pick-up similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was constantly in contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching and grooming. All three behavioural parameters were measured over consecutive 30-min. periods.

Rat test chambers. The locomotor activity of neonatal 6-OHDA-treated and sham-operated rats was measured in plexiglas test chambers (70 × 70 × 30 cm, Kungsbacka Mät & Reglerteknik AB, Fjärås, Sweden) which were placed within two series of infra-red photocell beams emerging from thirty-two photocells (one high-level and one low-level series, 3 and 13 cm, respectively, above the floor of the test chambers), as described previously (Archer and Fredriksson, 1992). For the purposes of the present study on the locomotion data, accummulated over the total 2-hr test period, was analysed.

Circular swim maze. The swim was a circular bath (diameter 150 cm), 50 cm deep, with depth of water maintained at 35 cm and thermostatically controlled at 25°C. Underwater within the bath a hidden platform (10 × 10 cm) was placed in a constant position 1 cm below the surface of the water. In general before testing, all the rats were allowed swim training during which the submerged platform was removed and each animals allowed to swim around the maze for a period of 60 secs, after which it was removed and allowed to dry over under the hot air blown from a fan-heater adapted for the purpose. On the Test days, the submerged platform was placed at its constant set position within the pool and each rat was given a test session consisting of five consecutive trials on each of four consecutive days. During each trial the rat was placed at the same point at the edge of the bath and allowed to swim around in order to locate the submerged platform and climb onto it. On reaching the platform, the rat was allowed to remain upon it for 30 secs (orientation period) before being placed in the water again for the next trial. If a rat failed to locate the submerged platform after 70 secs, it was removed from the water and placed on the platform for the stipulated 30 secs. On termination of all five trials, the rat was removed to the hot air drying chamber. Mean latencies to emerge onto the submerged platform, as well as failures to locate the submerged platform during the 70-sec peroid allocated, over all five trials within a test session were tabulated for each rat. Care was taken to ensure that each rat was allowed to dry itself completely before being replaced in its home cage.

Treatment and chemicals. MPTP (Research Biochemical Inc., Natick, MA. USA, 2 × 40 mg/kg, s.c., 24 hr interval, administered four to six weeks before behavioural testing, nine weeks in the case of the chronic administration of L-Dopa experiments).

Neonatal 6-OHDA treatment: On Day 1 or 2 after birth, the dams were removed from the home cage and the pups randomly divided into the different groups. All the animals were pretreated 30 min prior to the intracerebral (i.c.) injections (of 6-OHDA or vehicle) with the NA reuptake inhibitor, desipramine (DMI, 25 mg/kg, s.c.), to protect NA neurons. The pups were anesthetized thereafter by hypothermia (cooling on ice), and received the intracerebral (i.c.) injections using a 25 μl Hamilton syringe with a sharp 27-gauge needle equipped with a plastic stopper at 2.5 mm from the tip. Groups of rat pups were administered intracisternal (ic) injections of 6-OHDA, i.e. into the cisterna magna by penetrating the skin immediately caudal to the occipital bones with a rostral inclination until the stopper was reached. 6-OHDA (HCL base, AB Hässle, Sweden), dissolved in 0.9% physiological saline containing 0.1% ascorbic acid (vehicle) was administered then to groups of rat pups at a dose (free base) of 75 ug in a volume of 10 ul, whereas the control group (Vehicle) was administered an equal volume of the vehicle solution alone. All solutions (saline was used as vehicle) were injected subcutaneously in a standard volume of 5 ml/kg, s.c.. Dosages are expressed as the free base.

Postnatal MK-801 treatment: MK-801 (0.5 mg/kg, s.c., Research Biochemicals, USA) was administered in a volume of 2 ml/kg body weight on postnatal day 11 on three separate occasions, at 0800, 1600 and 2400 hrs. Saline was used as the vehicle and to prepare the dose of MK-801. This period of postnatal development coincides partially with the age of mice showing a "late-stage" profile (frontal, parietal, temporal, cingulate and retrosplenial cortices) of degenerating neuronal density that starts on postnatal day 3, peaks at around day 7 and is diminished by day 14 (cf. Ikonomidou et al., 2000).

L-Dopa (AB Hässle, Mölndal, Sweden), 1-aminoadamantane hydrochloride (amantadine, Aldrich, USA), 1-amino-3,5-dimethyladamantane hydrochloride (memantine, Merz + Co, Frankfurt/Mam, Germany) and (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate (MK-801, Research Biochemicals, USA) and CGP 40116 (Ciba-Geigy AG, Basel, Switzerland) were all dissolved in saline. Clozapine (Astra Arcus AB, Södertälje, Sweden) was dissolved in glacial acetic acid and upto volume in a final solution of 5.6% glucose and pH-value neutralized with a solution of K_2HPO_4, R(+)-SCH 23390 (Research Biochemicals Inc., Natick, USA), Amperozide (Kabi Pharmacia Therapeutics AB, Malmö, Sweden), Raclopride (Astra Arcus AB, Södertalje, Sweden), and Haloperidol (Jansson Pharmaceuticals, Belgium) were all dissolved in physiological saline. All solutions (saline was used as vehicle, except in the case of clozapine) were injected subcutaneously in a volume of 2 ml/kg, s.c.. Dosages are expressed as the free base. The dose of MPTP applied has not been found to affect food/water intake excessively. However, in all the experiments special precautions are taken to facilitate each animal's ability to acquire food/water by placing each appropriately on the floor of the cage for the first two days following the MPTP treatment.

I. Acute administration of subthreshold L-Dopa

Groups of mice (n = 12 mice in each group, unless where otherwise stated) injected s.c. with either MPTP (2 × 40 mg/kg) or saline (control) about four-to-six weeks prior to the behavioural testing. The doses of each compound were chosen in order to compare directly between each for relative potency.

MPTP-treated and control mice were pretreated with memantine (1, 3, 10 or 30 mg/kg, s.c.); amantadine (1, 3, 10 or 30 mg/kgs.c.); MK-801 (0.01, 0.03, 0.01 or 0.3 mg/kg, s.c.), CGP 40116 (0.003, 0.01,

0.03, 0.1 mg/kg, s.c) or saline immediately before placement in the activity test chambers. L-Dopa (5 mg/kg) or saline was injected after the 60-min period of habituation immediately prior to the 180-min motor activity registration period.

II. Effects of NMDA antagonists in neonatal 6-OHDA rats

Groups of either 6-OHDA-treated or vehicle-treated rats (n = 8 or 9) were injected with either memantine (0.7, 2.0 or 6.0 mg/kg, s.c.), CGP 40116 (0.01, 0.03, 0.1 or 0.3 mg/kg, s.c.), amantadine (2.0, 6.0 or 18.0 mg/kg, s.c.), or MK-801 (0.03, 0.1 or 0.3 mg/kg, s.c.) or saline immediately before placement in rat motor activity test chambers. Parameters of motor activity were registered over 180 min, of which the middle 120-min (from 30 min placement in the test cages to 30 min before removal from test cages) was submitted to analysis.

III. MK-801-induced alterations: Effects of neuroleptic compounds

Locomotion. To test the effects of MK-801 by itself upon motor activity parameters in the rat test chambers, groups of rats (n = 8) were injected MK-801 (0.01, 0.03, 0.1, 0.3 or 1.0 mg/kg, s.c.) or saline 60 min after placement in the activity test chambers (to allow habituation to the chambers) and motor activity was registered over a further 120 min.

Different neuroleptic compounds were injected 40 min after placement in the rat test chambers followed 20 min later by injection of MK-801 and motor activity was then registered over a further 120 min:

Amperozide (0.5 or 2.0 mg/kg, s.c.), haloperidol (0.01 or 0.1 mg/kg, s.c.), raclopride (0.01 or 0.1 mg/kg, s.c.), SCH 23390 (0.005 or 0.05 mg/kg, s.c.), clozapine (5.0 or 10.0 mg/kg, s.c.) or saline were administered 40 min after placement in the test chambers, and followed 20 min later by injection of either MK-801 (0.3 mg/kg, s.c.) or saline then left in the chambers for a further 120 min.

Circular swim maze acquisition. To test the effects of MK-801 by itself upon acquisition performance in the circular swim maze, groups of rats (n = 8) were injected MK-801 (0.01, 0.03 or 0.1 mg/kg, s.c.) 30 min before placement in the swim maze for the first of five trials on test day 1. Acquisition performance following these doses of MK-801 was tested over a further 3 test days.

Different neuroleptic compounds were injected 10 min before injection of MK-801, and 30 min later the rats were placed in the circular swim maze for the first of five trials on each of four test days:

Amperozide (0.5 or 2.0 mg/kg, s.c.), raclopride (0.01 or 0.1 mg/kg, s.c.), SCH 23390 (0.005 or 0.05 mg/kg, s.c.), clozapine (5.0 or 10.0 mg/kg, s.c.) or saline were administered 10 min before MK-801 (0.03 mg/kg, s.c.) or saline, 30 min before testing.

IV. Effects of postnatal MK-801 administration

Newborn male mouse pups were administered either MK-801 (0.5 mg/kg, s.c.) or vehicle (0.9% physiological saline) on postnatal day 11 at 0800, 1600 and 2400 hrs, and replaced with their mothers until weaning at day 25 after birth. Motor activity tests were performed when 70 days of age had been reached. Each mouse was placed in an ADEA test chamber and motor activity was registered over 2 × 30-min test periods after which it was removed from the test chamber injected either d-amphetamine (0.25 mg/kg) or saline s.c. and then replaced in the same test chamber. Motor activity parameters were then registered over a further 90 min (3 × 30-min test periods).

Neurochemical analysis. Mice were killed by cervical dislocation within two weeks of completion of behavioural testing. Determination of DA was performed using an high-performance liquid chromatograph with electrochemical detection (HPLC-EC), according to (Björk et al., 1991), as modified (Liu, 1995). Striatal regions were rapidly dissected out and stored at −80°C until neurochemical analysis. DA concentration was measured as follows: The frozen tissue samples were weighed and homogenized in 1 ml of 0.1 M perchloric acid, and alpha-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12,000 rpm, i.e. 18,600 g, 4°C, 10 min) and filtration, 20 μl of the supernatant was injected into the HPLC-EC to assay DA. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume: 20 μl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 μm), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 μm), and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and a MF-2000 cell) operating at a potential of +0.85V. The mobile phase, ph 2.69, consisted of K_2HPO_4 and citric acid buffer (pH 2.5), 10% methanol, sodium octyl sulphate, 40 mg/l, and EDTA. The flow rate was 1 ml/min, and the temperature of the mobile phase was 35°C.

