



Mechanisms of HIV-induced Neurodegeneration:

Role of NMDA Receptor Subtypes in Mediating Neuronal Cell Death

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BACKGROUND

- HIV infection of the central nervous system results in neuronal death and damage in the cerebral cortex, basal ganglia, and hippocampus
- Neurons in these regions are also relatively sensitive to excitotoxic insults that occur through overactivation of NMDA receptors (NMDAR)
- HIV-infected macrophages/microglia in the brain release neurotoxins, including glutamate or quinolinic acid that activate NMDARs

GOAL

To define the role of NMDARs in HIV-induced neurodegeneration, we examined effects of HIV-infected macrophages on survival of developing, primary, rodent hippocampal neurons, whose developmental expression of NMDAR subtypes is well established both *in vivo* and *in vitro*

RESULTS

Days *in vitro* (DIV)

■ 7 ■ 14 ■ 21

* p<0.05 versus same day Jago
** p<0.01 versus same day Jago
p<0.01 versus same day Mock

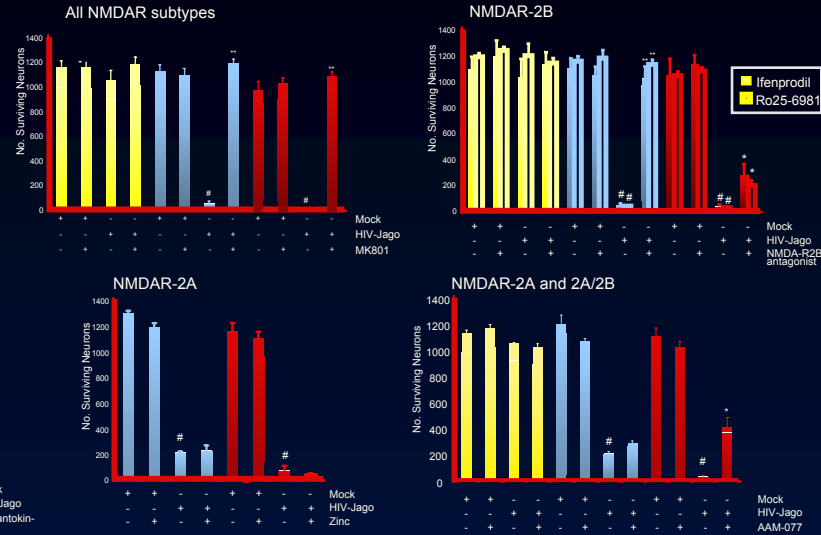
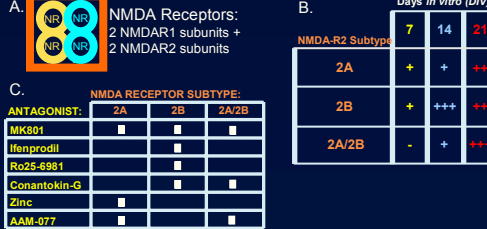


Figure 1. HIV-infected macrophages release neurotoxic factors.

A. P24 output from macrophages infected with the CNS HIV-1 isolate Jago is blocked by the non-nucleoside RT inhibitor efavirenz. B. Supernatants from Jago-infected macrophages are neurotoxic and neurotoxicity is blocked by inhibition of virus replication by Efavirenz.



NMDA Receptor Subtypes A. NMDA receptors are composed of two NR1 subunits, which bind glycine, and two NR2 subunits, which bind glutamate and quinolinic acid. There are 4 possible NR2 subtypes (NR2A-R2D), and expression of NR2 subtypes in hippocampal neurons changes over time (B). Various subtype specific, NMDA receptor antagonists were used in this study (C).

METHODS

Primary rodent cultures of hippocampal neurons were maintained for 7, 14, or 21 days *in vitro* (DIV). Cultures were preincubated with NMDAR antagonists for 1 hour and exposed for 24 hours to supernatants from macrophages infected with the HIV CNS-isolate, Jago. Cultures were then fixed and stained for MAP-2, a neuronal marker. The number of surviving neurons was determined for each condition and statistical comparisons were made by a paired t-test (n=6).

SUMMARY & CONCLUSIONS

NEUROPROTECTION

Antagonist	DIV14	DIV 21	NMDA-R subtype
MK801	+++	+++	2A, 2B, 2A/2B
Ifenprodil	+++	+	2B
Ro25-6981	+++	+	2B
Conantokin-G	+++	+++	2B, 2A/2B
Zinc	-	-	2A
AAM-077	-	++	2A, 2A/2B

- Developing hippocampal neurons acquire susceptibility to HIV-induced neurotoxicity dependent upon NMDA-receptor function
- Subtype expression of NMDA receptor subunits predicts neuronal susceptibility
 - NMDAR-2B and 2A/2B in combination, predominant subtypes in the forebrain and hippocampus, play a dominant role

IMPLICATIONS & FUTURE DIRECTIONS

- Neurotoxicity in maturing neurons is blocked by inhibitors of NMDA receptors containing 2B alone and 2A and 2B in combination, reflecting the emergence of 2A/2B hetero-receptors in mature hippocampal neurons.
- Confirm NMDA receptor subtype expression in our hippocampal model system
- Determine linkage to downstream cell death pathways triggered during HIV-induced neurotoxicity