Mobility of NMDA autoreceptors in presynaptic glutamate terminals in the rat entorhinal cortex

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We have demonstrated that glutamate release at synapses in the rat entorhinal cortex EC is tonically facilitated by presynaptic NR2B-containing NMDA autoreceptors (NMDAar; Woodhall, *et al*., 2001; Yang *et al*., 2006). NMDAr at postsynaptic sites are mobile, undergoing trafficking both within the membrane and between membrane and internal stores. In the present experiments we have attempted to determine whether presynaptic NMDAar are also mobile in presynaptic terminals and can move between active release zones and more distal sites in the terminals/axons.

Whole cell voltage clamp was used to record from layer V neurones in EC slices prepared from male Wistar rats (4-6 weeks). Excitatory postsynaptic currents (EPSCs) were evoked by electrically stimulating afferent synaptic pathways. In all studies, postsynaptic NMDAr were blocked from the inside by inclusion of the channel blocker, MK801 in the patch pipette. EPSCs evoked in this way are mediated by AMPA receptors, but show a frequency dependent facilitation when evoked at 3 Hz, which is mediated by released glutamate acting at presynaptic NMDAar (Woodhall et al., 2001). Thus the degree of frequency facilitation is a reliable reporter of the activity of presynaptic NMDAar.

Increasing stimulation frequency from 0.05 to 3Hz (n=10) resulted in a mean increase in EPSC amplitude of 36.2±6.4%. After 5 minutes of bath perfusion with MK801 (10 μM) the % change was -8.5±6.4%. MK801 is a use-dependent and essentially irreversible channel blocker, but 50 minutes after washing MK801 from the bath facilitation was restored to 46.6±14.4%. This anomalous recovery suggests that NMDAar that are activated by glutamate close to the release sites become blocked by MK801, but over time these are replaced by non-blocked receptors moving in from distal locations into the proximity of the active release zones. The recovery is unlikely to be due to activity use-dependent unblocking of the receptors near the release sites. In another group of neurones (n=6), facilitation was decreased from 53.2±6.5% to -1.8±2.8% 8 min after starting MK801 application. All stimulation was then halted but when it was recommenced after 30 min, facilitation had already recovered to 39.6±13.5%, showing that activation of the afferent pathways was not required for restoration of facilitation. Finally, in a further 8 neurones control facilitation was 58.3±13.6%. In these neurones, bath application of MK801 was combined with NMDA (250 μM). This will use-dependently block NMDAr at all sites. Facilitation was reduced to -1.1±17.3% after 5 minutes, but even when the agonist and blocker were washed for up to 65 minutes no recovery occurred (3.1±6.1%). Again, this supports the suggestion that NMDAar are mobile in the presynaptic terminals, that recovery is dependent on movement of NMDAar from distal locations to active zones, and that the mobility is dependent on lateral diffusion in the terminal membrane

Woodhall G.L. *et al*., (2001) J. Neurophysiol. 86, 1644-1651

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