Pharmacology Biochemistry & Behavior, Vol 27, pp 553–557 © Pergamon Journals Ltd 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Characterization of Motor Activity Patterns Induced by N-Methyl-D-Aspartate Antagonists in Gerbils

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BOAST, C A AND G PASTOR *Characterization of motor activity patterns induced by N-methyl-D-aspartate antagonists in gerbils* PHARMACOL BIOCHEM BEHAV 27(3) 553–557, 1987 —A computerized motor activity data collection and analysis system is described An example of the utilization of the Digiscan system is provided, in which motor activity patterns induced by three N-methyl-D-aspartate (NMDA) antagonists and the dissociative anesthetic, ketamine, are compared All of these compounds produce a distinct pattern of motor activity characterized by an increase in distance traveled, movement time, speed and perimeter walking, with a decrease in vertical activity Recently described links between NMDA and phencyclidine (PCP) binding sites may account for these findings The utility of computerized motor activity apparatus is clearly demonstrated

AP7 CPP Ketamine CGS 19755 Locomotor behavior Digiscan

EXCITATORY amino acid receptors have been classified into three categories, N-methyl-D-aspartate (NMDA), quisqualate and kainate types, on the basis of selective agonist activity [4] Selective antagonists at the NMDA type receptor have now been identified and characterized (e g , [8,15]) It has been proposed that these agents have potential therapeutic application as anticonvulsants [13], anxiolytics [1] and in reducing neuronal death after ischemia [2] Recently demonstrated links between NMDA and phencyclidine (PCP) binding sites (e g , [10,12]) have suggested the possibility that NMDA antagonists will share some of the behavioral effects of PCP In fact, AP5 and AP7 have been shown to produce PCP-like catalepsy in pigeons [8], and AP5 can produce PCP-like discriminative responding in rhesus monkeys [16] However, little has been reported in the literature on the effects of NMDA antagonists on spontaneous behavior in rodents, such as that measured using computer assisted motor activity apparatus

Previous work has suggested that a distinct pattern of motor activity follows the administration of selective NMDA antagonists Specifically, intracerebroventricular (ICV) administration of 2-amino-7-phosphonoheptanoic acid (AP7) to rats [11] induces a motor activity pattern of increased distance traveled, movement time and speed, accompanied by a decrease in vertical activity The increase in motor activity is manifested largely as continuous circumnavigation of the outer perimeter of the motor activity chamber (perimeter walking) These changes are observed to have a delayed onset, with a peak effect occurring one hour after drug administration Additional behavioral effects, such as ataxia, are seen as early as 5 minutes after drug administration and sometimes persist through the period of increased motor activity Circling about the body axis also occurs in some animals

When AP7 is administered intraperitoneally (IP) [11] to rats or mice, the pattern of motor activity seen after ICV treatment is not apparent, although ataxia is still observed In contrast to the lack of effect in rats and mice, IP AP7 in gerbils does cause motor activity changes similar to those seen after ICV administration to rats Specifically, distance traveled, speed and perimeter walking are increased Some ataxia is seen in gerbils but not more than 50% of the animals show this effect Circling about the body axis is not seen in rats, mice or gerbils following IP AP7 treatment These findings are interpreted to indicate that AP7 may cross the blood brain barrier in gerbils more readily than in rats or mice

The gerbil was selected for the comparison of additional NMDA antagonists because of the greater sensitivity to AP7 in this species [11] Two additional selective, more potent NMDA antagonists, 3-((±)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) [3, 7, 15] and *cis* 4-(phosphonomethyl)-2-piperidine-carboxylic acid (CGS 19755) [9] were assessed for motor activity effects and observed behavioral changes such as ataxia In view of the recent reports of links between NMDA and phencyclidine (PCP) binding sites [10,12], the dissociative anesthetic, ketamine, which acts at the PCP site, was also studied

