

Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury?

Chrysanthy Ikonomidou and Lechoslaw Turski

Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists (competitive receptor antagonists, ion channel blockers, and glycine antagonists)—such as selfotel, aptiganel, eliprodil, licostinel and gavestinel—failed to show efficacy in clinical trials of stroke or traumatic brain injury. This failure has been attributed to the deficient properties of the molecules that entered human trials and to inappropriate design of clinical studies. In this article we hypothesise that glutamate may be involved in the acute neurodestructive phase that occurs immediately after traumatic or ischaemic injury (excitotoxicity), but that, after this period, it assumes its normal physiological functions, which include promotion of neuronal survival. We propose that NMDA receptor antagonists failed stroke and traumatic brain injury trials in human beings because blockade of synaptic transmission mediated by NMDA receptors hinders neuronal survival.

Lancet Neurology 2002; **1**: 383–86

It has been known for at least 30 years that high concentrations of glutamate can destroy neurons. The theory of excitotoxicity (the neurodestructive potential of glutamate) was established by the demonstration that an overdose of systemic glutamate destroys hypothalamic nuclei in immature monkeys and rodents.^{1,2} Follow-up research confirmed that high concentrations of glutamate [100–500 μ M] induce cell death in vitro³ and that similar extracellular concentrations are present in the rodent brain and spinal cord during ischaemia or trauma.^{3,4} It was subsequently shown that NMDA receptors mediate glutamate-induced cell death in vitro⁵ and in vivo.^{5,7} These discoveries suggested that administration of NMDA antagonists in human beings could prevent cell death and confer neuroprotection after stroke and traumatic brain injury.

Chronic neurodegenerative disorders such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Alzheimer's disease were added to the list of disease candidates for neuroprotective therapy with NMDA antagonists,^{8,9} although experimental evidence for the involvement of excitotoxicity in the pathogenesis of these slowly progressing disorders was weak. To explain the role of glutamate in slow, ongoing neurodegeneration a hypothesis of "slow excitotoxicity", mediated by physiological concentrations of glutamate, was coined.¹⁰ Namely, if the postsynaptic membrane is partially depolarised because of changes in the ionic homeostasis of the cell, dysfunction of ion channels, or deficient energy supply, then even physiological concentrations of glutamate, acting via NMDA receptors, may destroy the cell.

As a result, the race to design effective NMDA receptor antagonists started, with high public expectations of the pharmaceutical industry.^{11–13} The chemical design of NMDA antagonists was successful; the compounds proved to be the most effective neuroprotective drugs ever tested both in vitro and on antecedent treatment regimens in animal models of stroke, traumatic brain injury, and spinal-cord injury.¹⁴

Clinical trials failed

Clinical trials of NMDA antagonists for stroke and traumatic brain injury were started despite the fact that the NMDA antagonists did not produce a significant post-insult neuroprotective time window in rodent models of stroke and trauma.^{14,15} However, discouraging news started to accumulate¹⁶ as one by one the clinical trials were terminated.^{17,18} Serious concerns began to emerge and the ability of the pharmaceutical industry to apply molecular neurobiology's progress was questioned.

Some researchers proposed that the clinical trials failed because the overall quality of the molecules was poor. In particular, they cited deficient pharmacokinetics, inability to reach effective concentrations in the penumbra, short neuroprotective time window, inappropriate receptor subunit selectivity, high drug toxicity in human beings precluding use of equivalent doses to those that were neuroprotective in rodents, and poor design of clinical trials.^{14,19} The alternative explanation—that the working hypothesis was flawed in some way—was not considered, however.

Subsequently, a second and third generation of NMDA receptor antagonists—such as aptiganel and gavestinel—were invented, but human trials with these compounds failed too.^{20,21} By 2001, all clinical trials of NMDA receptor antagonists in human beings with stroke or traumatic brain injury were considered unsuccessful because of lack of efficacy.¹⁹ Despite these developments, the theory of glutamate-induced excitotoxicity, the major power that forced NMDA receptor antagonists into human trials, has not been questioned and it continues to be advocated by scientists and clinicians.¹⁴

Cl is at the Department of Pediatric Neurology, Children's Hospital, Charite-Virchow Campus, Humboldt University, Berlin, Germany. LT is at Solvay Pharmaceuticals Research Laboratories, Weesp, The Netherlands.

