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MK-801 Prevents Hypobaric–Ischemic Neuronal Degeneration in Infant Rat Brain

J. W. Olney, C. Ikonomidou, J. L. Mosinger, and G. Frierdich

Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri 63110

Recent evidence implicates the endogenous excitatory amino acids, glutamate (Glu) and aspartate, in hypoxic/ischemic neuronal degeneration. In a preceding article (Ikonomidou et al., 1989) we described a new model for studying hypoxic/ischemic neuronal degeneration in the infant rat brain that entails unilateral common carotid artery ligation followed by exposure to a partial vacuum for 75 min. Promising features of this model include a low mortality rate and high incidence of acute brain damage disseminated over numerous brain regions. In addition, there is a striking similarity between the type of cytopathology characterizing this model of hypoxic/ischemic neuronal degeneration and that which has been described in infant animals treated with Glu. MK-801 is a powerful antagonist of the N-methyl-D-aspartate (NMDA) receptor ionophore complex (a subtype of Glu receptor). In the present study, after unilateral carotid artery ligation was performed on 10-d-old rat pups, they were treated either with MK-801 (1 mg/kg i.p.) or saline 15 min before exposure to the hypobaric condition. MK-801 exerted a strong neuroprotective effect without serious side effects; the majority of saline control animals sustained severe brain damage, whereas the majority of MK-801-treated pups had no brain damage. These and other recent findings suggest that the NMDA receptor may play an important role in hypoxic/ischemic neuronal degeneration in the immature brain and provide hope that NMDA antagonists such as MK-801 may be effective in preventing such degeneration.

Several lines of recent evidence suggest that the excitatory amino acids (EAA), glutamate (Glu) and aspartate, play a critical role in hypoxic/ischemic neuronal degeneration (Rothman and Olney, 1986). In the rat CNS, under hypoxic/ischemic conditions, EAA accumulate in high concentrations in the extracellular compartment (Benveniste et al., 1984). There they have access to postsynaptic EAA receptors through which they can act by an excitotoxic mechanism to destroy CNS neurons. Several EAA receptor subtypes have been identified (Watkins and Olverman, 1987), each being named by a potent EAA agonist [N-methyl-D-aspartate (NMDA), kainate, quisqualate] to which it is differentially sensitive. The NMDA receptor reportedly is the most abundant EAA receptor subtype in the mammalian CNS (Monaghan and Cotman, 1985) and has the highest affinity for endogenous Glu (Olverman et al., 1984). The participation of this receptor subtype in hypoxic/ischemic neuropathological processes therefore seems quite likely. Competitive NMDA receptor antagonists have been identified (Watkins and Olverman, 1987) but are of limited interest as neuroprotective agents, as they do not readily penetrate blood brain barriers. Lodge and colleagues (1988; Anis et al., 1983) made the important observation that phencyclidine (PCP) and related compounds, which do penetrate blood brain barriers, selectively antagonize the excitatory action of NMDA on CNS neurons. Subsequently it was shown that these compounds also protect neurons against the neurotoxic effects of NMDA, either *in vitro* (Olney et al., 1986b) or *in vivo* (Fuller et al., 1987).

MK-801, a dibenzocycloalkenimine that has PCP-like receptor binding properties (Wong et al., 1986), is the most powerful known antagonist of EAA neurotoxicity (Foster and Wong, 1987; Foster et al., 1987; Olney et al., 1987). Like other PCP analogs, its antagonist action is specific for NMDA receptor–mediated events, is noncompetitive, and entails blockade of an ion channel associated specifically with the NMDA receptor (Wong et al., 1986). Since MK-801 freely penetrates blood brain barriers, it is of potential interest as a neuroprotective agent in hypoxic/ischemic conditions. It has been reported that MK-801 prevents ischemic degeneration of CA1 hippocampal neurons in the adult gerbil (Gill et al., 1987; Lawrence et al., 1987) and mitigates loss of hemispheric mass in an infant rat model of hypoxia/ischemia (McDonald et al., 1987). On the other hand, while MK-801 is exceedingly powerful in preventing NMDA neurotoxicity in the chick embryo retina, it is relatively impotent in preventing ischemic neuronal degeneration in this preparation (Olney, 1988). In a companion article (Ikonomidou et al., 1989) we have described a new infant rat model of hypoxic/ischemic brain damage that is quite suitable for *in vivo* testing of neuroprotective drugs since acute neuronal necrosis occurs reliably in numerous brain regions and the mortality rate is low. The present study was undertaken to determine whether MK-801 can prevent nerve cells from degenerating in this infant rat model of hypoxia/ischemia.

