# DEVELOPMENT OF CLINICALLY-TOLERATED NMDA RECEPTOR ANTAGONISTS

**STUART A. LIPTON, POSINA V. RAYUDU**
**and H.-S. VINCENT CHEN**

**CNS RESEARCH INSTITUTE**
**HARVARD MEDICAL SCHOOL**
**BOSTON, MA 02115**

Keywords: glutamate, neurological disorders, excitatory post-synaptic current, open-channel blocker

Running title: Clinically-tolerated NMDA antagonists

## Abstract

The clinical potential of most *N*-methyl-D-Aspartate (NMDA) receptor antagonists as neuroprotectants is hampered by the fact that they block physiological NMDA receptor mediated activity at neuroprotective concentrations. However, unlike other NMDA antagonists, the open-channel blocker memantine displays relative sparing of excitatory post-synaptic currents (epsc) at neuroprotective levels, apparently due to its purely uncompetitive mode of antagonism. In contrast, the failure of MK-801 to spare epscs suggests that the clinical potential of an open-channel blocker is determined by its on- and off- rate constants. Is there a set of unique on- and off- rate constants that optimizes the mutually conflicting demands of neuroprotection and clinical tolerance? Using kinetic theory and exploiting the differences in temporal profile of glutamate concentrations under physiological and pathological conditions, we calculated optimal rate constants. The calculations were subject to the following three constraints: the antagonist should (i) block sustained NMDA receptor activity as much as possible, (ii) attain steady-state blockade within a reasonable time (seconds), and (iii) spare NMDA epscs to the greatest degree possible. Our calculations establish the best theoretical parameters for clinically-tolerated NMDA open-channel blockers and raise the possibility of using specific NMDA receptor channel blockers prophylactically for various neurologic disorders.

## Introduction

Glutamate is a major excitatory neurotransmitter in the brain. During synaptic transmission glutamate is released by pre-synaptic neurons and activates both NMDA and non-NMDA subtype of glutamate receptors of post-synaptic neurons. Calcium influx via NMDA subtype of ion-channels during epscs is crucial for various brain functions such as learning, memory, and perception. In contrast to the transient NMDA receptor activation that mediates normal physiological functions, sustained NMDA receptor activity has been implicated in various neurologic diseases. Excessive calcium influx during pathological NMDA receptor activation leads to neuronal degeneration and cell death in acute neurologic disorders such as stroke, trauma, epilepsy and has causative role in chronic diseases such as AIDS dementia, Huntington's disease, and Alzheimer's disease (Lipton and Rosenberg, 1994). One approach to neuroprotection is to block NMDA receptor activity. This strategy, though effective in offering neuroprotection, inevitably interferes with physiological NMDA epscs because NMDA antagonists, both competitive and non-competitive, cannot discriminate pathological activation of NMDA receptor from physiologic NMDA receptor activity (Lipton, 1993).

Memantine, an NMDA open-channel blocker, has been shown to be neuroprotective against excitotoxicity mediated by NMDA receptors (Chen et al., 1992). Unlike other classes of NMDA antagonists, memantine displayed relative sparing of epscs (Rayudu et al., 1996), apparently due to its purely uncompetitive mode of antagonism (Chen and Lipton, 1997). But MK-801, which is also an NMDA open-channel blocker, did not spare epscs. Compared to memantine, MK-801's inability to spare epscs may be due to its slow unblocking rate (Huettner and Bean, 1988). These experimental results suggest that on- and off- rates determine whether an open-channel blocker will spare epscs or not. Ideal NMDA antagonist should block pathologic NMDA activation in the disease locus while sparing NMDA epscs in other brain regions. Such ideal NMDA antagonists can be used prophylactically for various neurologic disorders because they spare NMDA epscs. Is

there a set of optimal on- and off- rate constants that satisfies the mutually conflicting demands of neuroprotection and physiological synaptic transmission?

During physiological synaptic release, peak glutamate concentration has been measured to be 1 mM and it decays with a time constant of 1 msec (Clements et al., 1992; Figure 1a). In pathological situations, glutamate concentration reaches 0.1 mM for prolonged duration on the order of minutes (Hagberg et al., 1985; Figure 1b). Exploiting these differences in the temporal profile of glutamate in physiological and pathological cases and using kinetic models of NMDA receptor, we obtained the optimal values for on- and off- rate constants of NMDA open-channel blocker. The calculations were subject to the following three constraints: the antagonist should (i) block sustained NMDA receptor activity as much as possible, (ii) attain steady-state blockade within reasonable timescale (seconds), and (iii) spare NMDA epscs to the greatest possible extent.