Correspondence: Prof Lechoslaw Turski, Solvay Pharmaceuticals Research Laboratories, CJ van Houtenlaan 36, 1381 CP Weesp, The Netherlands. Tel +31 294 479498; fax: +31 294 477109; email les.turski@solvay.com.

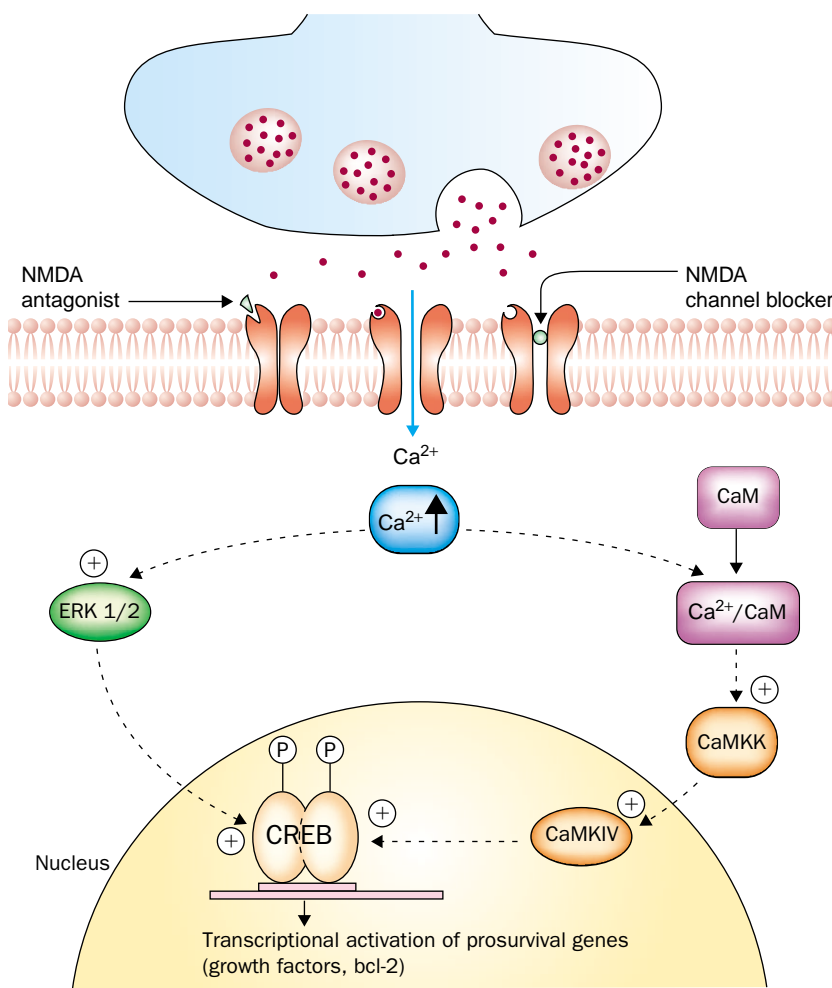


Figure 1. Activation of NMDA receptors leads to Ca²⁺ entry and an increased intracellular Ca²⁺-concentration. High concentrations of Ca²⁺ switches on extracellular signal regulated kinase (ERK1/2)-mediated synapse-to-nucleus signalling and phosphorylation of cAMP response element binding protein (CREB). In addition, calmodulin (CaM) binds and activates its target kinases including CaM kinase IV (CaMKIV) and CaM kinase kinase (CaMKK). CaMKK phosphorylates and fully activates CaM-KIV, which then phosphorylates and activates CREB. Both pathways lead, via CREB phosphorylation, to transcriptional activation of pro-survival genes, such as growth factors and bcl-2.

What was ignored by basic sciences?