Materials and Methods

As described in a companion article (Ikonomidou et al., 1989), unilateral carotid ligation was performed on 10-d-old mixed male and female rat pups (16–20 gm) under halothane anesthesia, and the pups were then subjected to hypobaric conditions (225 mm Hg for 75 min). The interval between carotid ligation and hypobaric exposure was 25 ± 10 min. Fifteen minutes before hypobaric exposure, experimental pups received an injection of MK-801 (1 mg/kg i.p.), and controls received an equivolume injection of normal saline. Ten experiments were performed,

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Correspondence should be addressed to John W. Olney, M.D., Department of Psychiatry, Washington University School of Medicine, 4940 Audubon Ave., St. Louis, MO 63110.

and the total number of infant rats used was 100 (50 experimental/50 control). In each experiment, a group of 10 pups (5 experimental/5 control) shared the hypobaric chamber at the same time, so that critical variables such as ambient temperature and oxygen pressure were identical for each animal. In some experiments all 10 pups were from the same litter. When more than one litter was used, pups from each litter were distributed evenly between experimental and control groups. We also distributed the pups as evenly as possible according to sex and body weight to ensure balance of these variables between experimental and control groups. During surgery the pups were kept on a warming pad that maintained body temperature at approximately 36°C as measured by a skin surface microprobe. At all other times during the experiment, the pups were in a glass jar submerged in a water bath that maintained the temperature inside the jar at 36°C. Two hours after hypobaric exposure, all pups were perfused with aldehyde fixatives under halothane anesthesia, and their brains were prepared for histopathological evaluation as described in a companion paper (Ikonomidou et al., 1989).

Transverse sections 1 μm thick were cut at 2 levels [2.3 mm and 5.6 mm anterior to the zero point according to the atlas of Sherwood and Timiras (1970)], which permitted visualizing brain regions known to be vulnerable to hypobaric/ischemic damage, viz., frontoparietal cortex, caudate/putamen, olfactory tubercle, islands of Calleja, hippocampus, septum, medial habenulum, ventroposterior, mediodorsal, and laterodorsal thalamic nuclei, and amygdala. The brains were evaluated for cytopathology by 2 experienced raters who were blind to the experimental conditions. For purposes of quantifying the damage in a given region, a count of acutely necrotic neurons (NN) was performed on a single section containing that region; then the counts for the several regions were added to give a total NN score for each brain. Counts were limited to "bull's-eye" profiles, i.e., neurons exhibiting vacuous swollen perikarya and pyknotic changes (chormatin clumping) in the nucleus. In some brain regions, primarily the cerebral cortex, where the lesions were sometimes too large to permit counting in this manner, the section was viewed on a video screen and the total lesioned (edematous) area measured; then a count was made on a subfield comprising a known fraction of the total area, and the total NN count was determined by extrapolation. The brains were assigned to one of 4 categories denoting the severity of damage as follows: Group 1 = no brain damage; Group 2 = mild damage (1–100 NN); Group 3 = moderate damage (101–200 NN); or Group 4 = severe damage (>200 NN). The difference between the distribution of scores for control and experimental animals was evaluated for statistical significance by the χ^2 test.

In the course of developing this hypobaric/ischemic model, we observed that we could obtain an incidence of brain damage in the range of 90% if the hypobaric chamber temperature was maintained in the 36–37°C range, whereas the incidence progressively declined as a function of declining temperature (Mosinger, J. L., C. Ikonomidou, and J. W. Olney, unpublished observations). For purposes of the present experiment, we were concerned that, although external temperature conditions were carefully regulated, the experimental drug (MK-801) might function as an independent variable causing the temperature of experimental pups to differ from that of controls. It would have been difficult to monitor body temperature of rat pups continuously while they were in the hypobaric chamber, and it might have confounded the interpretation of results regarding the neuroprotective properties of MK-801 if we had released the vacuum periodically to make temperature measurements. Therefore, we performed a pilot study on 10-d-old infant rats subjected to unilateral carotid ligation, following which one group (n = 29) was treated with saline and the other (n = 28) with MK-801 (1 mg/kg i.p.); we then measured temperatures before drug treatment and before, during (by interrupting the partial vacuum), and immediately after hypobaric exposure. Because of the release of vacuum in this study, these animals were not included in the quantitative analysis of brain damage described above. For statistical evaluation, these temperature data were subjected to a repeated measures analysis of variance (ANOVA).