Statistical analysis. The locomotion, rearing and total activity data for the total test period (120 min) from each of the experiments where each single-drug administration was combined with L-Dopa (either acute 5 mg/kg) in the MPTP experiments and where each compound was tested in the neonatal 6-OHDA experiments were submitted to a one-way ANOVA design in each case (Kirk, 1995). Locomotor counts and latency (secs) to emerge onto the submerged platform data from the neuroleptic + MK-801 experiments were submitted to split-plot ANOVA. Retention quotients and mean latency/test day were submitted to one-way ANOVA. Locomotion, rearing and total activity data from the neonatal MK-801 experiment was submitted to split-plot ANOVA. Pairwise testing between the different treatment groups was performed with the Tukey HSD test (ibid). Scheffé's test was used for nonpairwise comparisons between combinations of means. Dunnett's t-test was used to make comparisons between different dose-groups and saline. The 1% level of significance was maintained throughout, unless where otherwise stated.

Results

I. Acute, subthreshold dose of L-Dopa

Effect of CGP 40116

The co-administration of memantine (0.003, 0.01, 0.03 or 0.1 mg/kg) with L-Dopa (5 mg/kg) induced dose-related stimulatory effects upon locomotor and rearing behaviour in MPTP-treated, but not control mice.

Effect of memantine

The co-administration of memantine (1, 3, 10 or 30 mg/kg) with L-Dopa (5 mg/kg) induced dose-related stimulatory or suppressive effects upon locomotor and rearing behaviour, respectively, in MPTP-treated and control mice (Fig. 1). In hypokinetic MPTP-treated mice, locomotor activity was elevated at 3 mg/kg and rearing at 1 mg/kg. In control mice, locomotion was enhanced at 3, 10 and 30 mg/kg

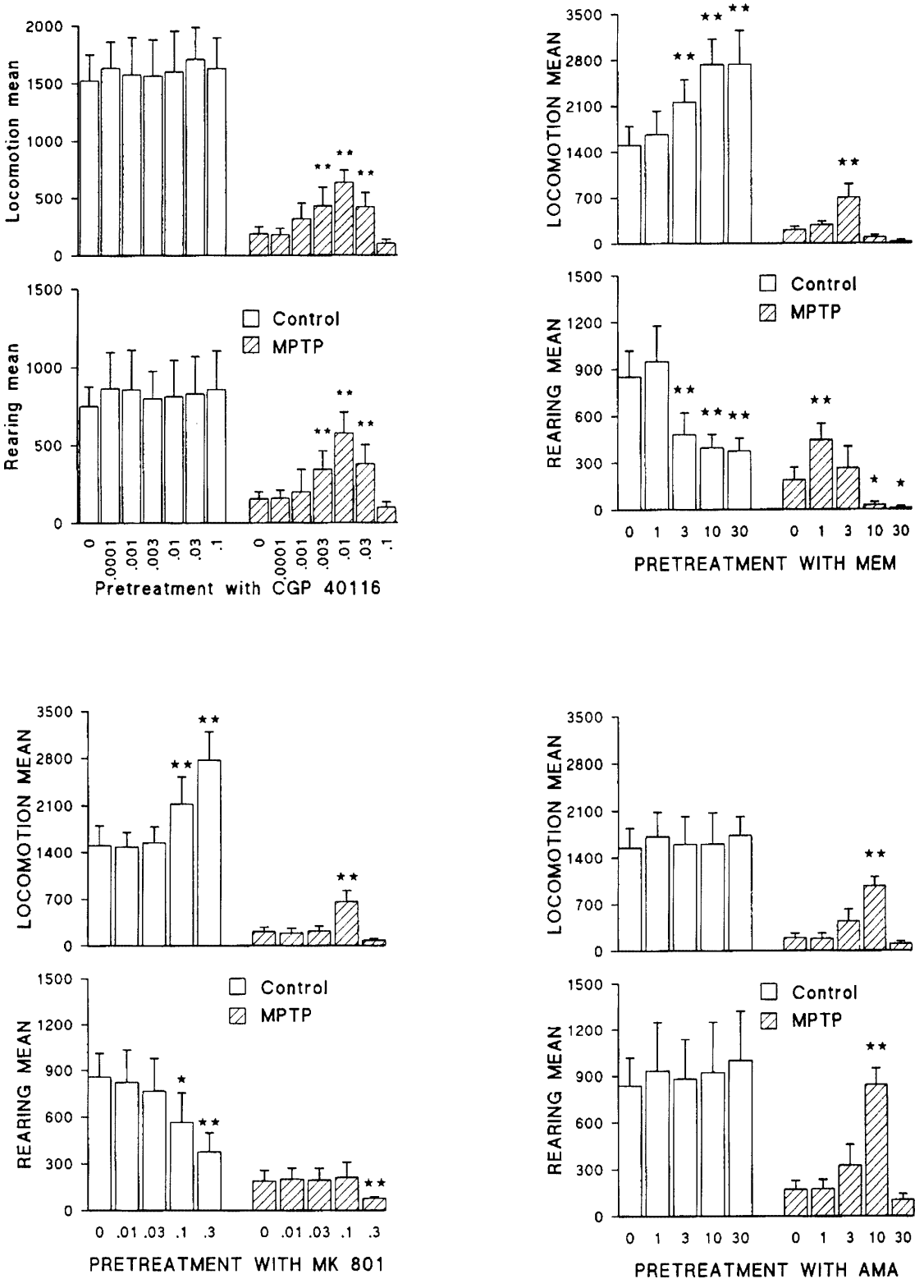

Fig. 1. Effects of the NMDA antagonists, CGP 40116, memantine, MK-801 and amantadine with an acute, subthreshold dose of L-Dopa. Locomotion and rearing counts by MPTP-treated and control mice administered CGP 40116 (0.003, 0.03, 0.01, 0.01 mg/kg, s.c.), memantine (1, 3, 10 or 30 mg/kg, s.c.), MK-801 (0.01, 0.03, 0.10 or 0.30 mg/kg, s.c.), amantadine (1, 3, 10 or 30 mg/kg, s.c.) or saline immediately before placement in the activity test chambers. L-Dopa (5 mg/kg, s.c.) was injected one hr later, i.e. 60 min after the start of activity recording. Each mouse was then tested over a total period of 180 min, as represented by each of the histogram bars. **$p < 0.01$, Tukey HSD tests, versus respective saline group. Values are expressed as means ± SD of 12 mice from the final 180 min

whereas rearing was suppressed at those doses. Thus, one-way ANOVA indicated significant Between-Groups effects for both Locomotion: $F(9, 110) = 176.51$, $p < 0.0001$, and Rearing: $F(9, 110) = 77.48$, $p < 0.0001$. Figure 1 presents the locomotion and rearing counts of MPTP-treated and control mice administered doses of memantine or saline, and L-Dopa at motor astivity testing. Tukey HSD testing for pairwise differences between groups indicated the following differences: In the MPTP-treated mice, co-administration of memantine (3 mg/kg) with L-Dopa (5 mg/kg) significantly enhanced locomotion whereas the 1 mg/kg dose + L-Dopa enhanced rearing, in comparison with the respective saline + L-Dopa group. At the same time, combination of memantine (either 10 or 30 mg/kg) with L-Dopa reduced rearing compared to the respective saline + L-Dopa group. In control, not MPTP treated mice, memantine (3, 10 or 30 mg/kg) + L-Dopa dose-dependently elevated locomotor activity and reduced rearing (exploratory) activity (see Fig. 1, top right-hand panel).

Effect of Amantadine

The co-administration of amantadine (1, 3, 10 or 30 mg/kg) with L-Dopa (5 mg/kg) induced a single dose facilitatory effect (10 mg/kg) upon locomotor and rearing behaviour in the MPTP-treated mice (Fig. 2), but no effects upon the motor behavior of control mice. Thus, one-way ANOVA indicated significant Between-Groups effects for both Locomotion: $F(9, 110) = 79.74$, $p < 0.0001$, and Rearing: $F(9, 110) = 37.32$, $p < 0.0001$. Figure 2 presents the locomotion and rearing counts of MPTP-treated and control mice administered doses of amantadine or saline, and L-Dopa at motor activity testing. Tukey HSD testing for pairwise differences between groups indicated that the MPTP-treated group administered amantadine (10 mg/kg) + L-Dopa performed significantly more locomotion and rearing counts than the MPTP-treated saline + L-Dopa group (see Figure 1, bottom left-hand panel). No differences were obtained in the control mice (see also Table 1).

Table 1. Effects of acute administration of either memantine (0.3, 1, 3 or 10 mg/kg, s.c.), amantadine (1, 3, 10 or 30 mg/kg), MK-801 (0.03, 0.1, 0.3 or 1 mg/kg, s.c.), or L-Dopa (5 mg/kg, s.c.) upon the motor activity of MPTP-treated and control mice. Locomotion and rearing counts after administration of either the noncompetitive NMDA antagonist agents (first 120 min after placement in the test cages) or L-Dopa (second 120 min after placement in the test cages). Memantine, amantadine and L-Dopa were injected immediately prior to placement in the activity test chambers. L-Dopa was injected after each mouse had spent 60 min (habituation) in the test chamber, and then immediately replaced. The 120-min period of activity measurement immediately after administration, in each case, was registered for each compound

Group		MPTP		Control	
		Locomotion	Rearing	Locomotion	Rearing
Saline[2]	(2 ml/kg) mg/kg	258 ± 106	205 ± 85	1567 ± 213	784 ± 141
CGP40116	0.003	271 ± 54	199 ± 33	1709 ± 201	812 ± 121
	0.01	256 ± 48	232 ± 58	1661 ± 261	779 ± 190
	0.03	266 ± 72	193 ± 67	1688 ± 171	808 ± 234
	0.10	239 ± 85	225 ± 61	1561 ± 134	791 ± 188
MK-801	0.03	265 ± 34	152 ± 32	1309 ± 227	975 ± 219
	0.10	378 ± 43	149 ± 41	1594 ± 323	769 ± 191
	0.30	149 ± 54	152 ± 48	2818 ± 296**	511 ± 249*
	1.00	96 ± 52**	91 ± 39**	3679 ± 303**	172 ± 288*
Amantadine	1	287 ± 37	191 ± 35	1601 ± 233	774 ± 173
	3	265 ± 41	180 ± 26	1398 ± 304	918 ± 222
	10	238 ± 43	161 ± 29	1588 ± 264	867 ± 184
	30	281 ± 39	149 ± 44	1473 ± 177	809 ± 162
Memantine	0.3	277 ± 46	181 ± 44	1473 ± 196	901 ± 217
	1	264 ± 51	177 ± 38	1525 ± 285	834 ± 277
	3	296 ± 39	94 ± 31**	1683 ± 163	781 ± 174
	10	271 ± 37	76 ± 43**	1897 ± 133**	472 ± 145**
L-Dopa	0[1]	213 ± 61	102 ± 54	1167 ± 213	684 ± 181
	5	201 ± 62	111 ± 48	1188 ± 196	659 ± 153

** $p < 0.01$, * $p < 0.05$, Tukey HSD tests, versus saline group. Values represent means ± SD of $n = 9$ mice. [1] Saline injected after 60-min habituation to test chambers. [2] Saline injected immediately prior to placement in the test chambers.