Synaptic transmission mediated by NMDA receptors is essential for neuronal survival; blockade of NMDA receptors triggers apoptosis in the developing brain.^{22,23} Environmental enrichment, which stimulates synaptic activity, inhibits spontaneous apoptosis in the hippocampus and is neuroprotective.²⁴ NMDA receptor antagonists, when administered during a critical period after traumatic brain injury or during slowly progressing neurodegeneration, markedly exacerbate damage in the adult brain.²⁵ In addition, NMDA-receptor antagonists cause apoptosis in primary hippocampal cultures and can exacerbate apoptosis induced by staurosporine.²⁶ By contrast, NMDA-receptor-mediated synaptic activation is neuroprotective in vitro and diminishes apoptosis induced by staurosporine.²⁶ Activation of pro-survival transcription factors, such as cAMP response element binding protein

(CREB), accompanies NMDA-receptor-mediated neuroprotection in vitro.²⁶

The Ca²⁺ pool in the immediate vicinity of synaptic NMDA receptors is the “on” switch for extracellular signal-regulated kinase (ERK1/2) mediated synapse-to-nucleus signalling (figure 1).²⁷ One important function of this Ca²⁺ microdomain, which is located near NMDA receptors, is to prolong CREB phosphorylation induced by synaptic stimulation, thereby enhancing CREB-mediated gene expression. CREB controls transcription of pro-survival genes such as brain derived neurotrophic factor (BDNF), vasoactive intestinal peptide (VIP), bcl-2, and mcl-1.²⁸⁻³¹ Thus the survival-promoting properties of NMDA-receptor-mediated synaptic activation could derive from the transcription of such pro-survival genes. Neurons that survive ischaemic insults (located in the penumbra) have high concentrations of the proteins BDNF, bcl-2, and activated CREB,³²⁻³⁵ which suggests sustained induction of pro-survival signals. The logical conclusion to draw from these findings is that suppression of synaptic NMDA-receptor-initiated survival-promoting signals with NMDA antagonists may facilitate the death of such cells.

In conclusion, there is evidence to suggest that synaptic activity mediated by NMDA receptors promotes survival of neurons. Blockade of NMDA-mediated synaptic transmission must therefore be detrimental in situations when support by endogenous measures is required, as occurs after stroke or traumatic brain injury or in chronic neurodegenerative disorders.

Glutamate concentrations after injury

Microdialysis of brain tissue immediately after brain injury in rodents showed that the extracellular glutamate concentration sharply and rapidly increases, exceeding preinsult concentrations 10–100 times.³⁶ However, this increase is only seen for 10–30 min after injury.³⁶ Microdialysis of human brain tissue after traumatic brain injury shows sustained (over days to weeks) but minor (in the range of 50–100%) increases in glutamate concentrations.³⁷⁻³⁹ Unfortunately, it is impossible to measure the increase in extracellular glutamate concentration in human beings immediately after traumatic brain injury.

The long-term (days to weeks) increase in glutamate concentrations in human brain after traumatic injury has been considered neurotoxic and interpreted as an opportunity for delayed therapy with NMDA antagonists (long time-window for therapy initiation). However, this interpretation may be wrong—such mild elevations of glutamate concentration may represent a self-defence mechanism of the injured brain, which may promote survival of endangered neurons and facilitate tissue repair.

The hypothesis

We hypothesise that glutamate may be involved in the acute neurodestructive phase that occurs immediately after traumatic or ischaemic injury, but that after this period it assumes its normal physiological functions, which include promotion of neuronal survival (figure 2). Others have suggested that the prolonged mild increases in glutamate concentrations that have been recorded in human brain after a traumatic injury promote neuronal death.^{37,40–42} We hypothesise here that such mild increases promote neuronal survival after the injury and help neurons to maintain their physiological functions.

Indeed, we have shown that neurons subjected to traumatic brain injury are harmed when NMDA antagonist administration starts after the initial rapid increase in extracellular glutamate concentration has subsided (ie, 1–7 h after trauma).²⁵ When NMDA antagonists were given prior to traumatic injury, neuronal death was prevented.²⁵ Other researchers have shown that delayed treatment with NMDA antagonists suppresses neurogenesis, triggered by focal cerebral ischaemia, in the hippocampus.⁴³ These findings suggest that glutamate kills neurons immediately after the injury, but starts to facilitate repair shortly thereafter. By contrast to its excitotoxic effect, repair mediated by glutamate appears to be long lasting. This is in agreement with the physiological function of glutamate in the nervous system during development (figure 2).

