

Activity dependent regulation of NMDA receptors in the rat substantia nigra pars compacta

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This study aimed to investigate NMDA receptor regulation in neurones of the substantia nigra pars compacta (SNc), one of the key brain regions that degenerate in Parkinson's Disease (PD). NMDA receptors are one type of glutamate receptor and have unique functional properties, including a high calcium permeability. Pathological over-activation of NMDA receptors, known as excitotoxicity, may contribute to the degeneration of SNc DA cells in PD.

Excessive calcium influx through NMDA receptors can trigger excitotoxicity and several protective processes have been identified that limit this. These include a negative feedback mechanism known as calcium dependent rundown, whereby elevations in cytosolic calcium activate calcium dependent proteins that negatively modulate NMDA receptors and so limit further calcium influx. Agonist binding can also promote receptor internalisation by inducing a conformational change in the receptor that promotes clathrin mediated endocytosis. This effect is independent of ion flux through the receptor channel.

Here, we used whole-cell patch-clamp electrophysiology to investigate these protective mechanisms in dopaminergic neurons of the SNc, using acutely isolated brain slices from 7 day old (P7) rats as a model system.

Repeated agonist applications (NMDA 200 μ M, glycine 10 μ M applied for 15 seconds, every 100 seconds) resulted in a significant decline ($65.6 \pm 3.1\%$; $n=8$; $P<0.05$, Students' *t*-test) in peak NMDA current over a period of 400 seconds. The mean NMDA current of the first response was -3873 ± 717 pA and was -1244 ± 162 pA for responses evoked 400s later. This decline in peak current was reduced to $14.9 \pm 11.9\%$ ($n=6$) by removing extracellular calcium (substituted with 1mM barium), enhancing intracellular calcium buffering (to $45.1 \pm 2.8\%$, $n=9$, $P<0.05$) using 10mM BAPTA, or clamping neurons at positive potential (+40mV; $14.8 \pm 5.8\%$, $n=7$) to greatly reduce calcium influx during receptor activation.

A small decline in peak NMDA current was observed when the neuron was clamped at positive potentials or when ion flux through the receptor was blocked with magnesium (10mM) during conditioning agonist applications. The rundown at +40 mV could be prevented by including a dynamin inhibitory peptide (QVPSRPNRAP; 50 μ M) in the pipette solution (but not when a control peptide of scrambled amino acid sequence was used (QPPASNPRVR; 50 μ M), consistent with a role for clathrin-dependent receptor internalisation in activity dependent receptor modulation.

Our results indicate that dopaminergic neurons of the SNc have robust mechanisms in place to negatively regulate NMDA receptors both in a calcium-dependent and calcium-independent manner.

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