## Results

### Pathological condition

The temporal profile of glutamate in disease states is pronouncedly different from that of normal synaptic transmission. To begin to model the problem of clinical tolerance of NMDA antagonists, we used a simplified 3-state kinetic scheme (Figure 2). Within the framework of this NMDA receptor model, we can analytically calculate NMDA current A(t) in response to pathological glutamate activation using the relation (see Appendix),

$$dA/dt = -A(k_1X + k_2) + k_1X \qquad \text{----- (1)}$$

With rate constants of glutamate activation of NMDA receptor $k_1 = 5 * 10^6\ M^{-1}sec^{-1}$ and $k_2 = 5\ sec^{-1}$ (Clements et al., 1992), we find that pathological concentrations of glutamate, X = 0.1 mM results in saturating NMDA current response (Figure 3a). NMDA current reaches steady-state with a time constant of 2 msec, consistent with experimental value (McBain and Mayer, 1994). In the presence of blocker, NMDA current A(t) is given by the solution of

$$d^2A/dt^2 + (k_1X + k_3Y + k_2 + k_4)dA/dt + (k_1k_3XY + k_1k_4X + k_2k_4)A = k_1k_4X \quad \text{-----(2)}$$

Assuming neuroprotection by the blocker is proportional to the fraction of NMDA current inhibited, we obtain a set of rate constants that give an arbitrary percentage block (v) of NMDA current. Thus, the degree of neuroprotection [f(v)] can be predicted from the on ($k_3$) and off ($k_4$) rate constants of the blocker,

$$100k_3Y = (k_3Y + k_4)v \quad \text{-----(3)}$$

Even though all points on the line given by equation (3) give v% block at steady-state; they differ in the time it takes to attain steady-state blockade. If the time constant of blockade is on the order of many minutes the blocker will not be neuroprotective because by the time blocker brings about steady-state inhibition of NMDA current, ionic gradients will be irreversibly dissipated (Rayudu et al., 1997). The blocking time constant (j in Figure 3a) is given by,

$$j = 1/(k_3Y + k_4) \quad \text{-----(4)}$$

For the blocker to be maximally neuroprotective j should preferably on the order of seconds.

**Physiological condition**

In response to synaptically released 1 mM glutamate transient, NMDA epsc decays from its peak value with a time constant of 200 msec (Figure 3b). In the presence of blocker NMDA epsc decay is governed by two time constants,

$$q_1 = 1/k_2 \quad \text{-----(5)}$$

$$q_2 = 1/(k_3Y + k_4) \quad \text{-----(6)}$$

We can minimize the inhibition of NMDA epsc by making $q_2$ small relative to $q_1$. Assuming clinical tolerance of the blocker is inversely proportional to the fraction of NMDA epsc blocked (r in Figure 3b), we obtain the locus of on- and off- blocker rate constants representing clinical tolerance constraint,

$$(k_3Y + k_4)r = 5 \qquad \text{-----(7)}$$

Taking blocker concentration Y = 1 μM (which is the order of magnitude concentration that an exogenous molecule can readily attain in the extracellular space of brain) and r = 0.01 we get the set of all on- and off- rate constants sparing NMDA epscs (Figure 4a). All the rate constants that block 90% (v = 90 in equation 3) of the pathological sustained NMDA current are shown in Figure 4b. Intersection of these two lines (Figure 4a and 4b) that lies beyond the dark region in Figure 4c (the set of all rate constants with blocking time constant of less than 1 sec) gives the optimal blocker. Subject to these constraints, we solve for ideal rate constants: $k_3 = 4.5 * 10^8\ M^{-1}sec^{-1}$ and $k_4 = 50\ sec^{-1}$. These rate constants give neuroprotection from excitotoxicity and also spare NMDA epscs. We find the affinity of optimal NMDA antagonist to be on the order of 0.1 μM.

## Conclusion

We developed a framework to systematically investigate clinical tolerance of NMDA antagonists. These results will help guide the current trail-and-error methods of drug design and also raise the possibility of using specific NMDA channel blockers prophylactically for various neurologic disorders because they spare NMDA epscs. More detailed models of NMDA receptor behavior (including additional receptor states such as desensitization) and synaptic transmission (train of epscs at physiologic frequencies) should provide added insight into the characteristics of clinically-tolerated NMDA antagonists.

## Appendix

According to the reaction scheme shown in Figure 2, in the absence of blocker

$$dA/dt = -Ak_2 + Ck_1X \quad \text{-----(1a)}$$

Similarly,

$$dc/dt = Ak_2 - Ck_1X \quad \text{-----(2a)}$$

Since the total number of receptors is constant, we can eliminate C from equation 1a,

$$dA/dt = -A(k_1X + k_2) + k_1X \quad \text{-----(3a)}$$

Solving for A(t) using the initial condition A(0) = 0, we get

$$A(t) = (k_1X/(k_1X + k_2))*(1 - e^{-(k_1X + k_2)t}) \quad \text{-----(4a)}$$

Thus, in response to a constant glutamate concentration, NMDA current reaches a steady state value $k_1X/(k_1X + k_2)$ with a time constant $1/(k_1X + k_2)$.