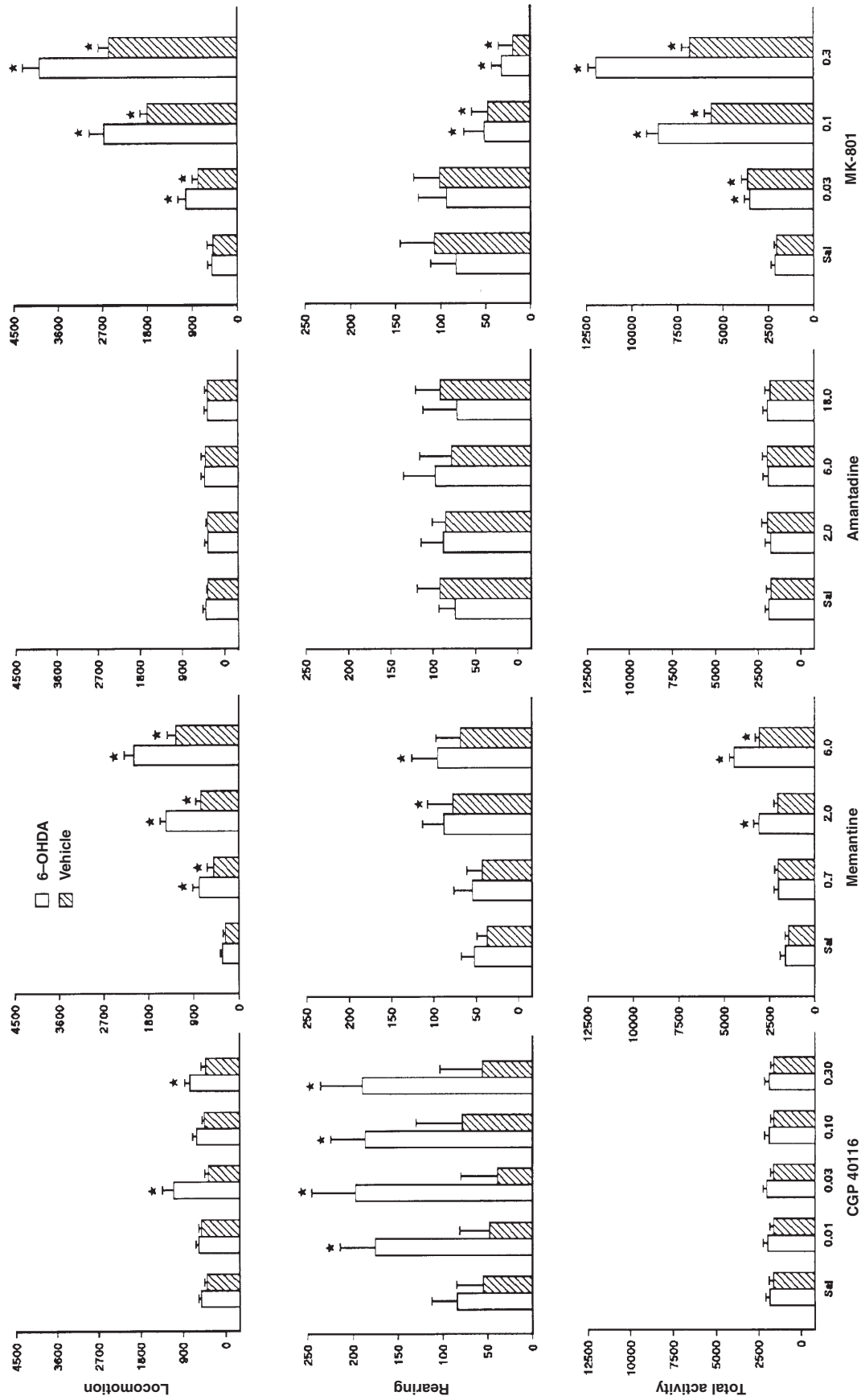

Fig. 2. Mean locomotion, rearing and total activity counts of neonatal 6-OHDA-treated and vehicle-treated rats administered either CGP 40116 (0.01, 0.03, 0.1 or 0.3 mg/kg), memantine (0.7, 2.0 or 6.0 mg/kg), amantadine (2.0, 6.0 or 18.0 mg/kg) or MK-801 (0.03, 0.1 or 0.3 mg/kg) or saline acutely. The rat pups were injected 6-OHDA (75 μg) intracisternally on Day 1 or 2 after birth, 30 min after i.p. injection of desipramine (25 mg/kg, s.c.). Vehicle-treated rats received desipramine injection followed by vehicle (0.9% physiological saline with 0.05 mg ascorbic acid). Values are expressed as means ± s.e.m. of 8 or 9 rats from the final 120 min

Effect of MK-801

The co-administration of MK-801 (0.01, 0.03, 0.1 or 0.3 mg/kg) with L-Dopa (5 mg/kg) induced a single dose facilitatory effect upon locomotor (0.1 mg/kg) and reduced rearing (0.3 mg/kg) behaviour in the MPTP-treated mice (Fig. 3). In control mice, locomotion was enhanced at 0.1 and 0.3 mg/kg whereas rearing was suppressed at those doses. Thus, one-way ANOVA indicated significant between-groups effects for both locomotion: $F(9, 110) = 146.77$, $p < 0.0001$, and Rearing: $F(9, 110) = 87.43$, $p < 0.0001$. Figure 3 presents the locomotion and rearing counts of MPTP-treated and control mice administered doses of MK-801 or saline, and L-Dopa at motor activity testing. Tukey HSD testing indicated that the MPTP-treated mice administered MK-801 (0.1 mg/kg) + L-Dopa (5 mg/kg) performed significantly more locomotion whereas the 0.3 mg/kg dose reduced rearing counts (see Figure 1, bottom right-hand panel). The only effects evident in the control mice were shown at the highest doses (0.1 and 0.3 mg/kg) of the compound that increased locomotion and reduced rearing behaviour, respectively (see also Table 1, below).

Effect of each compound by itself in MPTP and control mice

The effects of MK-801, amantadine, memantine, L-Dopa and saline upon the motor activity of MPTP-treated and control mice are presented in Table 1.

Note the complete lack of any effect of L-Dopa (5 mg/kg), by itself, in these mice. Amantadine, at the doses administered, did not affect the motor activity of MPTP-treated and control mice whereas both memantine and MK-801 did so at the highest doses employed.

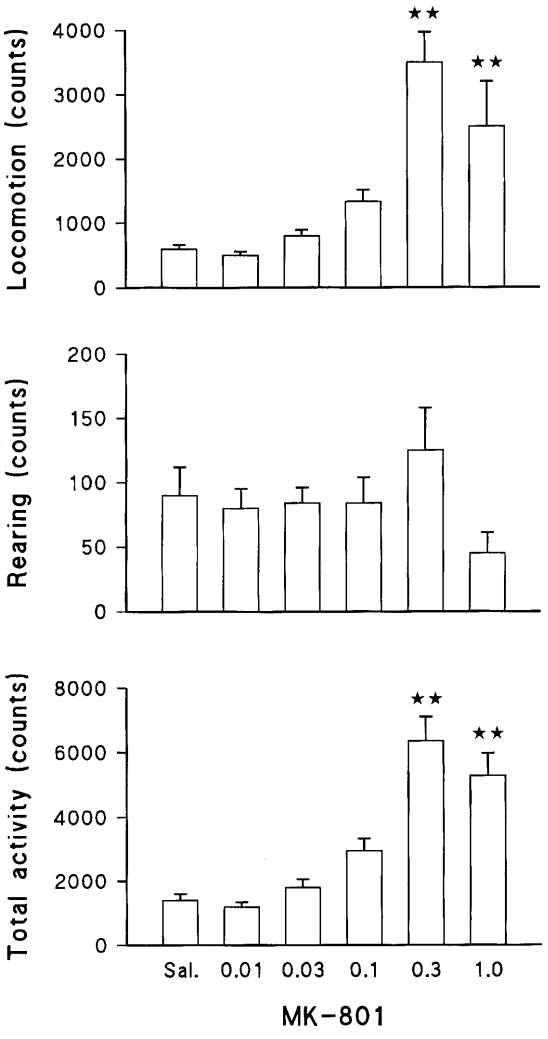

Fig. 3. Mean locomotion, rearing and total activity counts by groups of rats administered either MK-801 at the specified doses (0.01, 0.03, 0.1, 0.3 or 1.0 mg/kg) or saline following a 60-min habituation to the test chambers. MK-801 was injected 30 min prior to placement in the activity test chambers. Activity parameters were registered over 60 min (after habituation. Values are expressed as means ± SD of 8 rats. **$p < 0.01$, versus saline, Dunnett's t-test

II. Effects of NMDA antagonists in neonatal 6-OHDA rats

Effects of CGP 40116

Acute administration of CGP 40116 (0.03 and 0.3 mg/kg) increased locomotion and rearing activity in 6-OHDA-treated but not vehicle-treated mice; rearing activity was elevated even at the 0.01 and 0.1 mg/kg doses. Locomotion (0.03, 0.1 and 0.3 mg/kg) and rearing (0.01, 0.03, 0.1 and 0.3 mg/kg) activity in 6-OHDA rats was elevated above that of the vehicle rats. One-way ANOVA indicated significant Between-Groups effects for Locomotion: $F(9, 80) = 15.23$, Rearing: $F(9, 80) = 22.55$, Total activity: $F(9, 80) = 4.60$. Figure 2 presents the mean locomotion, rearing and total activity counts of neonatal 6-OHDA-treated and vehicle-treated rats administered either CGP 40116, memantine, amantadine or MK-801 or saline acutely.

Tukey HSD-testing indicated the following pairwise differences:

Locomotion: 6-OHDA-0.03,-0.3 < vehicle-0.03,-0.3.
Rearing: 6-OHDA-0.01,-0.03,-0.1,-0.3 > vehicle-0.01,-0.03,-0.1,-0.3.

Total activity: all 6-OHDA groups combined > all vehicle groups combined.
Dunnett's t-test indicated the following differences between dose groups and saline:
Locomtion: saline < 0.3 < 0.03. Rearing: saline < 0.01, 0.03, 0.1, 0.3. All in 6-OHDA-treated rats.