Results

In the experiments aimed at monitoring body temperature before and after drug treatment, measurements performed at 4 separate time points revealed that the mean body temperature of experimental and control pups was similar prior to and 15 min after treatment but was 0.7°C higher in MK-801 treated pups 50 min after treatment and 0.6°C higher 90 min after treatment. The 50-min interval was during the time in the hypobaric chamber, and the 90-min interval was immediately following the hypobaric episode. Repeated measures ANOVA revealed the following: a significant group effect [$F(1,52) = 5.34$, $p < 0.025$], a significant repeated measurement effect [$F(3,156) = 64.06$, $p < 0.001$], and a nonsignificant interaction [$F(3,156) = 2.55$, $p < 0.058$]. Subsequent pairwise comparisons yielded p values of <0.006 and <0.004, respectively, for the differences between the groups at 50 and 90 min and no significant differences between groups at the 2 earlier time points.

Immediately after exposure to the hypobaric condition, infant rat pups typically display transient hyperactivity, then become relatively quiescent except for periodic episodes, usually toward the end of the 75-min period, when they begin to squirm about and may display limb or total body clonus. In the present experiment, these signs were seen consistently in control but only occasionally in experimental pups. In general, MK-801-treated pups appeared moderately sedate; however, no differences were noted between control and MK-801-treated pups in respiratory rate or skin color. By χ^2 analysis the survival rate for MK-801-treated pups (42/50) was not significantly different from that for control pups (45/50).

The brain damage scores of the surviving pups revealed a potent neuroprotective effect of MK-801. The majority of the control pups (39/45) had brain damage, which in most cases was rated either severe (22/45) or moderate (11/45), and the vast majority of the MK-801-treated pups (40/42) had either no brain damage (31/42) or only mild damage (9/42). Only one MK-801-treated pup had moderate damage, and the score (NN = 107) for this animal barely missed falling in the mild category. Of the 23 pups that sustained severe damage, only one was an MK-801-treated pup, and the NN score for this pup (NN = 469) was at the lower end of the distribution for this category, in which NN scores ranged from 222 to 3012 and the mean was 724. The skewing of scores for MK-801-treated rats toward the no-damage category and the control scores toward the severe-damage category is depicted graphically in Figure 1. By χ^2 analysis, the distribution of scores for the experimental pups differed significantly ($p < 0.001$) from the distribution of control scores. Further analysis of the data revealed that the neuroprotective effect of MK-801 was uniformly distributed over all vulnerable brain regions, with no region being differentially resistant to the effect.

Discussion

In a separate report (Ikonomidou et al., 1989), we presented evidence that hypobaric/ischemic conditions cause an exceedingly acute neurodegenerative reaction in several regions of infant rat brain that is identical, both in time course and pathomorphological characteristics, to the neurodegenerative reaction seen in infant rodent or monkey brain following systemic administration of exogenous Glu. Here we demonstrate that MK-801, an antagonist of one subtype of Glu receptor, exerts a powerful neuroprotective effect against hypobaric/ischemic neuronal necrosis in the infant rat brain. These findings strongly support the hypothesis that an excitotoxic mechanism plays an important role in hypobaric/ischemic neuronal degeneration in the *in vivo* infant rat brain.

Interestingly, MK-801 treatment was associated with a mild elevation in body temperature. It is not clear whether this effect of MK-801 reflects an interaction of the drug with thermoreg-