METHOD

Animals

Female Mongolian gerbils (Tum (MON)) (Tumblebrook

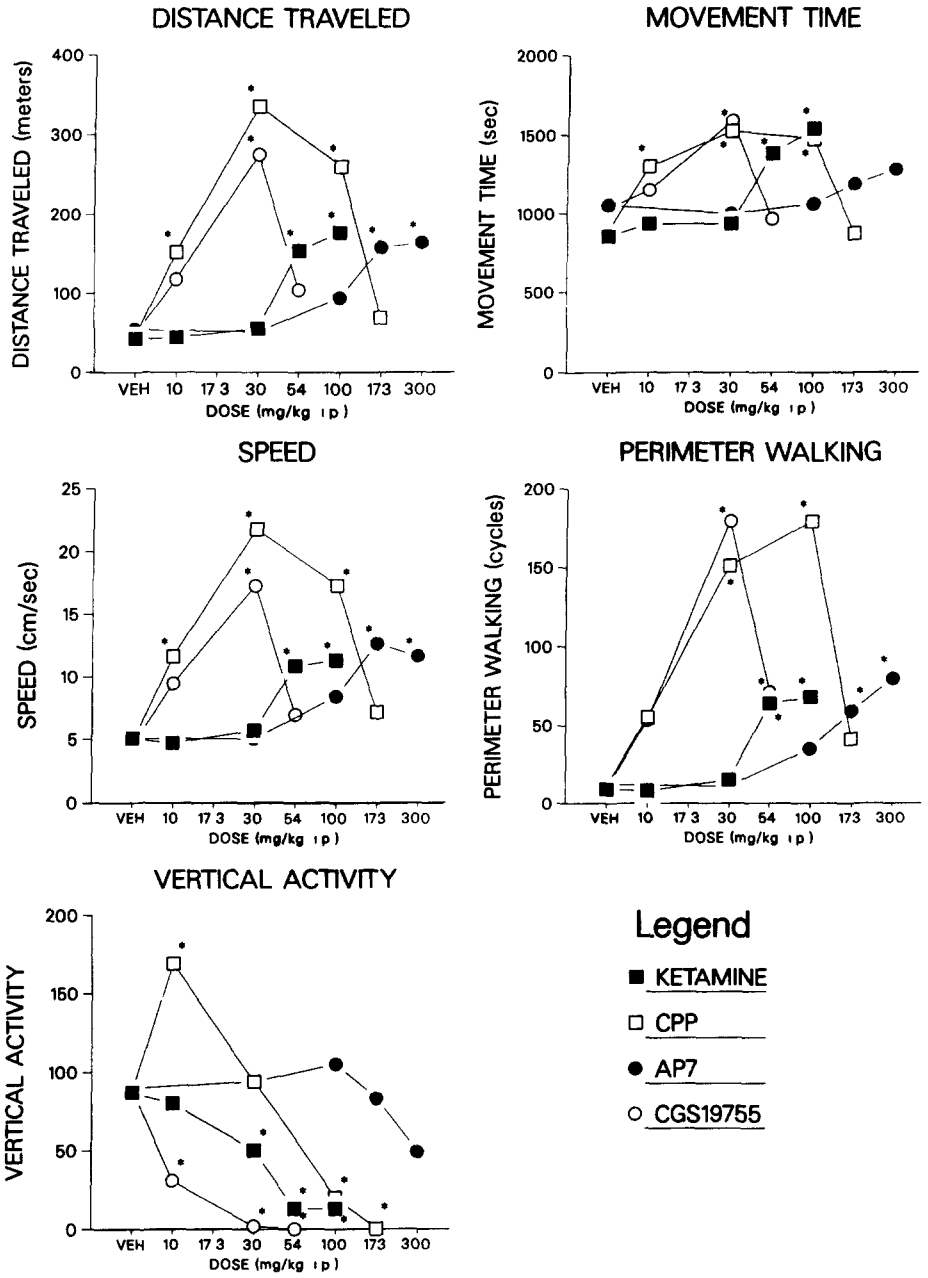

FIG 1 Motor activity changes in gerbils after treatment with AP7 (1 hr), ketamine (1 hr), CPP (2 hr) or CGS 19755 (2 hr) *$p<0$ 05 vs vehicle (Dunnett's t-test)

Farms, West Brookfield, MA) weighing 50–70 g were housed individually and maintained under a 12 hr light-dark cycle with lights on at 7 a m Food and water were available ad lib

Drugs

All drugs were dissolved in saline and administered IP (5 ml/kg) CPP (10, 30, 100 or 173 mg/kg) was synthesized by Dr J Schneider of CIBA-Geigy Ketamine HCl (Ketalar) (10, 30, 54 or 100 mg/kg) was obtained from Parke-Davis (Morris Plains, NJ) CGS 19755 (3, 10, 30, or 54 mg/kg) was synthesized by Dr A Hutchison of CIBA-Geigy Previously reported data [11] using AP7 (30, 100, 173 or 300 mg/kg) have been included to aid in making comparisons among the drugs