Effects of memantine

Neonatal 6-OHDA treatment potentiated the dose-dependent increments induced by memantine upon locomotor and total activity. One-way ANOVA indicated significant Between-Groups effects for Locomotion: $F(9, 80) = 27.73$, Rearing: $F(9, 80) = 3.24$, Total activity: $F(9, 80) = 19.65$. Tukey HSD-testing indicated the following differences: Locomotion: 6-OHDA-2.0 > vehicle-6.0: 6-OHDA-6.0 > vehicle-6.0 Total activity: 6-OHDA-2.0 > vehicle-6.0: 6-OHDA-6.0 > vehicle-6.0 Dunnett's test indicated saline < 0.7 < 2.0 < 6.0 for locomotion and total activity in both 6-OHDA and vehicle rats (see Figure 2, top right-hand panel).

Effects of MK-801

Neonatal 6-OHDA treatment potentiated the dose-dependent increments induced by MK-801 upon locomotor and total activity. Rearing was decreased in a dose-dependent manner in both 6-OHDA and vehicle rats. One-way ANOVA indicated significant Between-Groups effects for Locomotion: $F(9, 80) = 63.19$, Rearing: $F(9, 80) = 7.51$, Total activity: $F(9, 80) = 69.83$. Tukey HSD-testing indicated the following differences: Locomotion and total activity: 6-OHDA-0.1 > vehicle-0.1: 6-OHDA-0.3 > vehicle-0.3 Dunnett's test indicated saline < 0.003 < 0.1 < 0.3 for locomotion and total activity in both 6-OHDA and vehicle (see Figure 2, bottom right-hand panel). For rearing behaviour, saline > 0.3 in both 6-OHDA and vehicle rats.

Effects of Amantadine

No dose-dependent or other effects of acute amantadine and/or postnatal 6-OHDA were obtained. There were no significant effects of ANOVA.

Figure 2 (bottom, left-hand panel) presents locomotion, rearing and total activity of neonatal 6-OHDA and vehicle rats administered amantadine.

III. MK-801-induced alterations: Effects of neuroleptic compounds

Locomotion

Acute injection of MK-801 (0.01 to 1.0 mg/kg) increased locomotor and total activity but not rearing in the motor activity test cages. One-way ANOVA indicated significant Between-Groups for both Locomotion: $F(5, 42) = 16.54$, and Total activity: $F(5, 42) = 15.92$, but not rearing. Figure 3 presents mean locomotion, rearing and total activity counts by groups of rats administered either MK-801 at the specified doses of saline.

Tukey HSD-testing indicated significantly more locomotion and total activity by the 0.3 and 1.0 mg/kg dose groups.

Prior treatment with neuroleptic compounds differentially affected the psychostimulatory effects of MK-801 (0.3 mg/kg): Clozapine antagonised the effects of MK-801, as did SCH 23390 whereas raclopride and haloperidol induced a dose-specific antagonism at the higher doses of 0.1 mg/kg. Amperozide potentiated the effecs of MK-801 at the higher dose (2.0 mg/kg) but attenuated its stimulatory effect at the lower dose (0.5 mg/kg). One-way ANOVA indicated significant effects for Clozapine: $F(5, 42) = 148.22$, SCH 23390: $F(5, 42) = 169.54$, Raclopride: $F(5, 42) = 161.74$, Haloperidol: $F(5, 42) = 133.61$, and Amperozide: $F(5, 42) = 179.82$.

Figure 4 presents the mean locomotion counts of groups of rats administered MK-801 or saline after clozapine, SCH 23390, raclopride, haloperidol, amperozide or saline over a 120-min interval.

Tukey HSD-testing indicated the following pattern of differences:
Clozapine: cloz.(10) + MK(0.3) < cloz.(5) + MK(0.3) < MK(0.3)
SCH 23390: SCH(0.05) + MK(0.3) < SCH(0.005) + MK(0.3) < MK(0.3)
Raclopride: rac(0.1) + MK(0.3) < MK(0.3)
Haloperidol: hal(0.1) + MK(0.3) < MK(0.3)
Amperozide: amp(0.5) + MK(0.3) < MK(0.3) < amp(2) + MK(0.3)
Amp(0.5) > saline

Circular swim maze acquisition

Acute injection of MK-801 (0.01, 0.03 or 1.0 mg/kg, s.c.) caused a dose-dependent disruption of acqui-

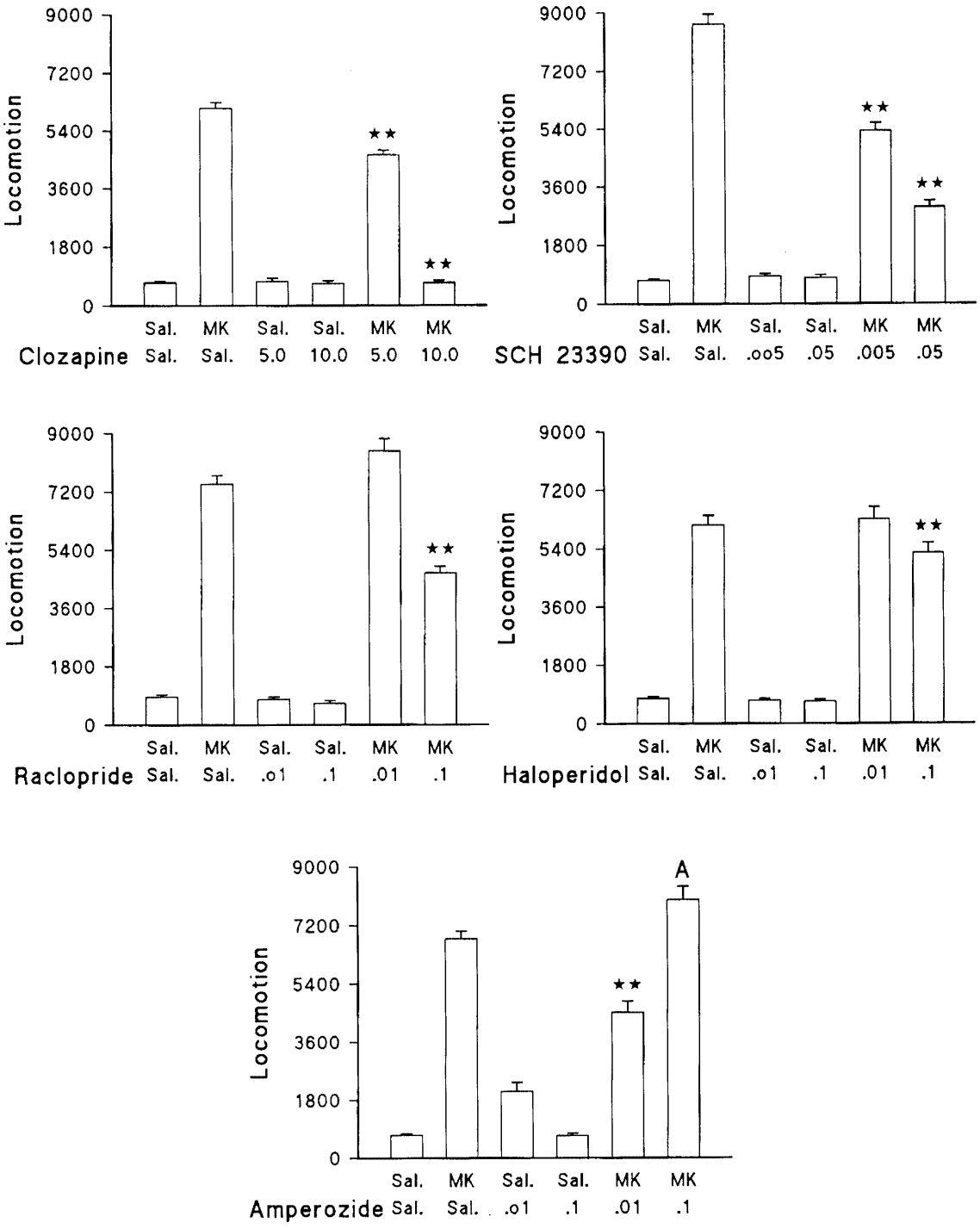

Fig. 4. Mean locomotion counts of groups of rats administered MK-801 (0.3 mg/kg, s.c.) or saline 20 min after administration of clozapine (5.0 or 10.0 mg/kg, s.c.), SCH 23390 (0.005 or 0.05 mg/kg, s.c.), raclopride (0.01 or 0.1 mg/kg, s.c.), haloperidol (0.01 or 0.1 mg/kg, s.c.), amperozide (0.5 or 2.0 mg/kg, s.c.) or saline injected 40 min after placement in the activity test chambers. Motor activity parameters were registered then over a 120-min interval. Values are expressed as means ± SD of 8 rats. **$p < 0.01$, versus MK-801, Tukey HSD-testing. A$p < 0.01$, more than MK-801, Tukey HSD-testing

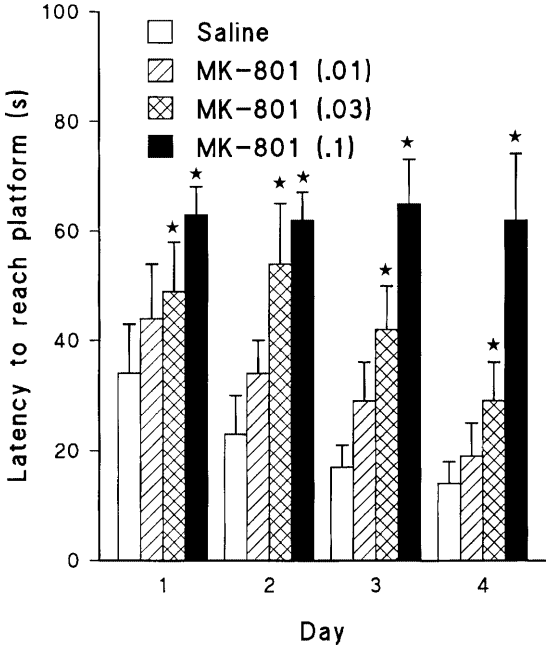

Fig. 5. Mean latencies to emerge onto the submerged platform by groups of rats administered either MK-801 at the specified doses (0.01, 0.03, 0.1, 0.3 or 1.0 mg/kg) or saline following placement at one side (constant) of the circular swim maze for the 1st trial of five trials during test day 1, 30 min after injection. Acquisition performance following these doses of MK-801 was tested over a further 3 test days. Values are expressed as means ± SD of 8 rats. *$p < 0.01$, versus saline, Dunnett's t-test

sitive performance over test days 1 to 4 in the circular swim maze. Split-plot ANOVA indicated a significant Groups main effect for latency to emerge onto the submerged platform: $F(3, 28) = 25.18$. Figure 5 presents the latencies to emerge onto the platform by different groups administered MK-801.