In the presence of blocker, there are 3 states and we get a second order differential equation

$$d^2A/dt^2 + (k_1X + k_3Y + k_2 + k_4)dA/dt + (k_1k_3XY + k_1k_4X + k_2k_4)A = k_1k_4X \quad \text{-----(5a)}$$

Solving for A(t), we find that the steady-state NMDA current in the presence of blocker is $k_1k_4X/(k_1k_3XY + k_1k_4X + k_2k_4)$. In order for the blocker to inhibit v % of NMDA current at steady state, the blocker rate constants must satisfy

$$100k_3Y = v(k_3Y + k_4) \quad \text{-----(6a)}$$

The blocking time constant is given by one of the two solutions of

$$j^2 + j(k_1X + k_3Y + k_2 + k_4) + (k_1k_3XY + k_1k_4X + k_2k_4) = 0 \quad \text{-----(7a)}$$

i.e.

$$j = 1/ (k_3Y + k_4) \quad \text{-----(8a)}$$

We can approximate the synaptic glutamate of 1mM decaying with 1msec time constant with 1 mM glutamate pulse of 1 msec duration. In the absence of blocker, in response to this glutamate pulse, NMDA current decays from the peak with a time constant of $1/k_2$, which is the epsc decay time constant.

In the presence of blocker, NMDA current following 1 msec glutamate pulse is governed by

$$d^2A/dt^2 + (k_3Y + k_2 + k_4)dA/dt + Ak_2k_4 = 0 \quad \text{-----(9a)}$$

Solving for NMDA epsc, we get

$$A(t) = ((k_3Y + k_4)/( k_3Y - k_2 + k_4))*e^{-t/q_1} - k_2/( k_3Y - k_2 + k_4)*e^{-t/q_2} \quad \text{-----(10a)}$$

The two time constants governing NMDA epsc decay are

$$q_1 = 1/k_2 \quad \text{-----(11a)}$$

$$q_2 = 1/(k_3Y + k_4) \quad \text{-----(12a)}$$

In order for the blocker to spare (total charge flux during epsc in the presence of blocker should be almost same as that of the control epsc) NMDA epscs, we make $q_2$ small relative to $q_1$.

## Acknowledgements

We thank Drs: P. Gaudiano, N. Sucher, and A. Przybyszewski for helpful discussions.

## References

Chen, H-S.V., et al. (1992). Open-channel block of *N*-methyl-D-Aspartate (NMDA) responses by memantine: Therapeutic advantage against NMDA receptor mediated neurotoxicity. J. Neuroscience 12 (11), 4427-4436.

Chen, H-S.V., and Lipton, S.A. (1997). Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. J. Physiology 499, 27-46.

Clements, J.D., et al. (1992). The time course of glutamate in the synaptic cleft. Science 258, 1498-1501.

Hagberg, H., et al. (1985). Ischemia-induced shift of inhibitory and excitatory amino acids from intra- to extracellular compartments. J. Cerebral Blood Flow and Metabolism 5, 413-419.

Huettner, J.E., and Bean, B.P. (1998). Block of *N*-methyl-D-Aspartate activated current by the anti-convulsant MK801: selective binding to the open-channels. Proc. Natl. Acad. Sci. 85, 1307-1311.

Lipton, S.A. (1993). Prospects for clinically tolerated NMDA antagonists: open-channel blockers and alternative redox states of nitric oxide. Trends in Neuroscience 16 (12), 527-532.

Lipton, S.A., and Rosenberg, P.A. (1994). Excitatory amino acids as a final common pathway for neurological disorders. New England Journal of Medicine 330, 613-622.

McBain, C.J., and Mayer, M.L. (1994). *N*-methyl-D-aspartic acid receptor structure and function. Physiological Reviews 74 (3), 723-760.

Rayudu, P.V., Chen, H.-S.V., Lipton, S.A. (1996). Memantine, a clinically-tolerated NMDA open-channel blocker, displays relative sparing of epscs. Soc. Neurosci. Abstr. 22:1537.

Rayudu, P.V., Demilia, D.M., Gaudiano, P., Lipton, S.A. (1997). Apoptosis vs. necrosis: role of $Ca^{2+}$ and energy failure. Soc. Neurosci. Abstr. 23, 2291.

## Figure Legends

**Figure 1**. Temporal profile of glutamate concentration. **a**. Physiological glutamate time course. During synaptic transmission, glutamate concentration X reaches a peak of 1 mM and decays with a time constant of 1 msec. **b**. Pathological glutamate concentration. In disease states glutamate concentration is 0.1 mM for prolonged duration on the order of minutes.

**Figure 2**. Three-state kinetic model. C, A, and B are closed, open, and blocked NMDA receptors respectively. $k_1$ and $k_2$ are glutamate rate constants. $k_3$ and $k_4$ are NMDA open-channel blocker rate constants. X and Y are the concentrations of glutamate and NMDA blocker, respectively.