If our hypothesis is correct, the failure of NMDA antagonists in human stroke and traumatic brain injury trials should prompt a re-evaluation of how long “cytoprotective” therapies, based on blockade of NMDA receptors, can be delayed after the onset of the ischaemic or traumatic insults and whether such therapies are justified for chronic neurodegenerative disorders.

Where does the theory of excitotoxicity belong?

For more than three decades the theory of excitotoxicity guided basic research and discovery of novel molecules to stop neurodegeneration.⁴⁴ This theory is based on the fact that glutamate, acting via NMDA receptors, kills neurons immediately after brain injury, but ignores the fact that glutamate preserves endangered neurons in the long term.

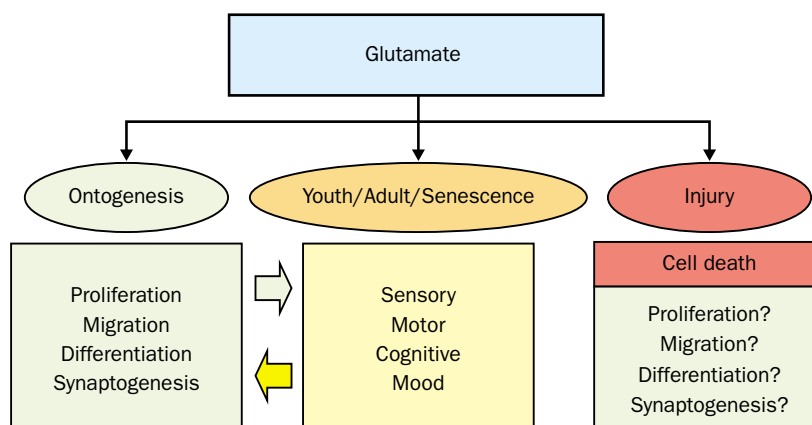


Figure 2. Synaptic activity mediated by NMDA receptors controls sensory, motor, and high cognitive functions (orientation, attention, memory, learning, and speech) and influences mood (yellow). During ontogenesis and early development (green), glutamate, acting via NMDA receptors, is essential for proliferation, migration, and differentiation of neuronal progenitors and immature neurons and for synaptogenesis. Excess of glutamate in the extracellular space in the immediate period after brain injury overactivates NMDA receptors and destroys cells (red). We hypothesise that after this initial neurodestructive phase, the neurotransmitter glutamate, acting via synaptic NMDA receptors, promotes survival of endangered neurons, possibly via the same mechanisms that operate during development. Decreased activation of synaptic NMDA receptors by administration of selective antagonists during a critical period after stroke or trauma may increase neuronal loss and worsen clinical outcome.

The interference with neuronal survival means that NMDA antagonists are unsuitable neuroprotective drugs for use in human emergency medicine. The only way to provide pharmacological neuroprotection with NMDA antagonists would be to administer them before the insult and for a very short period (minutes) after the injury, which is impossible in a clinical emergency setting.

The theory of glutamate-mediated excitotoxicity is correct with regards to the destructive function of glutamate. It should remain in textbooks of neurology and neurobiology as an explanation for acute neurodestruction after injuries to the nervous system, but it should not be regarded as a foundation for development of neuroprotective therapies aimed at delayed clinical application. When designing novel therapies, researchers need to consider and respect glutamate’s physiological role in the brain as well.

By focusing on the destructive effects of glutamate after injury and by ignoring its physiological functions, many patients were unnecessarily exposed to glutamate NMDA antagonists. In addition, many years of investigators’ time and pharmaceutical companies’ investment were wasted, resources that could have been used productively elsewhere.

Lessons to learn

Fundamental mistakes have been made while trying to understand the function of glutamate in the injured brain. Basic scientists must appreciate that drawing inaccurate conclusions can lead to the initiation of inappropriate clinical trials. Clinicians also need to be cautious when faced by enthusiastic “bench scientists”, and must not draw

premature conclusions as to the therapeutic usefulness of new principles even if the medical need is great.