Dunnett's t-testing for comparison of dose-groups against saline indicated the following pairwise differences: 0.01, saline < 0.03 < 0.1; 0.01 vs 0.03, not significant.

Retention quotients: Retention quotients pertaining to "latency to emerge onto the submerged platform" from Testing Day 1 to Testing Day 4 in the circular swim maze were derived by dividing the latency (in seconds), for each rat, during the first Testing Day by that obtained during the fourth Testing Day and multiplying the result by 100.

Mean latency to reach submerged platform/test trial over all four test days were calculated. Thus, the obtained retention quotients for acquisition performance

Table 2. Retention quotients and mean latency/test day for different dose-groups administered MK-801 acutely 30 min prior to placement in the circular swim maze for the 1st trial on the 1st of four test days

Groups	Retention quotient	Mean latency
Saline	279 ± 27	23.2 ± 9
0.01	231 ± 18*	31.5 ± 8
0.03	168 ± 23*	43.5 ± 11*
0.1	101 ± 17*	63.0 ± 16*

Retention quotients pertaining to "latency to emerge onto the submerged platform" from Testing Day 1 to Testing Day 4 in the radial arm maze were derived by dividing the latency (in seconds), for each rat, during the first Testing Day by that obtained during the fourth Testing Day and multiplying the result by 100. * $p < 0.01$, Dunnett's t-test, versus saline group

in the swim maze as well as mean latency per test day were subjected to one-way ANOVA that indicated significant Between-Groups effects: Retention quotient: $F(3, 28) = 19.88$; mean latency: $F(3, 28) = 7.63$. Table 2 presents the retention quotients and mean latency/test day for different groups administered MK-801. Dunnett's t-tests indicated the following differences:

Retention quotients: saline > 0.01 > 0.03 > 0.1
Mean latency/test day: 0.01, saline < 0.03 < 0.1

Prior treatment with neuroleptic compounds differentially affected the disruptive effects effects of MK-801 (0.3 mg/kg) upon acquisition performance in the circular maze. Clozapine and amperozide, at the higher doses (10.0 and 2.0 mg/kg, respectively) potentiated the MK-801-induced impairment whereas raclopride (0.01 mg/kg) blocked and SCH 23390 (0.05 mg/kg) just failed to attenuate it significantly. Split-plot ANOVA indicated significant Groups × test days interaction for amperozide: $F(15, 126) = 9.53$, but significant Groups main effects for clozapine: $F(5, 42) = 11.72$, SCH 23390: $F(5, 42) = 9.83$, and raclopride: $F(5, 42) = 13.45$. Figure 6 presents the mean latency/test day over all four days of testing to emerge onto the submerged platform groups of rats administered either clozapine (3.0 or 10.0 mg/kg), SCH 23390 (0.005 or 0.05 mg/kg), raclopride (0.01 mg/kg), amperozide (0.5 or 2.0 mg/kg) or saline 10 min before injection of MK-801 (0.03 mg/kg).

Tukey HSD-testing indicated the following significant differences [latency date] pertainent to the experiment:

Clozapine: saline, MK-801 < cloz(10) + MK
Raclopride: saline, rac(.01) + MK < MK-801
SCH 23390: saline < MK-801, SCH(.005) + MK
Amperozide: saline, MK-801 < amp(2) + MK

Scheffé's nonpairwise tests comparing the combined means of: (i) SCH(.05) and saline with that of MK-801, (ii) cloz(3) + MK and cloz(10) + MK with that of MK-801, and (iii) amp(.5) + MK-801 and amp(2) + MK, in each respective case, indicated significant differences.

Retention quotients: Retention quotients pertaining to "latency to emerge onto the submerged platform" from Testing Day 1 to Testing Day 4 in the circular swim maze and mean latency to reach submerged platform/test trial over all four test days were calculated for groups of rats administered either clozapine (3.0 or 10.0 mg/kg), SCH 23390 (0.005 or 0.05 mg/kg), raclopride (0.01 mg/kg), amperozide (0.5 or 2.0 mg/kg) or saline 10 min before injection of MK-801 (0.03 mg/kg). Thus, the obtained retention quotients for acquisition performance in the swim maze as well as mean

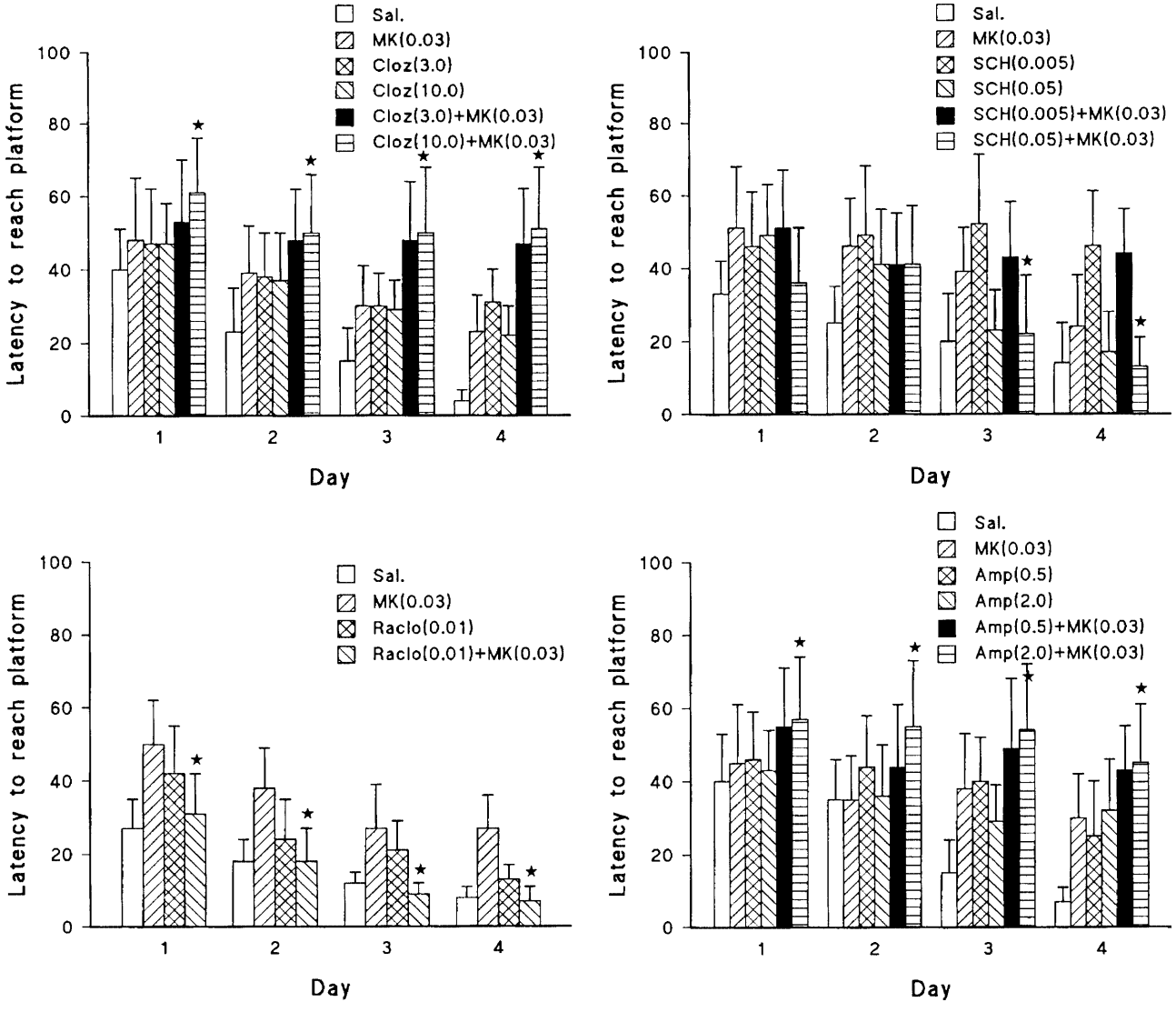

Fig. 6. Figure 6 presents the mean latency/test day over all four days of testing to emerge onto the submerged platform groups of rats administered either clozapine (3.0 or 10.0 mg/kg), SCH 23390 (0.005 or 0.05 mg/kg), raclopride (0.01 mg/kg), amperozide (0.5 or 2.0 mg/kg) or saline 10 min before injection of MK-801 (0.03 mg/kg) which was administered 20 min before placement at one side (constant) of the circular swim maze for the 1st trial of five trials during test day 1. Acquisition performance following these doses of the neuroleptic compounds followed by MK-801 was tested over a further 3 test days, with 5 trials/test day. Values are expressed as means ± SD of 8 rats. *$p < 0.01$, versus MK-801, Tukey HSD-testing. $^{A}p < 0.01$, combined versus MK-801, Scheffé's nonpairwise test

Table 3. Retention quotients and mean latency/test day over all four days of testing to emerge onto the submerged platform groups of rats administered either clozapine (3.0 or 10.0 mg/kg), SCH 23390 (0.005 or 0.05 mg/kg), raclopride (0.01 mg/kg), amperozide (0.5 or 2.0 mg/kg) or saline 10 min before injection of MK-801 (0.03 mg/kg) then placed in the circular swim maze for the 1st of five trials on each test day. The same treatment procedure was maintained on each test day

Groups:	Saline	MK-801	Cloz(3)	Cloz(10)	Cloz3 + MK	Cloz10 + MK
Retention Q.	444 ± 53	209 ± 41**	152 ± 40**	213 ± 45**	113 ± 42^{A}	120 ± 36^{A}
Mean laten.	21.7 ± 7.0	35.0 ± 10.2	36.5 ± 9.1	33.8 ± 8.0	49.0 ± 12.1^{o}	53.0 ± 8.6^{o,C}
	saline	MK-801	SCH(.005)	SCH(.05)	SCH.005 + MK	SCH.05 + MK
Retention Q.	236 ± 34	212 ± 29	100 ± 48*	288 ± 51	115 ± 41^{A}	277 ± 45
Mean laten.	23.0 ± 8.6	40.0 ± 9.2*	48.3 ± 13.6	32.5 ± 10.2	44.8 ± 10.3	28.0 ± 7.8
	saline	MK-801	Rac.(.01)		Rac.01 + MK	
Retention Q.	338 ± 24	185 ± 37**	323 ± 36		443 ± 47^{B}	
Mean laten.	16.2 ± 4.1	35.5 ± 8.5*	25.0 ± 7.1		16.2 ± 5.7^{D}	
	saline	MK-801	Amp(.5)	Amp(2)	Amp.5 + MK	Amp2 + MK
Retention Q.	571 ± 37	150 ± 55**	184 ± 54**	134 ± 49**	128 ± 46**	127 ± 43**
Mean laten.	23.7 ± 7.4	37.0 ± 11.0	38.8 ± 11.1	35.0 ± 9.8	47.8 ± 11.2^{o}	52.8 ± 9.6^{o,C}

Values represent means ± SD of 8 rats. Retention quotients pertaining to "latency to emerge onto the submerged platform" from Testing Day 1 to Testing Day 4 in the radial arm maze were derived by dividing the latency (in seconds), for each rat during the first Testing Day by that obtained during the fourth Testing Day and multiplying the result by 100

* $p < 0.05$, ** $p < 0.01$ versus saline group, Tukey HSD tests; ^{A} $p < 0.01$, RQ: less than MK-801, Latency: ^{C} $p < 0.01$, more than MK-801; ^{B} $p < 0.01$, RQ: more than MK-801, Latency: ^{D} $p < 0.01$, less than MK-801; ^{O} $p < 0.01$, combined means versus MK-801, Scheffé's nonpairwise tests.

latency per test day were subjected to one-way ANOVA that indicated significant Between-Groups effects: Retention quotient: $F(5, 42) = 16.18$; mean latency: $F(5, 42) = 13.27$. Table 3 presents the retention quotients and mean latency/test day for different groups administered MK-801 10 min after administration of either clozapine, raclopride, SCH 23390 or amperozide.