**Figure 3**. NMDA current time course. **a**. Sustained NMDA current A(t) in response to pathologic glutamate activation shown in figure 1b. In the presence of blocker, a fraction (v) of the sustained NMDA current is blocked with a time constant denoted by j. In order to maximize neuroprotection, v has to be large and j small. **b**. In response to synaptically released glutamate shown in figure 1a, NMDA epsc reaches a saturating peak and decays with 200 msec time constant. In the presence of blocker decay is faster and a fraction (r) of the total charge flux during NMDA epsc is blocked. For maximal clinical tolerance r has to be small.

**Figure 4**. Constraints and the corresponding rate constants. **a**. Clinical tolerance constraint. The set of all on- and off- rate constants of NMDA open-channel blocker that spare NMDA epsc is given by the line $k_3Y + k_4 = 500$. **b**. Neuroprotection constraint. The locus of on- and off- rate constants that block 90% of the sustained NMDA current is given by the line $k_3Y = 9k_4$. **c**. Time course of blockade. The black region denotes all blocker rate constants that give steady-state block with time constant greater than 1 sec.

**Figure 1**

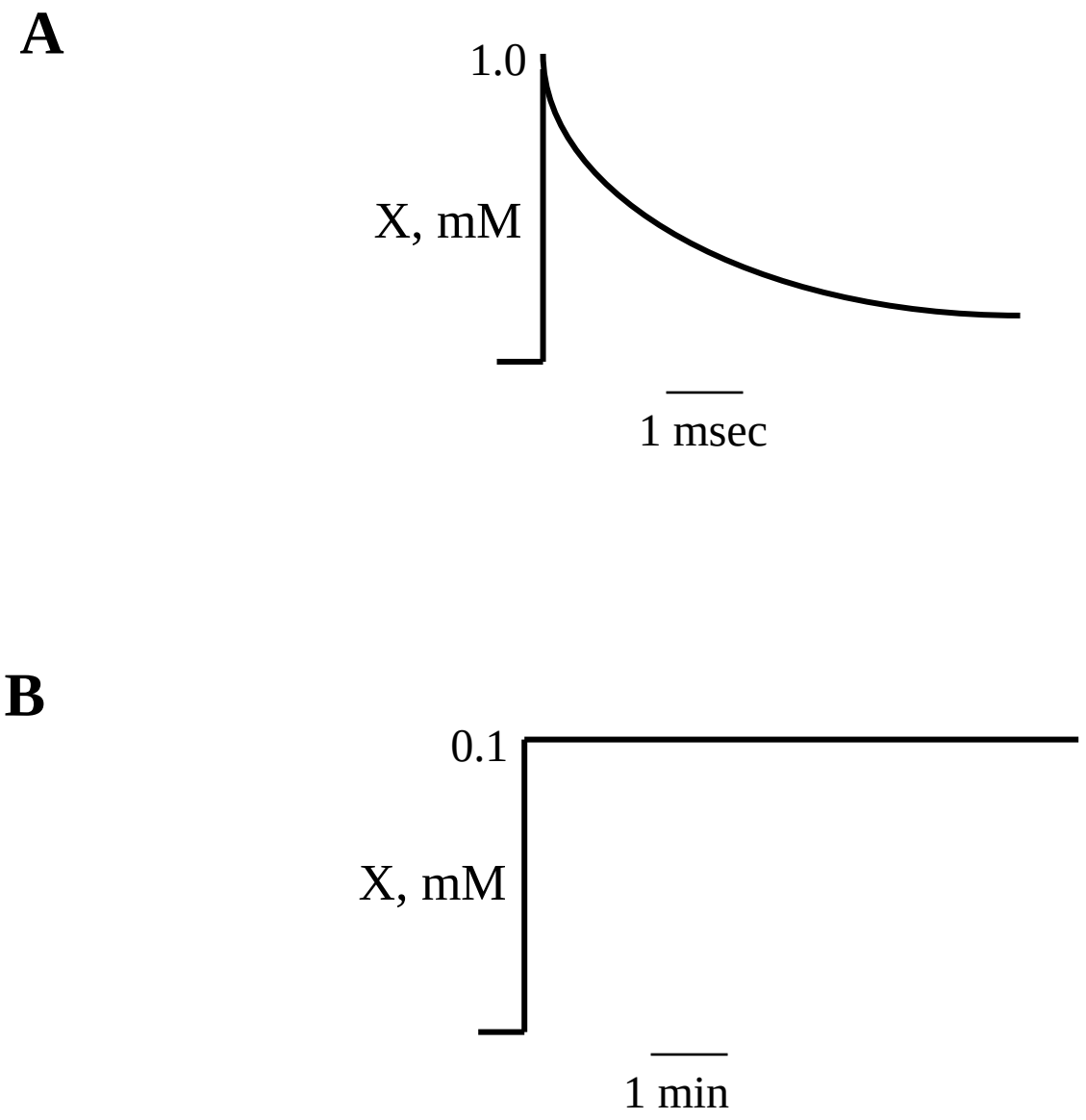


**Figure 2**

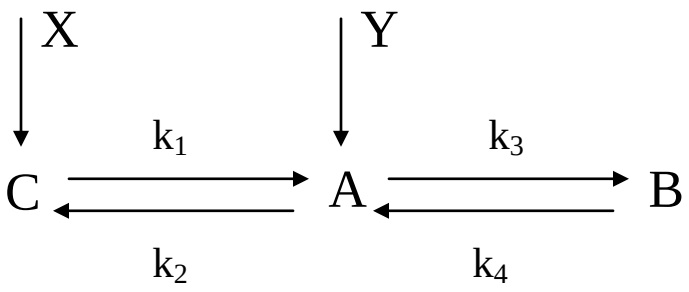
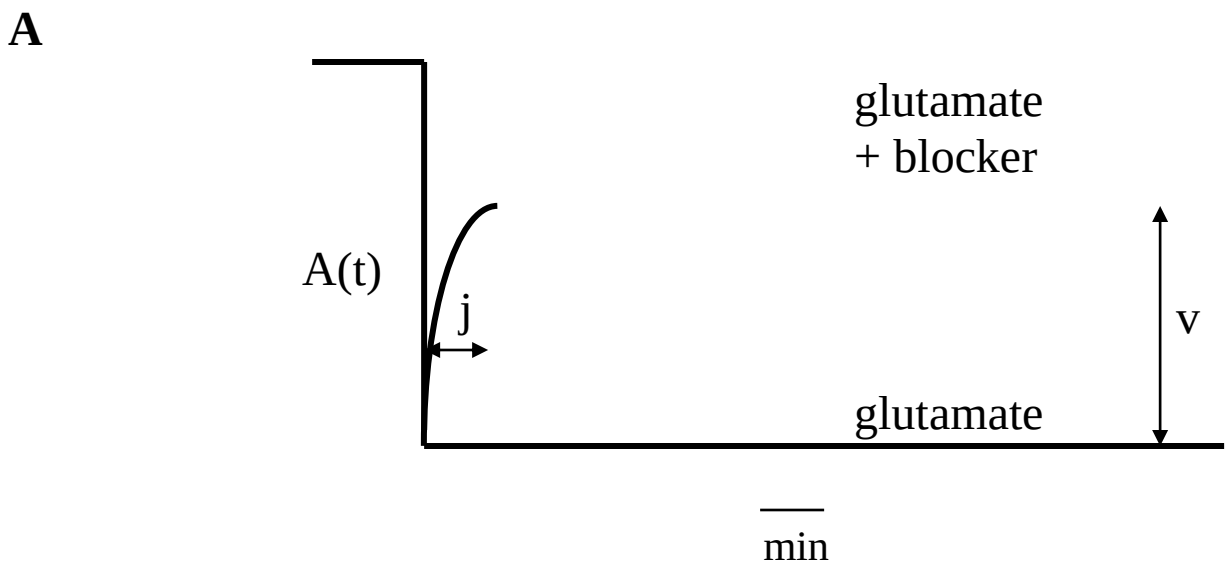


**Figure 3**


A
glutamate
+ blocker
A(t)
j
v
glutamate
min

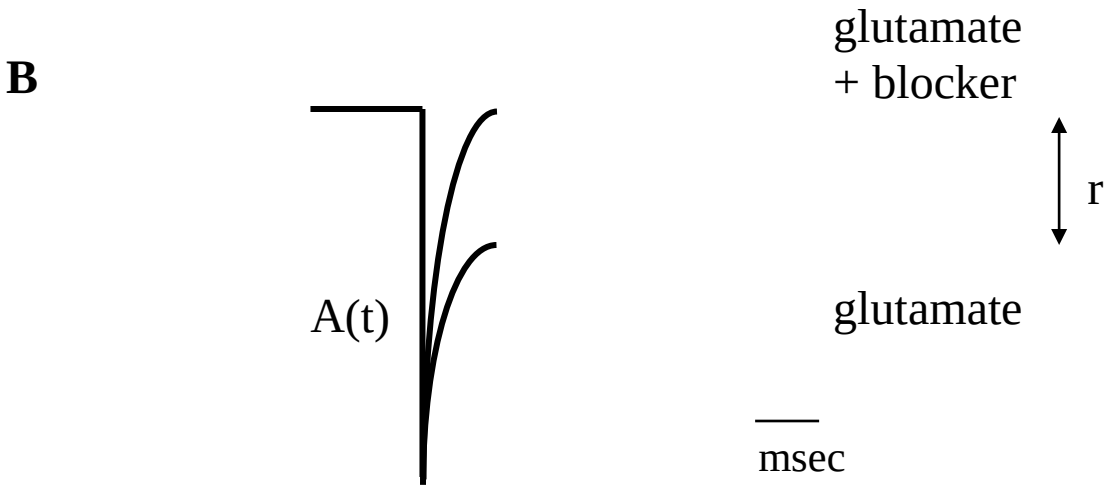
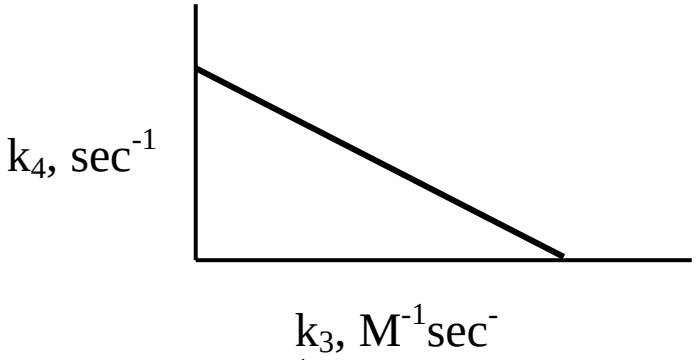
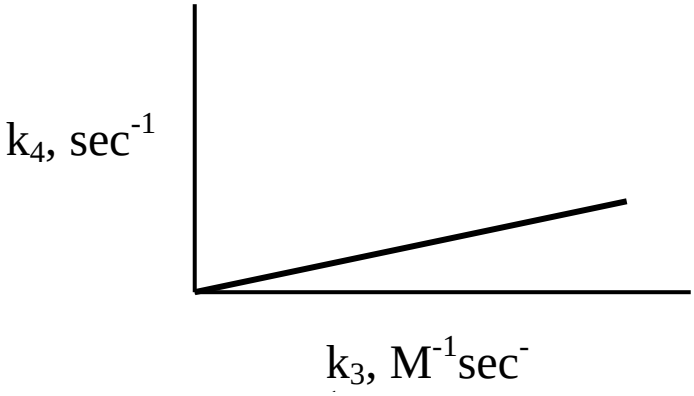