Procedures

Following drug administration, each gerbil was observed for the presence of a variety of behavioral measures (ataxia, loss of righting reflex, decreased righting reflex, hind limb paralysis, hind limb flail, circling and hyperactivity) every 30 minutes for a total duration of either 2 hr (ketamine, AP7) or 4 hr (CPP, CGS 19755) These observations were made by placing individual animals on a flat surface and observing spontaneous behaviors Each gerbil was then placed on its

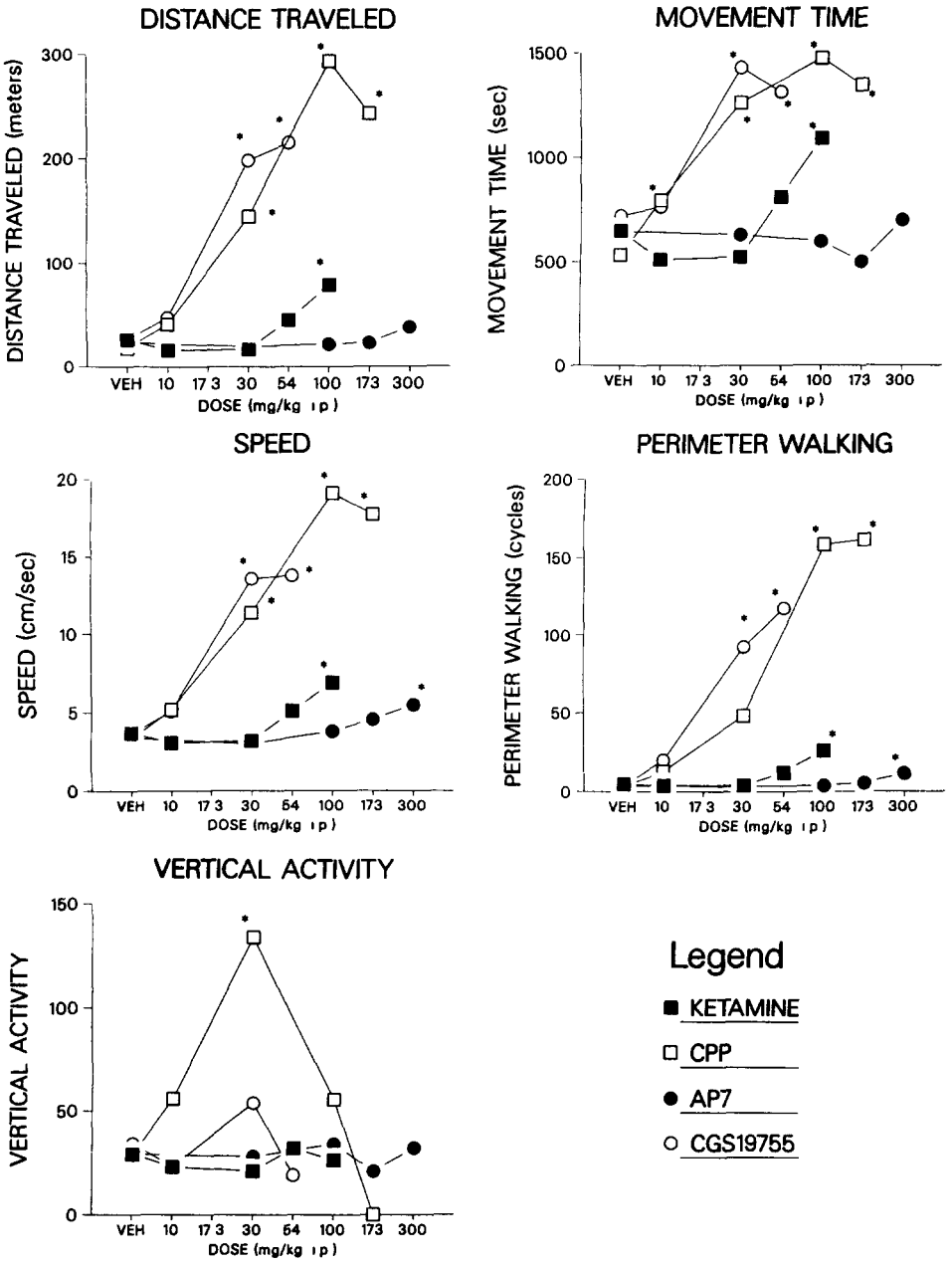

FIG 2 As for Fig 1 except data are from a later time period (AP7—2 hr, ketamine—2 hr, CPP—4 hr, and CGS 19755—4 hr)

