

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

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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.

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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.<sup>1,2</sup> Follow-up research confirmed that high concentrations of glutamate [100–500  $\mu$ M] induce cell death in vitro<sup>3</sup> and that similar extracellular concentrations are present in the rodent brain and spinal cord during ischaemia or trauma.<sup>3,4</sup> It was subsequently shown that NMDA receptors mediate glutamate-induced cell death in vitro<sup>5</sup> and in vivo.<sup>6,7</sup> 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,<sup>8,9</sup> 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.<sup>10</sup> 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.<sup>11–13</sup> 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.<sup>14</sup>

## 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.<sup>14,15</sup> However, discouraging news started to accumulate<sup>16</sup> as one by one the clinical trials were terminated.<sup>17,18</sup> 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.<sup>14,19</sup> 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.<sup>20,21</sup> 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.<sup>19</sup> 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.<sup>14</sup>

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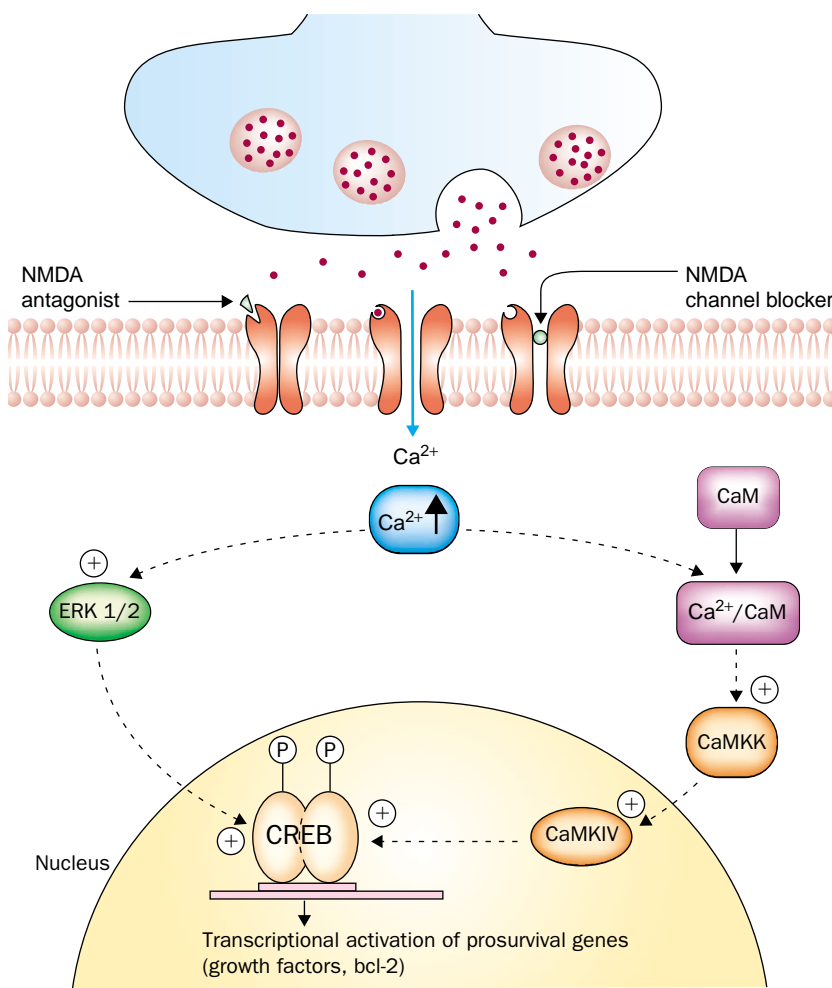


Figure 1. Activation of NMDA receptors leads to Ca<sup>2+</sup> entry and an increased intracellular Ca<sup>2+</sup>-concentration. High concentrations of Ca<sup>2+</sup> switches on extracellular signal regulated kinase (ERK1/2)-mediated synapse-to-nucleus signalling and phosphorylation of cAMP response 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.<sup>22,23</sup> Environmental enrichment, which stimulates synaptic activity, inhibits spontaneous apoptosis in the hippocampus and is neuroprotective.<sup>24</sup> 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.<sup>25</sup> In addition, NMDA-receptor antagonists cause apoptosis in primary hippocampal cultures and can exacerbate apoptosis induced by staurosporine.<sup>26</sup> By contrast, NMDA-receptor-mediated synaptic activation is neuroprotective in vitro and diminishes apoptosis induced by staurosporine.<sup>26</sup> Activation of pro-survival transcription factors, such as cAMP response element binding protein

(CREB), accompanies NMDA-receptor-mediated neuroprotection in vitro.<sup>26</sup>

The Ca<sup>2+</sup> 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).<sup>27</sup> One important function of this Ca<sup>2+</sup> 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.<sup>28-31</sup> 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,<sup>32-35</sup> 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.<sup>36</sup> However, this increase is only seen for 10–30 min after injury.<sup>36</sup> 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.<sup>37-39</sup> 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.<sup>37,40–42</sup> 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).<sup>25</sup> When NMDA antagonists were given prior to traumatic injury, neuronal death was prevented.<sup>25</sup> Other researchers have shown that delayed treatment with NMDA antagonists suppresses neurogenesis, triggered by focal cerebral ischaemia, in the hippocampus.<sup>43</sup> 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.<sup>44</sup> 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.

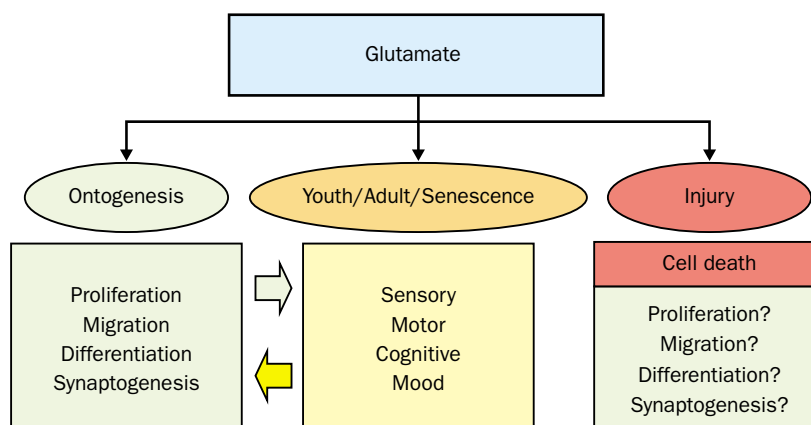


Figure 2. Synaptic activity mediated by NMDA receptors controls sensory, motor, and high cognitive functions (orientation, attention, memory, learning, and speech) and influences mood (yellow). During ontogenesis and early development (green), glutamate, acting via NMDA receptors, is essential for proliferation, 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.

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