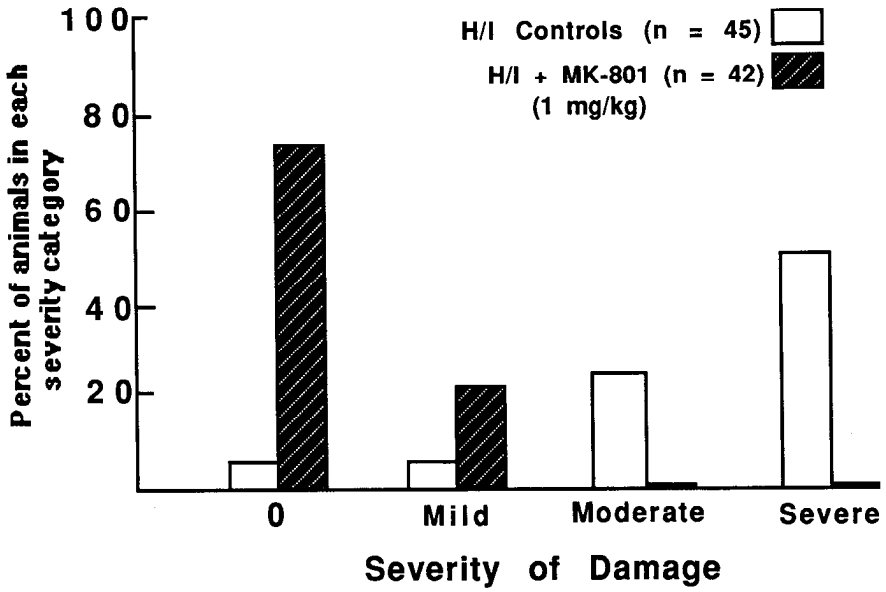

Figure 1. All rats were subjected to hypobaric/ischemic (*H/I*) conditions as described in Materials and Methods and were killed 2 hr later for brain examination. Fifteen minutes prior to hypobaric exposure, experimental pups received MK-801 (1 mg/kg i.p.) and controls received saline. An NN score for each brain was determined as described under Materials and Methods. The control and experimental brains were then assigned to one of 4 severity categories: 0 = no NN; mild = 1–100 NN; moderate = 101–200 NN; or severe = >200 NN. A skewed distribution of scores is clearly evident, with the preponderance of control scores falling in the severe or moderate categories and experimentals in the 0 or mild categories.

ulatory mechanisms or might be explained by the fact that the experimental pups, being sedate and immobile, often lay underneath the more active control pups. Since increased temperature would be expected to augment hypobaric/ischemic brain damage, it might be regarded as a handicapping factor that prevented MK-801 from providing more complete protection. In any event, the substantial degree of protection afforded by MK-801 under potentially unfavorable circumstances recommends it as a promising agent for protecting the immature CNS against hypoxic/ischemic brain damage.

MK-801 did not significantly influence mortality rate. It might be argued that because of its neuroprotective action, it should have reduced the mortality rate. However, this assumes that the severity of brain damage is a primary determinant of mortality rate, which seems not to be the case. In a separate study (Mosinger et al., unpublished observations), we evaluated the factors contributing to the death of pups while in the hypobaric chamber. We examined the brains of both survivors and nonsurvivors immediately after hypobaric exposure and found that early signs of brain damage were not more severe and sometimes were less severe in the nonsurvivors. Moreover, we systematically altered the time of exposure and degree of vacuum in the chamber and found that the mortality rate varied independently of these factors. Finally, we determined that only certain litters were subject to a high mortality rate and that the most effective way to control mortality was to eliminate unfavorable breeders from our colony.

MK-801-treated animals sustained less brain damage and also showed less tendency to develop motor symptoms (hypermotility and clonus) during hypobaric exposure than did control animals. Thus, a positive correlation exists between the manifestation of abnormal behavioral signs and damage to CNS neurons. Whether this signifies a causal link between neuronal injury and behavioral disturbances remains to be determined.

MK-801 is a powerful ligand of PCP receptors which are colocalized with and functionally linked to NMDA receptors in many regions of the mammalian forebrain (Maragos et al., 1986). Activation of the NMDA receptor opens an ion channel that has an exceedingly high Ca^{2+} conductance (MacDermott et al., 1986). The efficacy of MK-801 in blocking either the excitatory or the neurotoxic actions of NMDA stems from its activation of PCP receptors which results in blockade of the NMDA ion channel. Evidence has been presented for 2 types of mechanism by which excitotoxins may mediate neuronal death, one being a very acute process which is Na^+ but not Ca^{2+} dependent (Rothman, 1985; Olney et al., 1986a) and the other being a more slowly evolving process which is both Na^+ and Ca^{2+} dependent (Choi, 1987). Degeneration of CA1 hippocampal neurons, which occurs over a 4-d period in the adult gerbil model of transient cerebral ischemia, is considered an example of the slow mechanism of excitotoxin-mediated cell death. The ability of MK-801 to block this slow neurodegenerative process (Gill et al., 1987; Lawrence et al., 1987) is not surprising, given the exceedingly high density of NMDA receptors on CA1 hippocampal neurons (Monaghan and Cotman, 1985), the high Ca^{2+} conductance of the NMDA ion channel, the role of Ca^{2+} in the slow mechanism of excitotoxic cell death, and the ability of MK-801 to block NMDA ion channel function.