back to determine the presence of the righting reflex The animals were then returned to their home cage Observation times that coincided with motor activity assessment times were conducted just prior to, or just after, the beginning of a motor activity session Omnitech Digiscan (Columbus, OH) apparatus (see below) was used to measure motor activity over a 27 minute period either 1 and 2 hr (ketamine, AP7) or 2 and 4 hr (CPP, CGS 19755) after drug administration Thus, each group of animals was assessed twice in the motor activity apparatus and observed at multiple times during the experiment The effects of each dose of each drug were evaluated in separate groups of 8 animals

Motor Activity Data Handling

Data were obtained simultaneously from eight Digiscan-8 Animal Activity chambers Each chamber was equipped with arrays of infrared beam emitters and detectors in the x, y and z planes as described elsewhere [14] Interruption of any beam was monitored, recorded and mathematically manipulated by a control unit, to provide measures of distance traveled, movement time, number of movements, etc (a total of 22 parameters) These data were automatically transferred to a floppy disk via an Apple IIE microcomputer This computer also controlled the initiation of the experiment and

accepted identifiers such as group designations

Following the data acquisition phase, data were transferred to a DEC VAX 11/785 for tabulation, statistical analysis and graphing This allowed the Apple to be used exclusively for data acquisition and transfer, while further data analysis could be conducted simultaneously The transfer of data was accomplished using software (Poly-xfr) developed by Polygon Associates Inc (St Louis, MO), which required the addition of an Apple II Super Serial Card Interactions with the VAX were accomplished using a DEC VT220 terminal An Inmac A-B Switch facilitated the necessary cable switching between the terminal and the microcomputer The transfer process created VMS data files identical to the Digiscan original tables

In order to be able to combine data from different groups of eight animals (counterbalancing was used throughout these experiments), the VMS data files were electronically transferred into a data analysis system (RS/1) (Bolt, Beranek and Newman, Cambridge, MA) This process was facilitated by using the Research Programming Language (RPL) of RS/1 to create a procedure file (C Goeller, CIBA-Geigy), which automatically retrieved a designated VMS file and appended the data to a designated RS/1 file Included in this transfer step was the creation of several additional motor activity parameters derived from those already available Specifically, speed was calculated by dividing the total distance traveled by the total time spent moving for each animal Since the Digiscan control unit measures clockwise and counterclockwise revolutions about the center of the apparatus, these were combined to provide a measure of circumnavigation of the outer portion of the motor activity chamber, or perimeter walking Also calculated were the average distance per discrete movement and the average duration of each movement

Once the data for a complete experiment (i e , all doses for a given drug) were tabulated in an RS/1 table, group means and standard errors were generated Further, one-way analyses of variance (homogeneity of variance established first) followed by multiple comparisons (Dunnett's t-test) were conducted Using the results of these analyses, the data were then plotted using Tellagraf (Integrated Software Systems Corp , San Diego, CA)

RESULTS

All of the drugs tested produced a dose-related pattern of motor activity changes typified by an increase in distance traveled, movement time, speed, and perimeter walking In contrast, a decrease was seen in the amount of vertical activity These effects are shown for the first motor activity period in Fig 1, and for the second period in Fig 2

Specifically, AP7 at one hour showed significantly increased distance traveled at the 173 and 300 mg/kg doses Movement time was increased in a dose-related fashion but did not reach statistical significance at any dose of AP7 Speed and perimeter walking were significantly increased at the 173 and 300 mg/kg doses of AP7 Vertical activity was decreased dose dependently but not significantly by AP7 At one hour, the 54 and 100 mg/kg doses of ketamine significantly increased distance traveled, movement time, speed, and perimeter walking Vertical activity was significantly reduced by the 30, 54 and 100 mg/kg doses of ketamine The 10, 30 and 100 mg/kg doses of CPP, all significantly increased distance traveled, movement time and speed, with a peak effect after the 30 mg/kg dose Perimeter walking was significantly increased at the 30 and 54 mg/kg doses of CPP Vertical activity was significantly reduced by the 100 and 173 mg/kg doses of CPP Distance traveled, movement time, speed, and perimeter walking were all significantly increased by the 30 mg/kg dose of CGS 19755 In addition, the 54 mg/kg dose of CGS 19755 significantly increased perimeter walking Vertical activity was significantly reduced by the 10, 30 and 54 mg/kg doses of CGS 19755