Tukey HSD-testing indicated the following differences:

Retention Quotients:

Clozapine: Saline > MK, Cloz(3), Cloz(10). MK > Cloz(3) + MK, Cloz(10) + MK.

SCH 23390: Saline > SCH(.005), SCH(.005) + MK. MK > SCH(.005) + MK.

Raclopride: Saline > MK < Rac(.01) + MK

Amperozide: Saline > MK, Amp(.5), Amp(2), Amp(.5) + MK, Amp(2) + MK.

Mean latency per test day:

Clozapine: Saline < Cloz(3) + MK, Cloz(10) + MK > MK

SCH 23390: Saline < MK, SCH(.005), SCH(.005) + MK

Raclopride: Saline < MK > Rac(.01) + MK

Amperozide: Saline, MK < Amp(2) + MK.

IV. Effects of postnatal MK-801 administration

Mice postnatally administered MK-801 (0.5 mg/kg) showed hypoactivity during the 1st 30-min test period and hyperactivity during the 2nd 30-min test period. MK-801-treated mice administered d-amphetamine (0.25 mg/kg) showed markedly reduced levels of motor activity compared to MK-801 mice administered saline and vehicle-treated mice administered d-amphetamine, and was in fact comparable to vehicle mice injected saline. Split-plot ANOVA indicated significant Treatment Groups × Test periods interaction effects for Locomotion: $F(12, 144) = 68.52$, Rearing: $F(12, 144) = 26.35$, and Total activity: $F(12, 144) = 51.51$. Figure 7 presents the mean locomotion, rearing and total activity counts by mice injected MK-801 or vehicle neonatally and d-amphetamine or saline 60 min after placement in the activity test chambers during testing at 70 days of age.

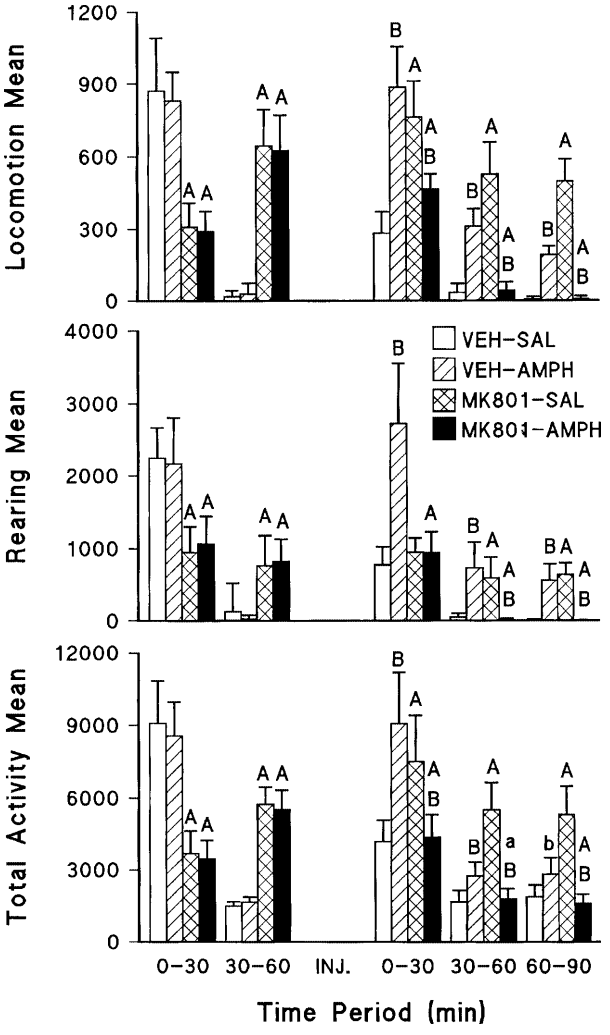

Fig. 7. Effects on neonatal administration of MK-801 upon motor activity of adult mice. Mean locomotion, rearing and total activity counts over successive 30-min test periods, before and after administration of d-amphetamine or saline. Newborn male mouse pups were administered either MK-801 (0.5 mg/kg, s.c.) or vehicle (0.9% physiological saline) on postnatal day 11 at 0800, 1600 and 2400 hrs, and replaced with their mothers until weaning at day 25 after birth. Motor activity tests were performed when 70 days of age had been reached. Motor activity parameters were registered over 2 × 30-min test periods after which each mouse was removed from the test chamber, injected either d-amphetamine (0.25 mg/kg) or saline s.c. and then replaced in the same test chamber. Values represent means ± SD of 10 mice. $^{A}p < 0.01$, $^{a}p < 0.05$, versus respective vehicle-treated (postnatal) group, Tukey HSD tests. $^{B}p < 0.01$, $^{b}p < 0.05$, versus respective saline-injected (acute) group, Tukey HSD tests

Tukey HSD-testing indicated the following pairwise differences:

Locomotion, rearing and total activity:

1st 30-min period: veh-sal, veh-amph > MK801-sal, MK801-amph

2nd 30-min period: veh-sal, veh-amph < MK801-sal, MK801-amph

Locomotion and total activity:

3rd 30-min period: veh-amph, MK801-sal > MK801-amph, veh-sal

4th and 5th 30-min periods: similar to 3rd, except that MK801-sal > veh-amph.

Rearing:

3rd 30-min period: veh-amph > MK801-sal, MK801-amph, veh-sal

4th and 5th 30-min periods: veh-amph, MK801-sal > veh-sal, MK801-amph.

Habituation to activity test chambers

Habituation is a relatively simple, nonassociative form of learning in situations where repeated measures of behaviour are monitored. In order to access the extent of habituation to the activity test chambers over each successive 30-min test period (1st period versus 2nd period; then after d-amphetamine, 3rd period versus 5th period) of testing over all five test periods, an habituation quotient for each rat was derived by dividing the numbers of counts (locomotion/rearing/total activity) during the 1st 30-min test period by that obtained during the 2nd 30-min test period, and dividing the number of counts obtained during the 3rd 30-min test period by that obtained during the 5th 30-min test period; the quotients referred to as Quotient[1] and Quotient[2], respectively. In each case the result of each division was multiplied by 100 to provide a quotient representing the reduction of activity counts from the first to the fifth day of testing for each rat (cf. Archer and Fredriksson, 2000; Fredriksson et al., 1992, 1999). Habituation quotients of 100 or less indicate the absence of any habituation. Data from Quotient[1] and Quotient[2] were submitted to one-way ANOVA and indicated significant Between-Groups effects:

Quotient[1]: Locomotion, rearing and total activity: $F(3, 36) > 7.53$

Quotient[2]: Locomotion, rearing and total activity: $F(3, 36) > 4.94$.

Table 4 presents the habituation quotients from the pre-amphetamine and post-amphetamine 30-min test periods for veh-saline, veh-amph, MK801-saline and MK801-amph treated mice.

Tukey HSD-testing indicated the following pairwise differences:

Quotient[1]:

Locomotion: Veh-saline > Veh-amph > MK801-saline, MK801-amph

Table 4. Habituation quotients of neonatal MK-801-treated and vehicle-treated mice for locomotion, rearing and total activity counts over successive 30-min test periods, before and after administration of d-amphetamine or saline. Newborn male mouse pups were administered either MK-801 (0.5 mg/kg, s.c.) or vehicle (0.9% physiological saline) on postnatal day 11 at 0800, 1600 and 2400 hrs, and replaced with their mothers until weaning at day 25 after birth. Motor activity tests were performed when 70 days of age had been reached. Motor activity parameters were registered over 2 × 30-min test periods after which each mouse was removed from the test chamber, injected either d-amphetamine (0.25 mg/kg) or saline s.c. and then replaced in the same test chamber

Groups	Quotient[1]			Quotient[2]		
	Locomotion	Rearing	T.Activ.	Locomotion	Rearing	T.Activ.
Veh.-saline	5118 ± 952	1725 ± 1088	610 ± 108	4014 ± 1128	11086 ± 2097	223 ± 106
Veh.-amph.	2964 ± 967^{o}	8022 ± 1916^{o}	546 ± 97	460 ± 211^{B}	489 ± 173^{B}	322 ± 162
MK801-saline	48 ± 59*	123 ± 52*	64 ± 39*	153 ± 96^{B}	148 ± 74^{B}	141 ± 61^{B}
MK801-amph.	46 ± 45*	128 ± 67*	62 ± 62*	5211 ± 1518^{A}	23650 ± 3363^{A}	271 ± 119^{A}

Values represent means ± s.e.m. of 10 mice
The extent of habituation to the activity test chambers was assessed over each successive 30-min test period (1st period versus 2nd period; then after d-amphetamine, 3rd period versus 5th period) of testing over all five test periods. An habituation quotient for each rat was derived by dividing the numbers of counts (locomotion/rearing/total activity) during the 1st 30-min test period by that obtained during the 2nd 30-min test period, and dividing the number of counts obtained during the 3rd 30-min test period by that obtained during the 5th 30-min test period; the quotients referred to as Quotient[1] and Quotient[2], respectively. In each case the result of each division was multiplied by 100 to provide a quotient representing the reduction of activity counts from the first to the fifth day of testing for each rat (cf. Archer and Fredriksson, 2000; Fredriksson et al., 1992, 1999). Habituation quotients of 100 or less indicate the absence of habituation. * $p < 0.01$, versus Veh.-saline; ^{o} $p < 0.01$, versus Veh.-saline, Tukey HSD tests. ^{A} $p < 0.01$, versus MK801-saline; ^{B} $p < 0.01$, Veh.-saline, Tukey HSD tests.

