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Involvement of Nitric oxide-signaling and presynaptic NMDA receptors in experience-dependent synaptic potentiation at *Drosophila melanogaster* neuromuscular junctions

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The developing neuromuscular junction (NMJ) of *Drosophila* larvae is used as a simple model system for analysis of activity-dependent processes at glutamatergic synapses. Experience-dependent strengthening in response to enhanced locomotor activity (2h of larval crawling) has been previously reported (1). Presynaptic *Drosophila* NMDA receptor (DNMDAR) activity has also been reported to be enhanced in larvae following this period of activity (2), however, the molecular mechanism of DNMDAR involvement is unknown but may involve activation of the nitric oxide (NO)/cGMP pathway. We therefore characterised the expression of DNMDAR and the role of nitric oxide in synaptic plasticity processes in the *Drosophila* larvae.
DNMDAR1 expression was assessed by Western blotting in 3rd instar larval brain and adult *Drosophila* head lysates. DNMDAR1 expression was also analysed by immunohistochemistry using formaldehyde-fixed larvae (1). Electrophysiological measurements of evoked excitatory junction potentials (eEJPs) were made from *Drosophila* muscle 6 (1,2) in the absence or presence of a soluble guanylyl cyclase inhibitor (ODQ) or in response to a NO donor (SNP).
Western blot analysis of DNMDAR1 expression in larval and adult brain showed a protein doublet of approximately 130kD, which increased in intensity upon overexpression. Immunohistological analysis of DNMDAR1 showed weak labelling of neuropil structures within the ventral cord of wild type larval brains. In transgenic larvae overexpressing DNMDAR1 in motorneurons, DNMDAR1 immunoreactivity increased in the cell bodies and axons. Furthermore, a weak DNMDAR1-specific immuoreactivity at type Ib boutons of wild type NMJs, which became stronger upon overexpression of wild type DNMDAR1 in neurons, was observed. Also, DNMDAR1 was found to be localised in close proximity to presynaptic active zones. These results show that DNMDAR1 is expressed in larval brain and at NMJs. Using larvae chronically grown at 29°C to increase locomotor activity (1), application of ODQ (20μM) attenuated eEJPs from 48.6±1.4mV to 39.6±1.7mV (mean±SEM, n=6, $P<0.05$, paired Student's t-test), whereas SNP (10μM) enhanced eEJPs in control larvae (25°C) from 41.2±1mV to 47.9±2.1mV (n=6, $P<0.05$, paired Student's t-test) suggesting that the NO/cGMP pathway can regulate eEJP at the *Drosophila* NMJ. A link between DNMDAR1 activation and the NO/cGMP pathway remains to be established. The present data offer new insights into the mechanisms mediating experience-dependent potentiation of glutamatergic signal transmission.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC71A

Development of short interfering RNAs (siRNA) for knock-down of TRPC channels in human cells

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There are seven structurally related canonical type transient receptor potential channels in mammalian cells (TRPC1-7), although TRPC2 is a pseudogene in man. The genes encoding these channels are differentially expressed in different cell types with many cells expressing at least 3 of the subtypes. Because of this heterogeneity of expression and the likelihood that channels may be formed of heteromultimers, the relative contribution of each individual channel subtype to calcium influx has been difficult to assess. To address this question we have attempted to generate small interfering RNAs (siRNAs) to serially deplete both individual, and combinations of TRPC channels. These siRNAs form a hairpin structure which are cleaved to generate 23 base duplexes. So as to provide an initial analysis of the efficacy and specificity of these siRNAs, we have cloned cDNAs encoding individual TRPC channels with carboxy terminal green fluorescent protein (GFP) tags. These cDNAs have been transiently transfected into HEK-293 cells and the correct membrane targeting has been assessed by imaging microscopy. We have begun to analyse the efficacy of each siRNA both by fluorescent activated cell sorting (FACS) and western blotting. At least two different siRNAs have been tested for each TRPC channel. TRPC1-GFP expression was repressed by 40% with TRPC1-siRNA1 and by over 90% with TRPC1-siRNA2 (Fig. 1; n=7). siRNAs directed against the remaining channels were partially effective when used individually, yielding repression ranging from between 15 and 53%. TRPC1-siRNA2 had no effect on expression of the other TRPC-GFP fusions indicating specificity for the desired target. We are currently testing the effects of use of combination of the partially active siRNAs as well as testing additional hairpins. Knockdown of endogenous TRPC mRNA will be tested using these siRNAs and functional experiments on calcium handling will also be carried out in cell lines and primary cultured airway smooth muscle cells.

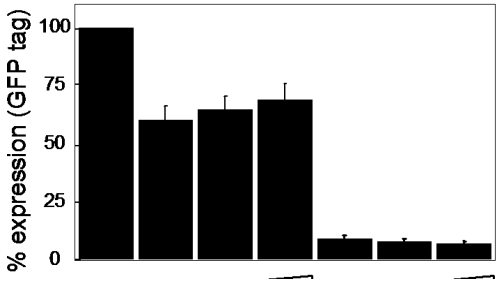

Figure 1. TRPC1 over-expression and knock-down with siRNAs. Amount of plasmid per $5x10^5$ cells shown below bars. Data derived from Western blots against GFP tag, and shown as mean ± SEM, n=7.

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