Examination of the second motor activity period (Fig 2) showed the same pattern of changes but with evidence that the drug effects were diminishing, as indicated by the right shift in the dose response curves Specifically, AP7 no longer significantly increased distance traveled or movement time, while speed and perimeter walking were only increased at the 300 mg/kg dose Vertical activity was not affected Similarly, ketamine increased distance traveled, movement time, speed and perimeter walking only at 100 mg/kg, with no effect on vertical activity Peak effects of CPP were now seen at 100 mg/kg, and the dose of 173 mg/kg now caused significant increases in distance traveled, movement time, speed and perimeter walking Vertical activity was increased by 30 mg/kg of CPP at this time period CGS 19755 also showed a right shift at the second time period indicated by peak increases in distance traveled and perimeter walking now seen at 54 mg/kg This dose also caused significant increases in movement time and speed Vertical activity was not altered by CGS 19755 at this time period

Despite the striking increases in distance traveled, movement time, speed and perimeter walking, ataxia was observed in 100% of those animals treated with ketamine (54 and 100 mg/kg), CPP (173 mg/kg) and CGS 19755 (54 mg/kg) Thus, although these animals exhibited more locomotor activity, their gait was abnormal Some ataxia was seen at lower doses of these drugs, i e , 83% at 100 mg/kg of CPP, and 33% at 30 mg/kg of CGS 19755 AP7 produced ataxia in 33% of the animals treated with the 300 mg/kg dose A decreased righting reflex was also seen in at least 50% of the animals treated with ketamine (54 and 100 mg/kg), CPP (100 and 173 mg/kg) and CGS 19755 (54 mg/kg) Other behaviors observed with relatively high frequency (at least 50%) included hind limb paralysis or flailing (ketamine at 54 mg/kg, CPP at 173 mg/kg), and circling (CPP at 173 mg/kg)

DISCUSSION

These data extend the earlier findings on AP7 [11] to include the additional NMDA antagonists, CPP and CGS 19755 As a class, these compounds produce a distinctive pattern of motor activity changes, typified by increases in distance traveled, movement time, speed and perimeter walking Vertical activity is often reduced The observation of this pattern of motor activity requires relatively high doses of these compounds compared to their effects in other test systems, such as convulsion [5, 9, 13], anxiety [1] or ischemia ([2,5], unpublished) models, however, the relative dose effectiveness in these models parallels the relative dose effectiveness for producing the motor activity effects Specifically, AP7 is considerably less effective than either CPP or CGS 19755, which are similar in effectiveness

This pattern of motor activity is also produced by the dissociative anesthetic, ketamine, a drug structurally related to phencyclidine (PCP) Ketamine is effective at doses intermediate between those for AP7 and those for either CPP or CGS 19755 Furthermore, the magnitude of the peak effects after treatment with either CPP or CGS 19755 is greater

than those seen after AP7 or ketamine Although different times of measurement of motor activity were used for the former two compounds compared to the latter two compounds, preliminary data suggest that the times selected were the peak times for activity changes to be seen

It is important to recognize the distinctiveness of the pattern of motor activity changes produced by these NMDA antagonists Other types of pharmacological agents produce patterns of locomotor behavior which have been described extensively (e g , [6]), and do not include the pattern described here One concern, of course, might be that the present studies were conducted using gerbils while those previously reported used rats Pharmacological studies in gerbils have verified the patterns induced by such agents as amphetamine (unpublished data) While amphetamine produces an increase in distance traveled and speed, movement time and perimeter walking are actually decreased Further, it has been previously reported that after central administration of AP7 to rats, a similar pattern to that reported here is observed [11] Thus, the pattern of motor activity described here and elsewhere [11] can be used effectively to characterize NMDA antagonists