Mistakes can be limited by changing the emphasis of preclinical neuroscience from mechanistic to more applied research. This could result in experimental work that mimics the clinical setting better, so that close conformity to requirements of clinical studies could be established at the research bench. The endpoints relevant for the evaluation of neuroprotective drugs in human stroke and trauma trials—such as the long-term assessment of functional outcome or of mortality after brain injuries—should be used, and not ignored, to determine the therapeutic potential of experimental drugs in rodent stroke or brain trauma models, as well as to assess the extent of the damage measured by histology or imaging. A drug that was effective in rodent stroke or trauma models only when given before the injury should not be promoted to human trials. Furthermore, publication of negative experimental results with putative neuroprotective drugs, although not highly appreciated by basic science, is helpful to clinicians and should be encouraged.

Therapeutic outlook

Although the disappointment regarding the failure of NMDA antagonists in recent clinical trials is high, the neuroprotective potential of NMDA isoform-specific antagonists and specific NMDA-receptor antagonists acting extrasynaptically remains to be explored. Such novel drugs should not yet be disregarded as potential neuroprotectants.

The search for new neuroprotective therapies continues and drugs are entering clinical trials. The lessons learnt will hopefully guide these new developments.

Authors' contributions

Both authors contributed equally to the article.

Conflict of interest

We have no conflict of interest.

Role of the funding source

CI is an employee of the Humboldt University in Berlin. Her research is supported by grants from the Bundesministerium für Bildung und Forschung (BMBF) and the Deutsche Forschungsgemeinschaft (DFG). LT is an employee of Solvay pharmaceuticals. None of the funding sources had a role in writing this review or in the decision to submit it for publication.