B
glutamate
+ blocker
r
A(t)
glutamate
msec

**Figure 4**

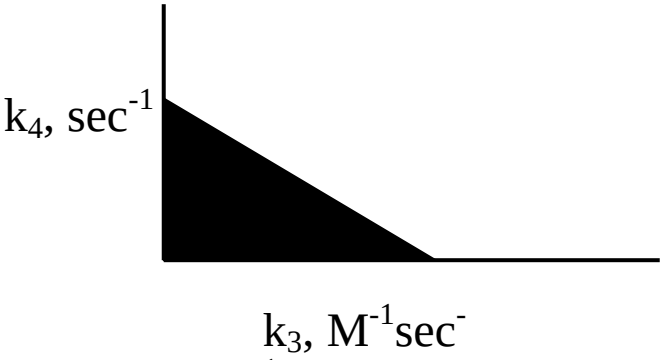
**A**




**B**




**C**



SOCIETY FOR NEUROSCIENCE

# ABSTRACTS

VOLUME 23, PART 2

**27TH ANNUAL MEETING**

**NEW ORLEANS, LA.**

**OCTOBER 25–30, 1997**

**688.7**

HETEROSYNAPTIC INTERACTION IN CEREBELLAR PURKINJE CELLS MEDIATED BY A METABOTROPIC GLUTAMATE RECEPTOR. F. Tempia*, M. C. Miniaci, D. Anchisi and P. Strata. Dept. of Neuroscience, University of Torino, I-10125 Torino, Italy

Information directed to the cerebellar cortex is combined and elaborated at the level of Purkinje cells. The presynaptic elements carrying such information are climbing and parallel fibers, both acting on glutamate receptors of the AMPA type. In addition, parallel fibers activate a metabotropic glutamate receptor (mGluR). Purkinje cells were whole-cell recorded in acute rat cerebellar slices in voltage-clamp conditions. Parallel fiber activation by brief bursts of stimuli of more than 10 Hz in the presence of ionotropic glutamate receptors blockers evoked a slow current with a mean latency of 0.3 s, a duration of 3.2 s and an amplitude of about 100 pA. In the same conditions climbing fibers were ineffective in evoking the inward current. The parallel fiber evoked current was reduced to half amplitude by the addition of 200 μM AIDA, a selective group I mGluR antagonist. Following a transient depolarization to 0 mV of the Purkinje cell, the first parallel fiber burst evoked a markedly potentiated response when it was delivered in a very broad time window of more than 90 s from the depolarization. The addition of 10 mM BAPTA to the internal solution abolished the response. This effect can be interpreted as a calcium triggered activation of a mechanism that acts as a switch setting the cell in a potentiated state. The first incoming parallel fiber burst gives a larger response and at the same time resets the switch to its default state. Such calcium dependent effect is a novel feature of synaptic signal elaboration that confers to Purkinje cells a response selectivity for the first parallel fiber burst following climbing fiber discharge while later signals, less related to climbing fiber activity, are discarded. Funded by MURST and CNR.

**688.8**

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION MODULATES EXCITATORY SYNAPTIC TRANSMISSION FROM TRIGEMINAL PREMOTONEURONS TO MOTONEURONS. C.A. Del Negro* and S.H. Chandler. Dept of Physiological Science, UCLA. Los Angeles, CA 90095-1568.

