

In Vitro Investigation of Synaptic Plasticity

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Abstract

A classical in vitro model for investigation of information storage in the brain is based on the acute hippocampal slice. Here, repeated high-frequency stimulation of excitatory Schaeffer collaterals making synapses onto pyramidal cells in the hippocampal CA1 region leads to strengthening of evoked field-recording responses—long-term potentiation (LTP)—in keeping with Hebb’s postulate. This model remains tremendously influential for its reliability, specificity, and relative ease of use. More recent plasticity studies have explored various other brain regions including the neocortex, which often requires more laborious whole-cell recordings of synaptically connected pairs of neurons, to ensure that the identities of recorded cells are known. In addition, with this experimental approach, the spiking activity can be controlled with millisecond precision, which is necessary for the study of spike-timing-dependent plasticity (STDP). Here, we provide protocols for in vitro study of hippocampal CA1 LTP using field recordings, and of STDP in synaptically connected pairs of layer-5 pyramidal cells in acute slices of rodent neocortex.

IN VITRO SYNAPTIC PLASTICITY: A MODEL OF LEARNING IN THE BRAIN

It is widely believed that the storing of information in the brain is accomplished by alterations in synaptic strength among connected neurons. This view is typically attributed to the Canadian neuroscientist Donald Hebb (1949), although many before him had advanced similar propositions for how learning in the brain could be accomplished (Markram et al. 2011). In Hebb’s postulate, he states that a way of storing information would be to increase synaptic strength of already connected cells if they are repeatedly and persistently activated simultaneously (Hebb 1949), a concept that has been summarized as “cells that fire together, wire together” (Shatz 1992).

The first evidence for Hebbian plasticity was reported by Bliss and Lømo (1973). They stimulated inputs to the dentate gyrus of rabbit hippocampus in vivo and found that response amplitudes would not only potentiate but also remain potentiated several hours after brief

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trains of high-frequency stimulation. As high-frequency stimulation drives postsynaptic cells, the persistent synaptic strengthening was in agreement with Hebb's postulate. However, a causal relationship between synaptic plasticity and learning still remains to be formally established in mammals. Tremendous progress has been made to link synaptic plasticity in the amygdala to fear conditioning (Stevens 1998; Pape and Pare 2010; Johansen et al. 2011; Nabavi et al. 2014). But memories are often distributed across multiple synapses, which makes it hard to establish clear-cut causal links between plasticity and behavior.

Here, we focus on two in vitro synaptic plasticity models that rely on acute slices from the rodent brain: field-recording LTP in the hippocampal CA1 region (see Protocol: **Long-Term Potentiation by Theta-Burst Stimulation using Extracellular Field Potential Recordings in Acute Hippocampal Slices**) [Abrahamsson et al. 2016]) and STDP in paired recordings of layer-5 pyramidal cells of visual cortex (Sjöström et al. 2001; see Protocol: **Using Multiple Whole-Cell Recordings to Study Spike-Timing-Dependent Plasticity in Acute Neocortical Slices** [Lalanne et al. 2016]). We provide tips and tricks as well as troubleshooting information. These protocols are easy to adapt to other brain regions or activity paradigms. Additional background on each of these methods is provided below.

LONG-TERM POTENTIATION BY THETA-BURST STIMULATIONS USING EXTRACELLULAR FIELD RECORDINGS IN ACUTE HIPPOCAMPAL SLICES

There are several advantages to using extracellular field recordings for the study of LTP. First, it is a relatively simple method that is suitable even for beginner electrophysiologists with little background or expertise. Second, it is a relatively noninvasive technique that does not disrupt the internal milieu of the neurons. This is in contrast to whole-cell recordings, where the experimenter runs the risk of washing out substances that are essential for LTP as the cell is dialyzed (Malinow and Tsien 1990; Isaac et al. 1996). Third, it is possible to generate stable recordings for a long period of time, up to several hours, which is technically more challenging with whole-cell recordings (Malinow and Tsien 1990; Watt et al. 2004; Sjöström and Häusser 2006). Finally, response variability is lowered with field potential measurements, which sample the activity of large numbers of neurons simultaneously. This averaging helps produce robust data sets rapidly. Figure 1 in Protocol: **Long-Term Potentiation by Theta-Burst Stimulation using Extracellular Field Potential Recordings in Acute Hippocampal Slices** (Abrahamsson et al. 2016) provides a comparison between field excitatory postsynaptic potentials (fEPSPs) and EPSPs from whole-cell recordings.