Rearing: Veh-amph > Veh-saline > MK801-saline, MK801-amph
Total activity: Veh-saline, Veh-amph > MK801-saline, MK801-amph
Quotient[2]:
Locomotion: MK801-amph, Veh-saline > Veh-amph > MK801-saline
Rearing: MK801-amph > Veh-saline > Veh-amph > MK801-saline
Total activity: Veh-amph, MK801-amph, Veh-sal > MK801-saline.

Neurochemical analysis

MPTP-treated mice tested in co-administrations of the above compounds + L-Dopa showed severe depletions of DA in the mouse striatum (% of saline group) in comparison with control mice (see Table 5). Neonatal 6-OHDA (75 ug) induced significant DA depletion in the rat striatum (% of vehicle).

Table 5. Striatal dopamine (DA) concentrations following treatment with either MPTP (2 × 40 mg/kg, s.c.), administered on two occasions separated by a 24-hr interval, of mice tested in the chronic, suprathreshold L-Dopa dose tests, or mice injected saline throughout the period of chronic administration and then injected saline at testing (Saline + Saline). MPTP-treated L-Dopa-tolerant mice were randomly assigned to neurochemical determination. Striatal dopamine (DA) concentrations of groups of rats treated neonatally with either 6-OHDA (75 ug in a volume of 10 ul vehicle, i.c.) or vehicle (0.9% physiological saline containing 0.1% ascorbic acid) administered on days 1 or 2 after birth (30 min after DMI 25 mg/kg, s.c.). (μg/g wet weight of tissue)

	MPTP	Saline
Dopamine (%)	4.09 ± 0.75* (21)	19.14 ± 0.95
	6-OHDA	Vehicle
Dopamine (%)	2.87 ± 0.66* (29)	9.74 ± 0.52

Values represent means ± s.e.m. of 6 mice or 6 rats. * $p < 0.01$, Student's t-test. () = percent of control values.

Discussion

The results presented above may be summarised as follows:

For the experiments employing the acute, subthreshold dose of L-Dopa (5 mg/kg) procedure in hypokinesic MPTP mice:

(1) CGP 40116 (mg/kg) increased both locomotion and rearing in co-administration with L-Dopa. (2) Both memantine (1 [rearing] and 3 [locomotion] mg/kg) and amantadine (10 mg/kg) co-administered with L-Dopa to hypokinesic MPTP-treated mice elevated motor activity. Memantine (10 and 30 mg/kg) + L-Dopa reduced further the rearing behaviour of the hypokinesic MPTP-treated mice. (3) Memantine (3, 10

and 30 mg/kg) + L-Dopa administered to the control mice enhanced locomotor activity, and reduced rearing activity, in a dose-dependent manner. MK-801 co-administered with L-Dopa (5 mg/kg) increased locomotion (0.1 mg/kg) and reduced rearing (0.3 mg/kg) in MPTP-treated mice; in control mice, MK-801 (0.1 and 0.3 mg/kg) + L-Dopa administered to the control mice enhanced locomotor activity, and reduced rearing activity, in a dose-dependent manner. (4) In control mice, without concomitant L-Dopa treatment, memantine (10 mg/kg) and MK-801 (0.3 and 1 mg/kg) elevated motor activity and reduced rearing, whereas in MPTP-treated mice memantine (3 and 10 mg/kg) reduced rearing and MK-801 (1 mg/kg) reduced both locomotion and rearing (see Table 1). Neither CGP 40116 nor amantadine induced any effects in the control mice whatsoever.

For the experiments testing glutamate antagonists in neonatal 6-OHDA-treated rats:

(1) CGP 40116 induced dose-related increases in locomotor (0.03 and 0.3 mg/kg), rearing (0.01, 0.03, 0.1 and 0.3 mg/kg) and total activity (0.03 mg/kg) in DA-denervated rats compared with vehicle rats. (2) Memantine (2.0 and 6.0 mg/kg) and MK-801 (0.1 and 0.3 mg/kg) increased locomotion and total activity, but not rearing, at the highest dose applied in comparison with vehicle rats. Amantadine did not induce any effects at the doses used. (3) Both MK-801 and memantine caused increased locomotor and total activity in vehicle-treated rats. Neither CGP 40116 nor amantadine induced alterations in vehicle-treated rats.

For the experiments testing the effects of neuroleptic compounds on behavioural alterations induced by acute MK-801:

(1) Clozapine (5.0 and 10.0 mg/kg), SCH 23390 (0.005 and 0.05 mg/kg), raclopride (0.1 mg/kg) and haloperidol (0.1 mg/kg) antagonised the stimulatory effects of MK-801 (0.3 mg/kg) upon locomotion in a dose-related manner. (2) Amperozide antagonised the effects MK-801 upon locomotion at the lower (0.5 mg/kg) dose but potentiated its effects at the higher (2.0 mg/kg) dose. (3) Raclopride (0.1 mg/kg) and to a lesser extent SCH 23390 (0.05 mg/kg) antagonised the disruptive effects of MK-801 upon learning acquisition in the circular swim maze. (4) Clozapine (10.0 mg/kg) and amperozide (2.0 mg/kg) potentiated the disruptive effects of MK-801. Analysis of retention quotients and mean latency/test day generally confirmed these findings. (5) MK-801 caused dose-dependent increments (0.3 and 1.0 mg/kg) of locomotor and total activity and disruptions (0.03 and 0.1 mg/kg) of circular swim maze acquisition.

For the experiment testing the effects of postnatal MK-801 to 11 day-old rat pups:

(1) Postnatally MK-801-treated mice (0.5 mg/kg) demonstrated a marked hypoactivity of locomotor, rearing and total activity during the 1st 30-min test period and hyperactivity during the 2nd 30-min test period of pre-amphetamine phase. (2) MK-801-treated mice administered d-amphetamine (0.25 mg/kg) showed markedly reduced levels of motor activity compared to MK-801 mice administered saline and vehicle-treated mice administered d-amphetamine, and was in fact comparable to vehicle mice injected saline. (3) Pre-amphetamine habituation quotients indicated a completion absence of any habituation to the test chamber by MK-801 mice. Post-amphetamine habituation quotients indicated continued lack of habituation in MK-801 mice injected saline and an upheaval of habituation in Vehicle mice injected d-amphetamine. MK-801 mice injected d-amphetamine displayed habituation levels in fact superior to that of the Vehicle mice injected saline.

Comparisons of the MPTP-treated and control mice that were injected saline 60 min before L-Dopa in the memantine, amantadine and MK-801 experiments (results described in Figure 1, Fredriksson et al., 1999a,b; Fredriksson et al., 2000), as well as several other experiments indicated that the MPTP-treated mice show only a percentage of the activity of control mice in each experiment as follows:

Compound	Locomotion	Rearing	Total activity
CGP 40116	11.7%	20.3%	32.2%
Memantine	12.5%	20.5%	36.1%
Amantadine	13.8%	22.3%	34.6%
MK-801	12.9%	23.2%	32.7%
Anticonvulsant drugs	10.8%	20.7%	30.6%
MAO-inhibitors	13.2%	21.0%	34.9%

These levels of hypoactivity shown by MPTP-treated mice agree well with those regularly obtained in these studies of functional changes induced by MPTP (cf. Archer and Fredriksson, 1999, 2000; Fredriksson and Archer, 1994). The synergistic and restorative (upon acute L-Dopa actions) effect of the NMDA-antagonist compounds are reflected against the above back-

ground of hypokinesis in MPTP mice. Despite this, it should be indicated that no *direct* evidence is provided here for this interpretation unrestrictedly, particularly in the case of the memantine and MK-801 (see Fig. 1) experiments, since the control animals also showed significant behavioural changes at the effective drug doses. On the other hand, the interpretations regarding the efficacy of amantadine for putative synergistic and restorative effects on co-administration with L-Dopa seem probable.

The present results confirm much other evidence of the activity-enhancing effects of NMDA antagonists with L-Dopa: e.g. elevations of motor activity with in combinations of MK-801 with L-Dopa (Goodwin et al., 1992), or MK-801 with dihydrexidine (Gossel et al., 1995). Neither MK-801 nor CGP 40116 restored motor activity in MPTP mice when injected with saline. In DA-depleted MPTP-treated animals either drug-naive or after chronic administration of L-Dopa, competitive/noncompetitive NMDA antagonists together with L-Dopa induced synergistic effects on motor activity (cf. Archer et al., 1996). Nevertheless, Crossman et al. (1989) have observed effects of MK-801 by itself in MPTP-treated monkeys. Several other investigations have documented synergistic effects of combining L-Dopa with NMDA antagonists but it should be noted that in these cases excessive concentrations of the DA-precursor were applied. Maj et al. (1993) showed that CGP 37849 (1.0 and 3.0 mg/kg), but not CGP 39551, plus L-Dopa (50 mg/kg, and benserazide, 100 mg/kg) caused a synergistic action on locomotor activity. Similarly, Greenamyre et al. (1994) found that the anticonvulsive agent, remacemide hydrochloride (5–40 mg/kg, p.o.) co-administered with a subthreshold dose of L-Dopa methylester dose-dependently increased motor activity. Remacemide is also an uncompetitive NMDA antagonist with neuroprotective properties (Palmer et al., 1995). In parkinsonian rhesus monkeys, remacemide (10 mg/kg, p.o.) potentiated the effects of suprathreshold doses of L-Dopa methylester (100–200 mg/kg, i.p.). Such positive interactions between memantine or amantadine and L-DOPA have been reported in several animal models of Parkinsonism (Gossel et al., 1995) but the present findings offer synergistic-restorative actions at remarkably lower doses those used previously (above). Despite dose considerations, the ability of amantadine and memantine to potentiate the antiparkinson actions of L-Dopa in the clinic has been demonstrated (Rabey et al., 1992).