Synaptic excitation of trigeminal motoneurons (TMNs) is accomplished by activation of ionotropic glutamate receptors. Metabotropic glutamate receptors (mGluRs) are also present on TMNs and their activation can modify synaptic transmission. Whole-cell recordings were obtained from visually identified TMNs in neonatal rat brainstem slices (age 0-7 days). Stimulation of individual trigeminal premotoneurons (TPMns) evoked unitary excitatory-amino acid (EAA)-mediated synaptic currents (eEPSCs) with NMDA and non-NMDA components. Amplitude histograms were generated from eEPSCs recorded in control conditions or during bath application of mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD, 100 μM). 1S,3R-ACPD application decreased the mean amplitude of the eEPSCs, without changing the amplitude of the smallest evoked response, and increased the rate of failures. Spontaneous miniature EPSCs (mEPSCs) were recorded under TTX (0.5 μM) conditions. The frequency of occurrence and mean amplitude of mEPSCs were reduced during 1S,3R-ACPD application. However, the frequency and mean amplitude of spontaneous EPSCs (sEPSCs) recorded in the absence of TTX showed either enhancement (n=3) or reduction (n=2) after 1S,3R-ACPD application, suggesting that soma-dendritic membranes of TPMns were excited in some cells causing increased synaptic transmission. Postsynaptically, 1S,3R-ACPD application increased the input resistance of TMNs by reducing a leakage $K^+$ current. These data indicate that mGluR activation presynaptically inhibits EAA-mediated synaptic transmission from TPMns to TMNs and increases postsynaptic excitability. Endogenous activation of mGluRs could serve as a mechanism for enhancement of the signal to noise ratio during relay of oral-motor commands to TMNs. Funded by NIH-NIDR DE 06193.

**688.9**

**DEVELOPMENT OF CLINICALLY-TOLERATED NMDA RECEPTOR ANTAGONISTS.** Stuart A. Lipton, Posina V. Rayudu and H.-S. Vincent Chen*. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The clinical potential of most NMDA antagonists as neuroprotectants is hampered by the fact that they block physiological NMDA receptor-mediated activity at neuroprotective concentrations (Lipton, *TINS* 12:527, 1993). However, unlike other NMDA antagonists, the open-channel blocker memantine displays relative sparing of EPSCs at neuroprotective levels (Rayudu et al., *Soc. Neurosci Abstr.* 22:606.17, 1996), apparently due to its purely uncompetitive mode of antagonism (Chen and Lipton, *J. Physiol.*, 499:27, 1997). In contrast, the failure of MK-801 to spare EPSCs suggests that the clinical potential of an open-channel blocker is determined by its on- and off-rate constants. Is there a set of unique on- and off-rate constants that optimizes the mutually conflicting demands of neuroprotection and clinical tolerance? Using kinetic theory and exploiting the differences in temporal profile of glutamate concentrations under physiological (Clements et al., *Science* 258:1498, 1992) and pathological conditions (Hagberg et al., *J. Cereb. Blood Flow Metab.*, 5:413, 1985), we obtained optimal values for these rate constants. The calculations are subject to the following 3 constraints: the antagonist should *(i)* block sustained NMDA receptor activity as much as possible, *(ii)* attain steady-state blockade within a reasonable time (seconds), and *(iii)* spare EPSCs to the greatest degree possible. Our calculations establish the best theoretical parameters for clinically-tolerated NMDA open-channel blockers and should help guide future clinical development of these drugs.

Funded by NIH grant P01 HD29587 (to S.A.L.).

**688.10**

NEUROPHARMACOLOGICAL CHARACTERIZATION OF NICOTINE INDUCED GLUTAMATE RELEASE IN THE RAT NUCLEUS ACCUMBENS Malcolm S. Reid* and S. Paul Berger. UCSF/VAMC, Substance Abuse Treatment Research 116W, 4150 Clement St., San Francisco, CA 94121

Previously, we have shown that an injection of cocaine or amphetamine stimulates glutamate release in the nucleus accumbens and that this effect is calcium dependent, mediated by dopamine$_1$ and becomes sensitized following chronic cocaine pretreatment. In the present study, nicotine stimulated glutamate release in the nucleus accumbens was studied in naive and chronically pretreated animals using *in vivo* microdialysis. All studies were done with freely moving, awake animals implanted 24 hrs prior to testing with 2 mm microdialysis probes (CMA 10) via guide cannulae. An acute injection of nicotine (free base (-) enantiomer, 0.6 mg/kg, s.c.) produced a short lasting increase in extracellular glutamate levels which was unaffected by removing calcium from the perfusion medium. Following local 6-OHDA lesions in the nucleus accumbens (2 wk prior to testing) the increase in glutamate levels was enhanced versus sham lesioned animals. Chronic nicotine pretreatment consisted of 15 daily nicotine (0.6 mg/kg, s.c) or saline injections, given in the test chamber, followed by testing with nicotine (0.6 mg/kg, s.c.) on day 16. Evidence for sensitization was observed with nicotine induced dopamine release and locomotor activity, however, nicotine induced glutamate release was reduced in the nicotine pretreated animals. These results demonstrate that nicotine stimulates extracellular glutamate levels in the nucleus accumbens. This effect is calcium and dopamine independent and becomes tolerant following chronic pretreatment. Further studies investigating the neuronal origin of the stimulated glutamate levels are indicated. (Supported by NARSAD)

