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Presentation Abstract

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Title: Fluorescence-assisted targeting of elusive synaptic connections

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Abstract: Paired recordings from multiple neurons have revealed a wealth of information about specific synaptic connections in the nervous system. Yet not all connections in the brain are readily studied with this method, typically due to the difficulty of preserving or visualizing the relevant neuronal structures within the acute slice preparation. Here we describe two complementary methods exploiting two-photon laser-scanning microscopy to target functional synaptic connections that are difficult to characterize with standard paired recording techniques. The first approach involves imaging neurons labelled with either a genetically encoded marker or by dye loading to identify those whose axons are preserved within the slice. These putative presynaptic axons are traced *in vitro*. Simultaneous laser-scanning Dodt contrast imaging allows identification and targeting of putative postsynaptic neurons (Wimmer *et al.*, 2004). Whole-cell recordings are then made from putative pre- and postsynaptic neurons to find functional synaptic connections. With this method, the yield of connected Purkinje cell pairs was increased by a factor of 20 in comparison with the standard paired recording approach (26%, 23/88 connections/attempts versus 1.3%, 2/154; $P < 0.001$). This targeting strategy more accurately estimates the functional connectivity, which can be underestimated when axons and dendrites are severed in the acute slice preparation. The second approach relies on a genetic label to enable targeted recordings of cell types that are otherwise difficult to identify. One such type is the Lugaro cell, which is not readily visualized with contrast-enhanced microscopy. To see if Purkinje cells make synapses onto Lugaro cells, we used two-photon laser-scanning microscopy of acute slices from mice that express GFP in glycinergic neurons including Lugaro cells (Zeilhofer *et al.*, 2005). We identified Lugaro

cells based on GFP expression, location within the cerebellar cortex, and morphology. After establishing targeted whole-cell recordings of Lugaro cells, we made extracellular recordings of spikes from multiple nearby Purkinje cells, and identified monosynaptically connected Purkinje - Lugaro pairs by spike-triggered averaging of IPSCs. With this technique, the connectivity rate (5%, 2/37 attempts) was still low because presynaptic Purkinje cell axon collaterals were often cut. Nonetheless, we established for the first time the presence of functional Purkinje - Lugaro cell connections in the mature cerebellum. These two complementary methods may be useful for probing the functional properties of rare synaptic connections in many mammalian neural circuits.

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