Some acute effects of NMDA antagonists have been studied in adult rats treated neonatally with 6-OHDA: Criswell et al. (1993) treated 2-day-old rat pups with 6-OHDA (100 ug ic in 100 ul), obtaining straital DA depletions of 99% in rats administered DMI prior to 6-OHDA. The behavioural response to MK-801, measured as activity counts over 150 min, was enhanced markedly in the neonatal 6-OHDA rats. In rats treated with 6-OHDA (200 ug, ic) as adults, the hyperactivity induced by MK-801 was substantially reduced (approx. 50%). CGS 19755, the competitive NMDA antagonist, induced a marginal effect in intact animals (significant increases coming first at 30 mg/kg) but a massive enhancement in the neonatal 6-OHDA rats. The present findings confirm the activity-enhancement (compared to vehicle-treated ras) of postnatal 6-OHDA in MK-801-injected rats (0.1 and 0.3 mg/kg) for locomotion and total activity, and demonstrate also enhancements after acute memantine (2.0 and 6.0 mg/kg). The competitive antagonist, CGP 40116, enhanced rearing behaviour in 6-OHDA rats at all the doses applied (0.01, 0.03, 0.1, 0.3 mg/kg), whereas the locomotion (0.03 and 0.3 mg/kg) and total activity (0.03 mg 7 kg) increases were more specific. Criswell et al. (1993) found that alpha-methyl-p-tyrosine (100 mg/kg, 50 min before) partially blocked the effects of MK-801 (by about 50%) in (i) postnatal 6-OHDA-treated, without DMI pretreatment, rats, (ii) postnatal DMI-pretreated 6-OHDA-treated rats to a greater extent (about 75%), (iii) adult 6-OHDA-treated rats, almost completely, and, (iv) control, unlesioned rats, marginally (about 14%). MK-801 dose-dependently reduced the self-injurious behaviours induced by acute L-Dopa. These results by Criswell et al. (1993) indicate that the behavioural effects of MK-801 to some extent a release of DA, as suggested elsewhere (Bowyer et al., 1991; Rao et al., 1990; Werling et al., 1990). Effects of MK-801 following regional microinjections of 6-OHDA to adult rats have been observed. Kronthaler et al. (1994) administered 6-OHDA (12 ug in 2 ul) into the lateral striatum and obtained increased MK-801-induced ambulatory behaviour in the 6-OHDA-lesioned rats accompanied by a decrease of head-dipping behaviour. These latter findings confirm with other results (e.g. Fredriksson et al., 2001) showing the predominance of hyperactive effects by MK-801 and memantine generally combined with reductions of behaviours associated with exploration whereas CGP 40,116 and amantadine also elevate the latter class (see also Figure 2, CGP 40116).

Ikonomidou et al. (2000) demonstrated that MK-801, administered at a dose of 0.5 mg/kg on postnatal day 7, on three occasions, markedly increased the rate of apoptosis in several different brain regions. Those regions found to be most drastically affected were: the parietal cortex, layer II (26.13% degenerating cell density as percentage of total cell density versus 1.08% of saline-injected: 24.19-fold), frontal cortex, layer II (22.65% versus 1.55%: 14.61-fold), cingulate cortex, layer II (15.49% versus 1.54%: 10.05-fold), laterodorsal thalamus (11.91% versus 0.30: 39.70-fold), retrosplenial cortex, layer II (11.49% versus 0.89%: 12.91-fold) and subiculum (10.70% versus 0.59%: 18.14-fold). Ethanol (2.5 g/kg $\times$ 2) and diazepam (30 mg/kg $\times$ 1) induced comparable rates of accelerated apoptosis (Ikonomidou et al., 2000). The authors indicated that exposure to the NMDA-antagonist over some hours during the specific developmental period encompassing synaptogenesis destroyed large numbers of neurons from several major brain regions through an apoptotic neurodegenerative reaction.

The mechanism of potentiation of L-Dopa action by NMDA antagonism might involve inhibition of overactive descending glutamatergic input from the cortex to striatum (28, 39) although other possible drug-neurotransmitter-receptor site interactions should be taken into account. Recently, Fisher et al. (1998) examined the acute effects of NMDA and non-NMDA antagonists on the activity of aromatic l-amino acid decarboxylase (AADC) in the striatum and substantia nigra of rats. MK-801 (0.01, 0.1 and 1.0 mg/kg) and phencyclidine (4 mg/kg) elevated AADC in both regions (2-to-3 fold), and even more so amantadine, 40 mg/kg (striatum: 3.8 fold; substantia nigra: 9.0 fold), and memantine, 40 (striatum: 3.4 fold; substantia nigra: 3.1 fold). Interestingly, CGP 40116 at 1 and 5 mg/kg, had no effect upon AADC activity but it should be noted that the compound affects L-Dopa responses in MPTP-treated mice at much lower doses, i.e. 0.01 and 0.03 mg/kg (Archer and Fredriksson, 1999). It was suggested that the ability of amantadine and memantine to potentiate the antiparkinson actions of L-Dopa may be due to facilitated decarboxylation of L-Dopa by the brain (Fisher et al., 1998). The novel aspect of such a potentially theraputic combination is believed to consist of an attenuation of the motor fluctuations, seen after prolonged exposure to L-Dopa, by administration of NMDA receptor antagonists (Bravi et al., 1994).

Locomotion-enhancing effects of MK-801 may be antagonists by DA receptor antagonists acting at both DA D_1 and D_2 sites (e.g. Ouagazzal et al., 1993). Ögren and Goldstein (1994) showed that the locomotor stimulatory effects of MK-801 (0.2 mg/kg) were reduced by haloperidol and raclopride at cataleptogenic doses (1.0 and 3.0 umol/kg, respectively), whereas the effects of a lower dose of MK-801 (0.1 mg/kg) were antagonists at the 0.3 and 3.0 umol/kg doses, respectively, of the neuroleptics. Remoxipride (40 umol/kg) also antagonised the higher, 0.2 mg/kg, dose of glutamate antagonist. Tiedtke et al. (1990) blocked the enhanced locomotion and stereotypic sniffing behaviour of MK-801; clozapine antagonised locomotion at a much lower dose (1.0 mg/kg) than it antagonised sniffing behaviour (Hoffman, 1992). Also in agreement with the present findings (raclopride), MK-801-induced stimulation of locomotor behaviour was antagonised by YM 09151-2, a D2 antagonist (Dall'Olio et al., 1992), as well as by similar doses of haloperidol (0.1–0.25 mg/kg) applied to blockade of apomorphine-induced stereotypy in male and female rats (Löscher and Hönack, 1992).

Glutamatergic-DA D_1 interactions may modulate both MK-801-induced locomotor hyperactivity (0.3 mg/kg) and the disruption of swim maze acquisition learning (0.03 mg/kg). SCH 23390 (0.005 and 0.05 mg/kg) antagonised the hyperlocomotion effect of MK-801, a confirmation of the Dall'Olio et al. (1992) study. Wolf et al. (1993) indicated that a 0.25, but not 0.1, mg/kg dose of SCH 23390 antagonised the stimulatory effects of an higher dose of MK-801, 0.25 mg/kg. Behavioural sensitization to MK-801-induced ambulatory behaviour was attenuated by the 0.25 mg/kg dose, but not the 0.1 mg/kg dose, of the D_1 antagonist. SCH 23390 (0.05 mg/kg) antagonised the disruption of performance in the circular swim maze.

Clozapine antagonised dose-dependently the hyperlocomotion effects of MK-801 but potentiated its disruptive effects in the circular swim maze. Amperozide potentiated both the former as well as the latter. Clozapine interacts widely with neurotransmitter binding sites particularly alpha$_1$, 5-HT$_{1C}$, 5-HT$_2$, muscarini and histamine-H_1 (Coward, 1992), and the putative DA D_4 receptors (Van Tol et al., 1991). Moderate binding was obtained to other DA sites: D_1, D_2, D_3 (Sokoloff et al., 1990). Clozapine appears to act differentially upon D_1 and D_2 receptors by antagonising grooming behaviour induced by the D_1 agonist, SKF 77434, to a greater extent than D_2 agonist, LY 163502, induced hyperactivity (Murray and Waddington, 1990), suggesting a preferential D_1 blockade

(Coward et al., 1989; Imperato and Angelucci, 1988). The blockade of MK-801 hyperlocomotion by clozapine confirms that of Tiedtke et al. (1990) and Hoffman (1992): stereotyped sniffing was antagonised by a dose of 10 mg/kg and hyperlocomotion by doses of 1.0–10.0 mg/kg (clozapine) whereas haloperidol and eticlopride were equally effective upon sniffing behaviour and locomotion at doses of 0.05–0.5 mg/kg and 0.01–0.05 mg/kg, respectively. It is possible that the differential action of clozaline on MK-801-induced alterations, i.e. antagonism of hyperlocomotion and potentiation of swim maze impairment, reflects a DA D_1 modulation in the former case and a 5-HT-receptor mediation in the latter. Amperozide potentiated both effects of the NMDA antagonist.

In other studies (cf. Archer et al., submitted), all four compounds, CGP 40116, amantadine, memantine and MK-801, partially or completely restored in a dose-related manner the antihypokinesic effect of the suprathreshold dose of L-Dopa following previous chronic administration ("wearing off"). This confirms results showing that MK-801 and prolongs the duration of L-Dopa-induced contralateral rotations in rats chronically administered L-Dopa over three or four weeks (Engber et al., 1994; Papa et al., 1995). Similar effects were described for amantadine (50 mg/kg) (Karcz-Kubicha et al., 1998), in agreement with positive effects for amantadine and memantine in L-Dopa tolerant patients (Rabey et al., 1992; Shannon et al., 1987) form the clinic. Interestingly, amantadine (and some other agents having antagonistic effects on NMDA receptors) inhibits also the dyskinesias evoked by prolonged L-Dopa treatment both in MPTP-treated monkeys and in Parkinsonian patients (Blanchet et al., 1997; Verhagen Metman et al., 1998a, b). Hence, it has been suggested that both dyskinesias and "wearing-off" involve similar mechanisms, namely an oversensitivity of NMDA receptors (mainly NR1/NR2B) in the striatum due to enhanced phosphorylation, resulting from tonic activation of the DA receptors (Oh et al., 1998). Thus, as suggested recently (Danysz et al., 1997; Lange et al., 1997), the aminoadamantanes, memantine and amantadine, show an antiparkinsonian action in animal models and in Parkinsonian patients possibly through NMDA receptor blockade, although this mechanism remains less certain in the case of amantadine.

Out of all the agents tested, only amantadine is administered on regular basis for the treatment of Parkinson's disease. The first indications that the substance may possess an antiparkinsonian action date from the early 1970s when Strömberg and Svensson (1971) found that amantadine increased motor activity in mice and ipsilateral rotational behavior in unilaterally striatomized rats. These authors suggested that the antiparkinsonian action of amantadine could be explained by catecholamine release in the brain. Note however, that in those studies substantially greater concentrations of the compound were applied: certainly at those concentrations (50–150 mg/kg) catecholamine release may be expected. In the clinic, Blomberg et al. (1972) demonstrated that amantadine had an assured effect in two-thirds of PD cases. Hypokinesia and rigidity were most affected whereas tremors were reduced to a lesser extent. These studies confirmed the positive results of other studies (e.g. Hughes et al., 1971; Kannari and Markstein, 1991; Laitinen and Vilkki, 1971). However, the finding that amantadine inhibits both dyskinesias and "wearing off" effects in Parkinsonian patients following long lasting exposure to L-Dopa opens new avenues for therapeutic use of this "old agent", particularly at the dose levels used in the present study.

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