In contrast to the above, the primary focus of the present study is on what appears to be an example of the acute mechanism of excitotoxin-mediated cell death that is not Ca^{2+} dependent and is not limited to CA1 hippocampal neurons nor even to neurons with an exceedingly high density of NMDA receptors. The finding that MK-801 potently protects against this exceedingly acute, presumably excitotoxic cell death process affecting many neurons in the infant rat brain confirms a recent study pertaining to a similar infant rat model of hypoxia/ischemia (McDonald et al., 1987) but is inconsistent with evidence we have generated in the *ex vivo* chick embryo retina (Olney, 1988). In the chick retina, MK-801 is extremely powerful in protecting against acute NMDA neurotoxicity but is relatively impotent in blocking acute neuronal degeneration induced either by Glu or ischemia. Since thiamylal, a broad spectrum antagonist of all EAA receptor subtypes, does prevent either Glu- or ischemia-induced neuronal degeneration in the chick retina, we have attributed the impotence of MK-801 in this situation to its specificity for NMDA receptors; i.e., endogenous Glu released under ischemic conditions can act at other EAA receptor types to destroy neurons even if MK-801 is effectively blocking NMDA receptors.

A possible explanation for the efficacy of MK-801 in preventing hypoxic/ischemic damage in the 10-d-old infant rat brain would be that during this stage of development the NMDA receptor is relatively hypersensitive compared to other EAA receptor subtypes and therefore is preferentially activated under hypoxic/ischemic circumstances. This interpretation is supported by evidence that when NMDA is injected directly into the rat brain it exerts much more potent neurotoxic effects in neonatal than in adult brain (Ikonomidou et al., 1988; McDonald et al., 1988), whereas the opposite is true for kainic acid (Campochiaro and Coyle, 1978). Moreover, examining this pheromenon in greater detail, we have observed that during the first week of neonatal life many neuronal groups in rat brain become increasingly hypersensitive to the neurotoxic effects of NMDA and to hypobaric/ischemic brain damage (Ikonomidou, et al., 1988) and remain hypersensitive to each condition for a period that varies from days to weeks depending on the type of neuron. Our observation that the NMDA-specific antagonist MK-801 showed no regional selectivity in its ability to protect the immature rat brain against hypoxic/ischemic damage (all regions were protected in most animals and no region was differentially resistant to protection) supports the hypothesis that it may be a characteristic of many CNS neurons to undergo a phase in development during which they are hypervulnerable to hypoxic/ischemic damage because of the relative hypersensitivity of their NMDA receptors.

It is relevant to consider whether the efficacy of MK-801 in protecting the infant rat brain against hypoxic/ischemic brain damage might have therapeutic implications for the human. This may depend to some extent on whether there is a stage in human ontogenesis during which NMDA receptors are relatively hypersensitive and other EAA receptor subtypes relatively hyposensitive to excitotoxic stimulation. If there is, we propose that during this interval the immature brain might be transiently hypervulnerable to hypoxic/ischemic damage, that NMDA receptors might play a singularly important role in mediating such damage, and that it might be prevented by the timely administration of NMDA antagonists such as MK-801. Determining whether there is such a stage in human ontogenesis and delineating the time during which therapeutic intervention with NMDA antagonists might be particularly beneficial is an important challenge for future research.

References

Anis, N. A., S. C. Berry, N. R. Burton, and D. Lodge (1983) The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methylaspartate. Br. J. Pharmacol. *79:* 565.

Benveniste, H., J. Drejer, A. Schousboe, and N. H. Diemer (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. *43:* 1369–1374.

Campochiaro, P., and J. T. Coyle (1978) Ontogenetic development of kainate neurotoxicity: Correlates with glutamatergic innervation. Proc. Natl. Acad. Sci. USA *75:* 2025–2029.

Choi, D. W. (1987) Ionic dependence of glutamate neurotoxicity. J. Neurosci. *7:* 369–379.

Foster, A. C., and E. H. F. Wong (1987) The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. Br. J. Pharmacol. *91:* 403–409.

Foster, A. C., R. Gill, J. A. Kemp, and G. N. Woodruff (1987) Systemic administration of MK-801 prevents N-methyl-D-aspartate-induced neuronal degeneration in rat brain. Neurosci. Lett. *76:* 307–311.