References

- Olney JW. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 1969; **164**: 719–21.
- Olney JW, Sharpe LG. Brain lesions in infant rhesus monkey treated with monosodium glutamate. *Science* 1969; **166**: 386–88.
- Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu Rev Neurosci* 1990; **13**: 171–82.
- Beal MF. Mechanisms of excitotoxicity in neurologic diseases. *FASEB J* 1992; **6**: 3338–44.
- Rothman SM. Synaptic activity mediates death of hypoxic neurons. *Science* 1983; **220**: 536–37.
- Simon RP, Swan JH, Griffiths T, Meldrum BS. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* 1984; **226**: 850–52.
- Faden AI, Demediuk P, Panter SS, Vink R. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* 1989; **244**: 798–800.
- Turski L, Bressler K, Rettig K-J, Löschnann P-A, Wachtel H. Protection of substantia nigra from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature* 1991; **349**: 414–18.
- Lipton SA, Rosenberg PA. Excitatory amino acids as a common pathway for neurologic disorders. *N Engl J Med* 1994; **330**: 613–22.
- Albin RL, Greenamyre JT. Alternative excitotoxic hypotheses. *Neurology* 1992; **42**: 733–38.
- Olverman HJ, Jones AW, Watkins JC. L-Glutamate has higher affinity than other amino acids for [³H]-D-AP5 binding sites in rat brain membranes. *Nature* 1984; **307**: 460–62.
- Cavalheiro EA, Lehmann J, Turski L. Frontiers in Excitatory Amino Acid Research. New York: Alan R Liss, 1988.
- Herrling PL. Excitatory amino acids—clinical results with antagonists. New York: Academic Press, 1997.
- Lee J-M, Zipfel GJ, Choi DN. The changing landscape of ischaemic brain injury mechanisms. *Nature* 1999; **399**: A7–A14.
- Meldrum BS. Protection against ischemic neuronal damage by drugs acting on excitatory neurotransmission. *Cerebrovasc Brain Metab Rev* 1990; **2**: 27–57.
- Morris GF, Bullock R, Marshall SB, Marmarou A, Maas A, Marshall LF. Failure of the competitive N-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two phase III clinical trials. *J Neurosurg* 1999; **91**: 737–43.
- Albers GW, Clark WM, Atkinson RP, Madden K, Data JL, Whitehouse MJ. Dose escalation study of the NMDA glycine-site antagonist licostinel in acute ischemic stroke. *Stroke* 1999; **30**: 508–13.
- Davis SM, Lees KR, Albers GW, et al. Selfotel in acute ischemic stroke: possible neurotoxic effects of an NMDA antagonist. *Stroke* 2000; **31**: 347–54.
- Birmingham K. Future of neuroprotective drugs in doubt. *Nature Med* 2002; **8**: 5.
- Sacco RL, DeRosa JT, Haley EC Jr, et al. Glycine antagonist in neuroprotection for patients with acute stroke. GAIN Americas: a randomized controlled trial. *JAMA* 2001; **285**: 1719–28.
- Albers GW, Goldstein LB, Hall D, Lesko LM. Aptiganel hydrochloride in acute ischemic stroke: a randomized controlled trial. *JAMA* 2001; **286**: 2673–82.
- Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 1999; **283**: 70–74.
- Pohl D, Bittigau P, Ishimaru MJ, et al. NMDA antagonists and apoptotic cell death triggered by head trauma in developing rat brain. *Proc Natl Acad Sci USA* 1999; **96**: 2508–13.
- Young D, Lawlor PA, Leone P, Draganow M, Durning MJ. Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Med* 1999; **5**: 448–53.
- Ikonomidou C, Stefovskva V, Turski L. Neuronal death enhanced by N-methyl-D-aspartate receptor antagonists in injured brain. *Proc Natl Acad Sci USA* 2000; **97**: 12885–90.
- Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci* 2002; **5**: 405–14.
- Hardingham GE, Arnold FJL, Bading H. A calcium microdomain near NMDA receptors: on switch for ERK-dependent synapse-to-nucleus communication. *Nature Neurosci* 2001; **4**: 565–66.
- Fink JS, Verhave M, Walton K, Mandel G, Goodman RH. (1991) Cyclic AMP- and phorbol ester-induced transcriptional activation are mediated by the same enhancer element in the human vasoactive intestinal peptide gene. *J Biol Chem* 1991; **266**: 3882–87.
- Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca²⁺ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* 1998; **20**: 709–26.
- Riccio A, Ahn S, Davenport CM, Blendy JA, Ginty DD. Mediation by a CREB family transcription factors of NGF-dependent survival of sympathetic neurons. *Science* 1999; **286**: 2358–61.
- Wang JM, Chao JR, Chen W, Kuo ML, Yen JJ, Yang-Yen HF. The antiapoptotic gene mcl-1 is up-regulated by the phosphatidylinositol 3-kinase/Akt signaling pathway through a transcription factors complex containing CREB. *Mol Cell Biol* 1999; **19**: 6195–206.
- Chen J, Graham SH, Chan PH, Lan J, Zhou RL, Simon RP. BCL-2 is expressed in neurons that survive focal ischemia in the rat. *NeuroReport* 1995; **6**: 394–98.
- Tanaka K, Nagata E, Suzuki S, Dembo T, Nogawa S, Fukuuchi Y. Immunohistochemical analysis of cyclic AMP response element binding protein phosphorylation in focal cerebral ischemia in rats. *Brain Res* 1999; **818**: 520–26.
- Tanaka K, Nogawa S, Ito D, et al. Activated phosphorylation of cyclic AMP response element binding protein is associated with preservation of striatal neurons after focal cerebral ischemia in the rat. *Neuroscience* 2000; **100**: 345–54.
- Walton MR, Draganow M. Is CREB a key to neuronal survival? *Trends Neurosci* 2000; **23**: 48–53.
- Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 1984; **43**: 1369–74.
- Baker AJ, Moulton RJ, MacMillan VH, Shedden PM. Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J Neurosurg* 1993; **79**: 369–72.
- Person L, Hillered L. Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. *J Neurosurg* 1992; **76**: 72–80.
- Bondoli A, Barbi S, Camaioni D, Della Morte F, Magalini SJ. Plasma and cerebrospinal fluid free amino acid concentration in post-traumatic cerebral oedema in patients with shock. *Resuscitation* 1981; **9**: 119–24.
- McCulloch J. Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man. *Brit J Clin Pharmacol* 1992; **34**: 106–14.
- Grotta JC. Acute stroke therapy at the millennium: Consummating the marriage between the laboratory and bedside. *Stroke* 1999; **30**: 1722–28.
- Clausen T, Bullock R. Medical treatment and neuroprotection in traumatic brain injury. *Curr Pharm Des* 2001; **7**: 1517–32.
- Arvidsson A, Kokaia Z, Lindvall O. N-Methyl-D-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. *Eur J Neurosci* 2001; **14**: 10–18.
- Olney JW, Ho OL, Rhee V, DeGubareff T. Neurotoxic effects of glutamate. *N Engl J Med* 1973; **289**: 1374–75.