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Short communication

Paired recordings from distant inhibitory neuron pairs by a sequential scanning approach

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ABSTRACT

Simultaneous recordings from connected neuron pairs have brought important insights into synaptic communication between neurons. However, patch clamp recordings from neuron pairs have been largely restricted to brain areas in which connections among nearby neurons exist at a relatively high probability. In the case of more distant connections or in areas in which neurons are connected with low probability, recordings from synaptically connected neuron pairs have remained scarce. Here, we present a method that allows dual recordings from remotely connected neuron pairs by scanning potential presynaptic neurons. The applicability of this new approach was tested in the inhibitory pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), a sound localization pathway in the auditory brainstem. Using a three-step approach that sequentially combines focal uncaging of glutamate, pressure application of glutamate, and loose patch recordings allowed us to reliably achieve recordings from distant, synaptically connected GABA/glycinergic MNTB–LSO neuron pairs. Our results demonstrate that single MNTB neurons evoke highly variable mono-synaptic responses in developing LSO neurons, and heterogeneous short term synaptic dynamics, suggesting local variations in the refinement of these inhibitory connections. Paired recordings, enabled by scanning of remotely connected pairs, will be highly useful to perform detailed investigations of the synaptic function and plasticity from these circuits during the period of developmental refinement. In general, this method should provide a valuable tool to find connected neurons in other brain areas in which recording from candidate pairs has a low success rate.

1. Introduction

Simultaneous intracellular recordings from connected neuron pairs allow detailed investigation of synaptic transmission between identified pre- and postsynaptic neurons. Patch clamp recordings of connected pairs have been obtained in neuronal culture (Bi and Poo, 1998; Tao et al., 2000) and in brain slice preparations from a variety of brain regions (e.g., Debanne et al., 1996; Markram et al., 1997; Pouzat and Hestrin, 1997; Petersen and Sakmann, 2000; Sjöström et al., 2001). Most of these paired recordings have been performed in brain areas in which nearby neurons make synaptic connections with a relatively high probability (e.g., mammalian neocortex). Recordings from connected neuron pairs that are farther apart or sparsely connected are rather difficult to obtain.

The chance of achieving recordings from connected neuron pairs can be increased by screening for pre- or postsynaptic partners. In cortical slices, screening of postsynaptic target neurons has been achieved using calcium imaging (Peterlin et al., 2000; Kozloski et al., 2001). With this method, spikes are evoked in a presynaptic neuron to reveal monosynaptic responses in postsynaptic neurons, which are detected as a rise in intracellular Ca^{2+} levels. This method, however, has the limitation that it is restricted to identify excitatory connections because activation of inhibitory synapses generally does not elicit a calcium signal in the postsynaptic neuron. In this study, we present a new method that can be used for finding inhibitory as well as excitatory connected pairs by scanning for presynaptic neurons at multiple spatial scales.

We applied this method to an auditory brainstem pathway in which inhibitory synaptic connections are present between nuclei that are separated by hundreds of microns. In the lateral superior olive (LSO), a binaural sound localization nucleus, neurons receive synaptic inputs from the contralateral ear via the medial nucleus of the trapezoid body (MNTB) (Boudreau and Tsuchitani, 1968; Cant

Abbreviations: MNTB, medial nucleus of the trapezoid body; LSO, lateral superior olive; PSC, postsynaptic current.

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and Casseday, 1986; Sanes and Rubel, 1988; Bledsoe et al., 1990; Sommer et al., 1993). The mature MNTB–LSO pathway is glycinergic, but during development MNTB neurons also release GABA and glutamate (Kotak et al., 1998; Gillespie et al., 2005). The developing MNTB–LSO pathway undergoes extensive refinement (Sanes and Friauf, 2000; Kandler et al., 2009), which involves the silencing of most initial MNTB inputs and the strengthening of maintained connections (Kim and Kandler, 2003, 2010). Recordings with minimal stimulation techniques indicated the presence of highly variable responses by single MNTB inputs in developing LSO neurons (Kim and Kandler, 2003, 2010; Gillespie et al., 2005; Noh et al., 2010). However, in these experiments activation of more than one axon could have contributed to this variability, and in addition some responses may have been elicited by fibers that did not originate in the MNTB. Recordings from unambiguously identified pairs with known cell body locations could help resolve these issues.

By prescreening potential presynaptic neurons in the MNTB using the sequential application of focal uncaging of glutamate, pressure application of glutamate, and loose patch recordings, we were able to establish recordings from connected MNTB–LSO pairs. Our results from neonatal rats and mice show that the synaptic inputs from a single MNTB neuron are highly variable in respect to amplitude and short-term synaptic dynamics. Our methods will enable detailed investigations of these inhibitory synaptic connections and their developmental plasticity. Because the prescreening approach can be easily applied to a variety of other brain areas, the method described here will be of general use to increase the success rate of achieving paired recordings.