Fuller, T. A., J. J. Lawrence, and J. W. Olney (1987) Blockade of N-methylaspartate-induced arcuate nucleus damage by PCP, MK-801 and related compounds. Soc. Neurosci. Abstr. *13:* 1475.

Gill, R., A. C. Foster, and G. N. Woodruff (1987) Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. J. Neurosci. *7:* 3343–3349.

Ikonomidou, C., J. Mosinger, K. Shahid Salles, J. Labruyere, and J. W. Olney (1988) Parallel patterns of hypersensitivity to NMDA toxicity and hypobaric/ischemic damage in developing rat brain. Soc. Neurosci. Abstr. *14:* 501.

Ikonomidou, C., M. T. Price, J. L. Mosinger, G. Frierdich, J. Labruyere, K. Shahid Salles, and J. W. Olney (1989) Hypobaric-ischemic conditions produce glutamate-like cytopathology in infant rat brain. J. Neurosci. *9:* 1693–1700.

Lawrence, J. J., T. A. Fuller, and J. W. Olney (1987) MK-801 and PCP protect against ischemic neuronal degeneration in the gerbil hippocampus. Soc. Neurosci. Abstr. *13:* 1079.

Lodge, D., J. A. Aram, J. Church, S. N. Davies, D. Martin, J. Miller, and S. Zeman (1988) Sigma opiates and excitatory amino acids. In *Excitatory Amino Acids in Health and Disease,* D. Lodge, ed., pp. 237–259, Wiley, London.

MacDermott, A. B., M. L. Mayer, G. L. Westbrook, S. J. Smith, and J. L. Barker (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. Nature *321:* 519–522.

Maragos, W. F., D. C. M. Chu, J. T. Greenamyre, J. B. Penney, and A. B. Young (1986) High correlation between the localization of [^{3}H]TCP binding and NMDA receptors. Eur. J. Pharmacol. *123:* 173–174.

McDonald, J. W., F. S. Silverstein, and M. V. Johnston (1987) MK-801 protects the neonatal brain from hypoxic/ischemic damage. Eur. J. Pharmacol. *140:* 359–361.

McDonald, J. W., F. S. Silverstein, and M. V. Johnston (1988) NMDA neurotoxicity in 7 D.O. rats offers an *in vivo* model to rapidly screen neuroprotective drugs: Neuroprotective effects of MK-801 and related compounds. Neurochem. Int. *12:* 37.

Monaghan, D. T., and C. W. Cotman (1985) Distribution of N-methyl-D-aspartate sensitive L-^{3}H-glutamate-binding sites in rat brain. J. Neurosci. *5:* 2909–2919.

Olney, J. W. (1988) Endogenous excitotoxin and neuropathological disorders. In *Excitatory Amino Acids in Health and Disease,* D. Lodge, ed., pp. 337–351, Wiley, London.

Olney, J. W., M. T. Price, L. Samson, and J. Labruyere (1986a) The role of specific ions in glutamate neurotoxicity. Neurosci. Lett. *65:* 65–71.

Olney, J. W., M. T. Price, T. A. Fuller, J. Labruyere, L. Samson, M. Carpenter, and K. Mahan (1986b) The anti-excitotoxic effects of certain anesthetics, analgesics and sedative-hypnotics. Neurosci. Lett. *68:* 29–34.

Olney, J., M. T. Price, K. Shahid Salles, J. Labruyere, and G. Frierdich (1987) MK-801 powerfully protects against N-methyl aspartate neurotoxicity. Eur. J. Pharmacol. *141:* 357–361.

Olverman, H. J., A. W. Jones, and J. C. Watkins (1984) L-Glutamate has higher affinity than other amino acids for ^{3}H-D-AP5 binding sites in rat brain membranes. Nature *307:* 460–462.

Rothman, S. M. (1985) Excitatory amino acid neurotoxicity is produced by passive chloride influx. J. Neurosci. *6:* 1483–1489.

Rothman, S. M., and J. W. Olney (1986) Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann. Neurol. *19:* 105–111.

Sherwood, N. M., and P. S. Timiras (1970) *A Sterotaxic Atlas of the Developing Rat Brain,* University of California Press, Berkeley.

Watkins, J. C., and H. J. Olverman (1987) Agonists and antagonists for excitatory amino acid receptors. TINS *10:* 265–272.

Wong, E. H. F., J. A. Kemp, T. Priestley, A. R. Knight, G. N. Woodruff, and L. L. Iversen (1986) The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc. Natl. Acad. Sci. USA *83:* 7104–7108.