**688.11**

OXOTREMORINE ATTENUATES L-TRANS-PDC-EVOKED GLUTAMATE LEVELS IN RAT STRIATUM. S.M. Rawls* and J.F. McGinty. Dept. of Anat. and Cell Biol., East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Activation of muscarinic receptors attenuates glutamatergic transmission in striatal slices[1]. Moreover, muscarinic receptor stimulation decreases psychostimulant-induced behavior and striatal gene expression[2], which depend on glutamatergic and dopaminergic transmission. Therefore, we investigated whether local activation of intrastriatal muscarinic receptors would reduce extracellular glutamate levels evoked by L-trans-pyrrolidine-2,4-dicarboxylic acid (L-trans-PDC), a competitive inhibitor of glutamate reuptake. The nonselective muscarinic agonist, oxotremorine, and L-trans-PDC were co-perfused by reverse microdialysis into the striatum of conscious rats. Dialysate samples were collected every 15 min. Glutamate was assayed using HPLC coupled with diode array detection. Basal glutamate levels were unaffected by oxotremorine. However, oxotremorine (5, 0.5, and 0.05 μM) significantly attenuated L-trans-PDC-evoked extracellular glutamate levels in a concentration-dependent manner. Previous work from this laboratory has shown that reduced calcium and tetrodotoxin perfusion decreases L-trans-PDC-evoked glutamate levels by 50%[3]. The present study also demonstrated that muscarinic receptor activation attenuates L-trans-PDC-evoked extracellular glutamate levels by 50%. Based on these data, we hypothesize that presynaptic muscarinic receptor activation attenuates calcium- and/or action potential-dependent glutamate efflux evoked by L-trans-PDC from corticostriatal and thalamostriatal terminals by attenuating transsynaptic activity. Supported by DA03982.

1. Hernandez E. et al., 1996, Soc. Neurosci. Abs., 410.
2. Wang J.Q. and McGinty J.F., 1996, *Neuroscience* **75**, 43-56.
3. Rawls S.M. and McGinty J.F., 1997, *J. Neurochem.* **68**, 1553-1563.

**688.12**

ENHANCEMENT OF *N*-METHYL-D-ASPARTATE-INDUCED $[Ca^{2+}]_i$ RESPONSE BY SULPIRIDE: INVOLVEMENT OF PROTEIN KINASE AND MODULATION BY SIGMA LIGANDS. T. Hayashi[1,2], T.-P. Su[1*], A. Kagaya[2], T. Oyamada[2] and S. Yamawaki[2]. [1]Unit on Pathobiology, Molecular Neuropsychiatry Section, IRP, NIDA/NIH, Baltimore, MD 21224, U.S.A. and [2]Department of Psychiatry and Neurosciences, Hiroshima University School of Medicine, Hiroshima 734, Japan.

The effects of sulpiride, a selective dopamine D2 antagonist, on *N*-methyl-D-aspartate (NMDA)-induced changes in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) were investigated using the primary culture of rat frontal cortical neurons. Frontal cortical neurons of rat fetuses at embryonic day 18 were cultured for 6-7 days in DMEM containing 5% FCS and 5% horse serum. Cells were loaded with fura2-AM and the $[Ca^{2+}]_i$ in single neurons was measured using a fluorescence video microscope. A subacute treatment with sulpiride (10 nM-1μM, >2 days) enhanced NMDA (100 μM)-induced increase in $[Ca^{2+}]_i$ in a dose-dependent manner. S(-)Sulpiride enhanced the $[Ca^{2+}]_i$ responses induced by NMDA, whereas R(+)-sulpiride was inactive. Subchronic treatment with phorbol-12-myristate-13-acetate also enhanced the NMDA-induced increase in $[Ca^{2+}]_i$. Subchronic treatments with protein kinase inhibitors, H-7 and H-89 (selective for A kinase), abolished the enhancement. *Sigma* ligand DTG (10 nM-1μM), when added into the medium, potentiated the $[Ca^{2+}]_i$-enhancing effect of sulpiride. Haloperidol, a putative *sigma* receptor antagonist, at concentrations without effect on its own (10 nM-1μM), did not affect the action of sulpiride. Haloperidol, however, antagonized the DTG's potentiating effect on sulpiride. Our results therefore suggest that the effect of sulpiride on NMDA-induced $[Ca^{2+}]_i$ changes is mediated *via* the activation of protein kinase and that *sigma* receptors can modulate this mechanism in an agonist-antagonist fashion.