Considerable evidence now exists to indicate that NMDA and PCP binding sites are linked, possibly as receptor site (NMDA) and associated ion channel (PCP) (e g , [10,12]) Consistent with this evidence, similar behavioral effects of PCP and NMDA receptor antagonists have been shown in pigeons [8] and rhesus monkeys [15] The present experiments have shown that a PCP analogue, ketamine, produces effects on motor activity in gerbils similar to those seen after administration of NMDA antagonists Future research will determine whether NMDA antagonists that do not show these properties can be identified

The value of utilizing automated motor activity analysis which demonstrates that patterns of motor activity changes, not just increases or decreases, are important to characterize compounds and classes of compounds, is evident from these studies The volume of data that results, necessitates the use of fully computerized data acquisition and analysis

REFERENCES

1 Bennett, D A and C L Amrick Antagonists at the N-methyl-D-aspartate receptor produce anticonflict effects In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24* edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 213–216

2 Boast, C A , S C Gerhardt and P Janak Systemic AP7 reduces ischemic brain damage in gerbils In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24* edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 249–252

3 Davies, J , R H Evans, P L Herrling, A W Jones, H J Olverman, P Pook and J C Watkins CPP, a new potent and selective NMDA antagonist Depression of central neuron responses, affinity for [^{3}H]D-AP5 binding sites on brain membranes and anticonvulsant activity *Brain Res* **382** 169–173 1986

4 Foster, A C and G E Fagg Acidic amino acid binding sites in mammalian neuronal membranes their characteristics and relationship to synaptic receptors *Brain Res Rev* **7** 103–164, 1984

5 Gerhardt, S C , P Bernard, G Pastor and C A Boast Effects of systemic administration of the NMDA antagonist, CPP, on ischemic brain damage in gerbils *Soc Neurosci Abstr* **12** 59, 1986

6 Geyer, M A , P V Russo and V L Masten Multivariate assessment of locomotor behavior Pharmacological and behavioral analyses *Pharmacol Biochem Behav* **25** 277–288, 1986

7 Harris, E W , A H Ganong, D T Monaghan, J T Watkins and C W Cotman Action of 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) a new and highly potent antagonist of N-methyl-D-aspartate receptors in the hippocampus *Brain Res* **382** 174–177, 1986

8 Koek, W , E Kleer, P J Mudar and J H Woods Phencyclidine-like catalepsy induced by the excitatory amino acid antagonist D,L-2-amino-5-phosphonovalerate *Behav Brain Res* **19** 257–259, 1986

9 Lehmann, J , A J Hutchison, S E McPherson, C Mondadori, M Schmutz, C M Sinton, C Tsai, D E Murphy, D J Steel, M Williams and P J Wood CGS 19755, a selective and competitive NMDA-type receptor antagonist *J Pharmacol Exp Ther*, in preparation

10 Lehmann J , P Loo, S McPherson, J M H French-Mullen, D Steel, A Braunwalder, M Williams and P L Wood NMDA- and PCP-type receptor interactions In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24*, edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 91–98

11 Liebman, J M , C L Amrick, P S Bernard, G Pastor, C A Boast and D A Bennett Distinctive behaviors induced in rodents by high doses of 2-amino-7-phosphonoheptanoic acid In *Excitatory Amino Acid Transmission, Neurology and Neurobiology Vol 24* edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 261–264

12 Lodge, D , J A Aram, J Church, S N Davies, D Martin, C T O'Shaugnessy and S Zeman Excitatory amino acids and phencyclidine-like drugs In *Excitatory Amino Acid Transmission, Neurology and Neurobiology Vol 24*, edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 83–90

13 Meldrum, B Excitatory amino acids and epilepsy In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24* edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 189–196

14 Sanberg, P R , S H Hagenmeyer and M A Henault Automated measurement of multivariate locomotor behavior in rodents *Neurobehav Toxicol Teratol* **7** 87–94, 1985

15 Watkins, J C , R H Evans, K N Mewett, H J Olverman and P C Pook Recent advances in the pharmacology of excitatory amino acids In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24*, edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 19–26

16 Woods, J H , W Koek and P Ornstein A preliminary study of PCP-like behavioral effects of 2-amino-5-phosphonovalerate in rhesus monkeys In *Excitatory Amino Acid Transmission, Neurology and Neurobiology, Vol 24*, edited by T P Hicks, D Lodge and H McLennan New York Alan R Liss Inc , 1987, pp 205–212