Theta-Burst stimulation is a highly influential LTP induction paradigm that is commonly used because it resembles physiological theta activity and because it is quite robust in brain regions as different as neocortex and hippocampus (Kirkwood et al. 1993). Another standard LTP induction paradigm uses uninterrupted high-frequency stimulation—tetanization—rather than theta-burst stimulation, e.g., the original LTP study by Bliss and Lømo (1973).

USING QUADRUPLE WHOLE-CELL RECORDINGS TO STUDY SPIKE-TIMING-DEPENDENT PLASTICITY IN ACUTE NEOCORTICAL SLICES

Using extracellular field recordings, electrophysiologists have made great strides in the study of synaptic plasticity in hippocampus (see Protocol: **Long-Term Potentiation by Theta-Burst Stimulation using Extracellular Field Potential Recordings in Acute Hippocampal Slices**) [Abrahamsson et al. 2016]). With the neocortex, however, it has not been quite as straightforward. Although a considerable amount has been learned about neocortical plasticity by stimulating in the white matter or in layer 4 (Kirkwood et al. 1993, 1995; Kirkwood and Bear 1994), neocortical extracellular stimulation experiments often suffer from the shortcoming that it is difficult to know which synapse types were recorded from.

With paired recordings, however, the experimenter knows precisely what neuronal types are being stimulated and recorded (Miles and Poncer 1996; Debanne et al. 2008). Paired recordings are thus particularly suited for the study of neocortical circuits, where multiple cell types exist side by side and where plasticity is known to be synapse and cell-type specific (Buchanan et al. 2012; Blackman et al. 2013; Larsen and Sjöström 2015). To benefit maximally from paired recordings, they should ideally be combined with morphological reconstruction and classification, either from biocytin histology or 3D imaging stacks obtained with two-photon laser-scanning microscopy (2PLSM) (Blackman et al. 2014; Ferreira et al. 2014). In addition, paired recordings provide pharmacological access to both the pre- and postsynaptic cell, thus enabling wash-in of drugs or dyes into the transmitting or recipient neuron (Kaiser et al. 2004; Koester and Johnston 2005; Rodriguez-Moreno and Paulsen 2008; Buchanan et al. 2012). Finally, paired recordings also enable precise timing of spikes in connected neurons, which is absolutely essential for the STDP experimental paradigm (Markram et al. 1997; Sjöström et al. 2001).

Unfortunately, neocortical connectivity is sparse—typically only 10%–50% of neighboring excitatory cells are monosynaptically connected (Song et al. 2005; Lefort et al. 2009; Ko et al. 2011)—which makes paired recordings slow and painstaking. Fortunately, the number of connections tested scales favorably with the number of cells recorded: with n neighboring cells simultaneously patched, the number of connections tested is $n(n - 1)$. As n increases, more manipulators are required, resulting in considerable spatial and financial constraints. Still, several studies have been reported with 7–12 simultaneous whole-cell recordings (Lefort et al. 2009; Perin et al. 2011). Because quadruple recordings sample twelve possible connections simultaneously with reasonable spatial constraints and at a relatively realistic cost, we suggest that $n = 4$ recordings represent an ideal choice for many electrophysiology laboratories. However, care should be taken when identifying the cells that are connected. In multiple simultaneous patching, the nonconnected cells are typically also labeled with the same dye and visualized. One possible way to avoid this problem is to use different dyes in the different recording pipettes (Kaiser et al. 2004; Koester and Johnston 2005).

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