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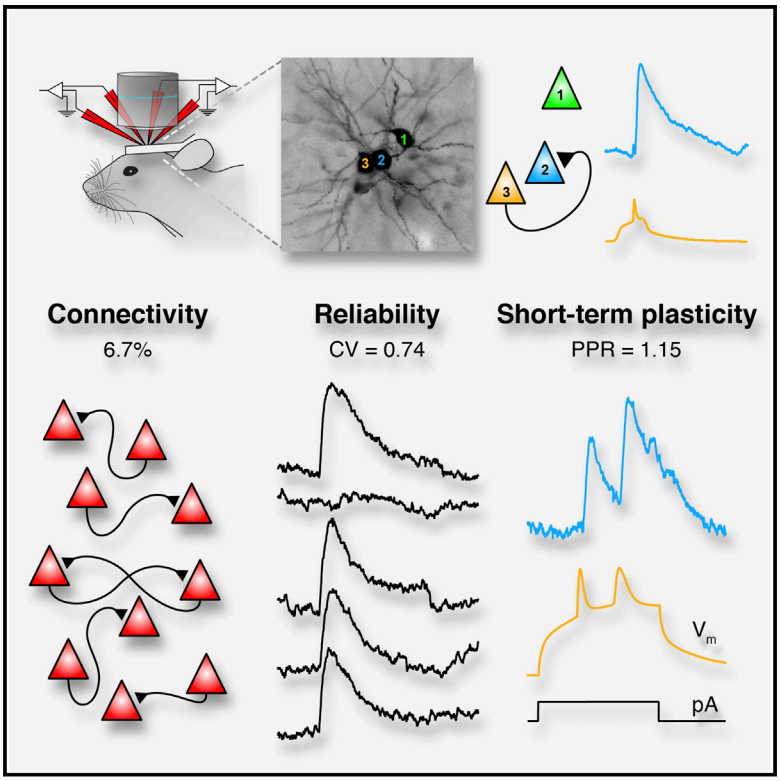
# In Vivo Monosynaptic Excitatory Transmission between Layer 2 Cortical Pyramidal Neurons

## Graphical Abstract

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## In Brief

Jouhanneau et al. use multiple in vivo two-photon targeted whole-cell recordings to measure the rates of connectivity, amplitude, kinetics, reliability, and short-term plasticity of monosynaptic excitatory connections between layer 2 cortical pyramidal neurons.

## Highlights

- Targeted whole-cell recordings from monosynaptically connected neurons in vivo
- Sparse but non-random connectivity between layer 2 pyramidal neurons in vivo
- Reduced failure rate and trial-by-trial reliability with increasing uEPSP amplitude
- Weak short-term synaptic plasticity

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# In Vivo Monosynaptic Excitatory Transmission between Layer 2 Cortical Pyramidal Neurons

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## SUMMARY

**Little is known about the properties of monosynaptic connections between identified neurons in vivo. We made multiple (two to four) two-photon targeted whole-cell recordings from neighboring layer 2 mouse somatosensory barrel cortex pyramidal neurons in vivo to investigate excitatory monosynaptic transmission in the hyperpolarized downstate. We report that pyramidal neurons form a sparsely connected (6.7% connectivity) network with an overrepresentation of bidirectional connections. The majority of unitary excitatory postsynaptic potentials were small in amplitude (<0.5 mV), with a small minority >1 mV. The coefficient of variation (CV = 0.74) could largely be explained by the presence of synaptic failures (22%). Both the CV and failure rates were reduced with increasing amplitude. The mean paired-pulse ratio was 1.15 and positively correlated with the CV. Our approach will help bridge the gap between connectivity and function and allow investigations into the impact of brain state on monosynaptic transmission and integration.**

## INTRODUCTION

Local excitatory synaptic connections between cortical pyramidal neurons are critical for sensory perception, cognition, and memory and form the backbone of massive-scale modeling and mapping efforts of the mammalian brain. Fundamental properties of monosynaptic excitatory glutamatergic transmission have been well characterized in cortical slice studies (Feldmeyer et al., 2006; Holmgren et al., 2003; Lefort et al., 2009; Markram et al., 1997; Silver et al., 2003; Song et al., 2005; Thomson and Lamy, 2007). In vitro work has demonstrated that monosynaptic connections between neighboring pyramidal neurons are typically small in amplitude, are highly reliable with little synaptic failure, and show short-term depression. The probability of finding a connection between two neighboring pyramidal neurons is low, about 10% in somatosensory cortex (Holmgren et al., 2003; Lefort et al., 2009; Markram et al., 1997) and 10%–20% in visual cortex (Cossell et al., 2015; Ko et al., 2011; Song et al., 2005; Yoshimura et al., 2005), and previous studies have identified non-random patterns of connectivity between pyramidal neurons in some cortical regions (Cossell et al., 2015; Ko et al., 2011; Markram et al., 1997; Perin et al., 2011; Song et al., 2005). However, because of axonal slicing and dendritic spine growth (Kirov et al., 1999), as well as differences in firing rates, extracellular calcium (Borst, 2010), and neuromodulator concentrations in brain slices, it is unclear whether these features of cortical excitatory monosynaptic connections are also found in vivo.

Little is known about properties of cortical synaptic transmission in vivo, in part because of the technical difficulty of performing membrane potential ($V_m$) recordings from multiple, neighboring neurons. Cortical slice work has shown that the probability of identifying a connected pair of pyramidal neurons is higher if the cells' somata are within ~200 μm of each other (Holmgren et al., 2003; Perin et al., 2011). Thus, to identify a connection without prior knowledge of which cells are connected, recordings should ideally be targeted to nearby cell somata. Previous in vivo studies of synaptic connectivity, however, have used blind recording methods including: dual extracellular recordings (Fujisawa et al., 2008; Reid and Alonso, 1995; Swadlow and Gusev, 2002), a combination of extracellular and intracellular recordings (Bruno and Sakmann, 2006; London et al., 2010; Matsumura et al., 1996; Yu and Ferster, 2013), or sharp microelectrode recordings (Crochet et al., 2005). More recently, optogenetics with targeted whole-cell recordings was used to identify excitatory connections to cortical GABAergic interneurons (Pala and Petersen, 2015).

Here, we used in vivo two-photon targeted whole-cell recordings from two to four neighboring, layer 2 (L2) pyramidal neurons in primary somatosensory cortex of anesthetized mice. Our approach allowed us to test for unidirectional and bidirectional connections and examine fundamental properties of unitary excitatory connections between pyramidal neurons.

## RESULTS

### In Vivo Two-Photon Targeted Whole-Cell Recordings from Monosynaptically Connected L2 Excitatory Pyramidal Neurons

We made 185 dual, 84 triple, and 2 quadruple $V_m$ recordings from 630 neighboring (horizontal distance between soma centers, 41.01 ± 0.74 μm; n = 878 tested connections) pyramidal