Glutamate alters the morphology of dendritic spines in motoneurons

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Dendrites are the primary receptive component of neurons. In motoneurons (MNs), for example, the dendritic tree comprises ~90% of the membrane surface area (Cullheim *et al*., 1987), up to 60% of which is covered with presynaptic terminals (Örnung *et al*., 1998). Dendrites therefore play a primary role in determining neuronal information processing. The first opportunity for dendritic signal processing is at the synapse. In a variety of neuron types, synapses occur on spines, specialised membrane projections where the effect of a synaptic input on the cell can be dynamically regulated by altering spine morphology. Spines have only rarely been reported on MNs. As an initial step toward understanding the functional significance of spines for MN function, we combined laser-scanning fluorescence microscopy, whole-cell recording and image processing techniques to characterise the density, distribution and dynamic morphology of dendritic spines in hypoglossal (XII) MNs from neonatal mice.

Medullary slice preparations (200 μm thick) were prepared from anaesthetised Swiss CD-1 mice (postnatal day 0-3) and XII MNs were labelled during whole-cell recording using pipettes filled with avidin-conjugated biocytin (Molecular Probes). For morphological experiments, tissue was fixed (4% paraformaldehyde), and recorded cells were visualised using Alexa 488-avidin conjugate (Molecular Probes) and imaged using confocal microscopy.

Dendritic segments displayed two distinct morphological patterns: (Type I) smooth, uniformly tapered segments with spines at low density (0.09 ± 0.01 spine.μm^{-1}, $n = 3$), or (Type II) periodically swollen segments that lacked spines. Spines also displayed a range of morphologies, including the classical mushroom-headed (pedunculated) form (site of synaptic inputs in other neurons) and those characterised by long filopodia but no head (proposed to be important for synaptogenesis; Cailliau Portera & Yuste, 2001).

We then tested the hypothesis that spine structure is not fixed but can be dynamically modulated in response to glutamate receptor activation. Moreover, based on observations in cultured cortical neurons (Hasbani *et al*., 2001), we hypothesised that the Type II morphology results from retraction of spines and local swelling of dendrites at these sites in response to high concentrations of glutamate. XII MNs were labelled with Alexa Fluor 350 or 488 Hydrazide (Molecular Probes) under voltage-clamp conditions. Spines were identified and their morphologies recorded before, during and after bath-application of glutamate (125 - 500 μM for 5 - 10 minutes), using two-photon excitation microscopy. Glutamate application was associated with membrane depolarisation (from -59 ± 7 mV, to -5 ± 0.7 mV, $n = 2$), spine retraction and dendritic swelling, which transformed dendrites from Type I to Type II as defined in fixed preparations. These changes partially reversed upon glutamate wash-out. Membrane potential returned to -52 ± 2 mV and dendritic swelling was reduced.

These data confirm the presence at low density of dendritic spines on XII MNs, and establish that their morphology is not fixed but can be modified by glutamate receptor activation. Whether these changes in spine morphology represent a physiological mechanism important for synaptogenesis, for modulating synaptic strength (Hasbani *et al*., 2001) or reflect the pathological consequence of glutamate-induced excitotoxicity remains to be determined.

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