2. Materials and methods

2.1. Slice preparation

All experimental procedures were in accordance with NIH guidelines and approved by the IACUC at the University of Pittsburgh. Acute brain stem slices were prepared from Sprague Dawley rats aged postnatal day (P) 4 and 6, and CD1 mice aged P6 and 7. Animals were anesthetized with isoflurane before decapitation and brainstem slices were prepared as described previously (Kim and Kandler, 2003; Kullmann and Kandler, 2008). In brief, transverse slices (300 μm) were cut on a vibrating microtome (DTK-1500E, Ted Pella, Redding, CA, USA) in ice-cold ACSF (artificial cerebrospinal fluid (in mM): 124 NaCl, 26 $NaHCO_3$, 10 Glucose, 5 KCl, 1.25 KH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 1 kynurenic acid, pH = 7.4 when infused with 95% O_2/5% CO_2). Slices that contained the MNTB and the LSO were selected and incubated at room temperature in an interface-type chamber. For recordings, slices were transferred to a recording chamber and continuously perfused with oxygenated ACSF (without kynurenic acid) at room temperature at a rate of 3–4 ml/min.

2.2. Electrophysiology and data analysis

Recordings of MNTB and LSO neurons were made using standard patch clamp techniques. Recording electrodes (2–3 MΩ) contained (in mM): 76 Cs-methanesulfonate, 56 CsCl, 10 EGTA, 1 $MgCl_2$, 1 $CaCl_2$, 2 ATP–Mg, 0.3 GTP–Na, 5 Na_2–phosphocreatine, and 10 HEPES (pH = 7.4, 290 mOsm). With the internal and external solution, E_{Cl} was −20 mV and LSO neurons were held at −70 mV (corrected for −5 mV liquid junction potential), resulting in 50 mV driving force for Cl^-. Presynaptic recordings were made with an Axoclamp 2B amplifier, and postsynaptic recordings were made with an Axopatch 1D amplifier. The signals were filtered at 2 kHz and digitized at 10 kHz using custom-written programs in the Labview environment (Kullmann and Kandler, 2001). After establishing a whole cell configuration in an LSO neuron, the objective was switched from 40× to 4× and an optical fiber for glutamate uncaging was placed over the MNTB. Switching between 40× (water immersion) and 4× objectives was done using the standard manual toggle system installed on an Olympus BX50 upright microscope, and this switching did not disturb our whole cell recordings. The extracellular solution contained caged glutamate (200–400 μM, γ-(α-carboxy-2-nitrobenzyl) ester L-glutamic acid) and 50–100 ms long UV flashes were delivered via an optical fiber (20 μm core diameter) for focal glutamate uncaging (Kim and Kandler, 2003). The MNTB was scanned with uncaging in small steps (~50 μm) to identify the location of presynaptic neurons. A successful uncaging that evoked a synaptic response defined a relatively small area (<100 μm diameter) that contained a presynaptic neuron. To further constrain the area, a pipette filled with 1–2 mM glutamate was placed within the target region for pressure application of glutamate (Picospritzer, 10 ms pulse at 10 psi). At 40× magnification, the puffing pipette was moved in small steps to define a search area (<50 μm diameter). Typically, the search area was defined as a small elongated area along the long axis of the puffing pipette. In the final step, individual MNTB neurons inside this elongated area were scanned one cell at a time using a loose-seal configuration with a patch pipette filled with ACSF. For each candidate presynaptic neuron, a gentle suction was applied to form a loose seal (~100 MΩ). Current pulses (0.5–2 nA for 4–5 ms) were applied to elicit spikes. The spikes were visible as a small peak riding on top of the voltage response to the current pulses (Fig. 1C: Petersen and Sakmann, 2000). If no response was seen in the LSO neuron, the pipette was pulled away from the current candidate MNTB neuron and re-used to test a next, nearby neuron. Although we did not systematically keep track of the number of neurons we scanned before we found a connected neuron, typically we did not have to scan more than 10 candidate neurons. Synaptic responses were evoked at 0.1 Hz. In a subset of experiments, pairs of presynaptic pulses separated by 50 ms were applied to investigate paired-pulse responses.

Custom routines written in MATLAB (Mathworks) were used for data analysis. The peak amplitude of a synaptic current was measured with respect to the base line (average over 5 ms) before a presynaptic spike. Paired pulse ratio was measured as the peak amplitude of the second response divided by the first.

3. Results

To find monosynaptically connected MNTB–LSO pairs, we scanned the MNTB in three sequential steps (Fig. 1). In the first step, we focally uncaged glutamate in the MNTB using an optical fiber, a method that has been used previously to map functional MNTB–LSO connectivity (Kim and Kandler, 2003; Noh et al., 2010). The tonotopic axis runs mediolaterally in the MNTB, and it has been shown that in neonatal animals, the MNTB input area tends to span the entire dorsoventral extent (Kim and Kandler, 2003). Therefore, we scanned the MNTB along the mediolateral axis at about the dorsoventral midpoint so as to intersect the input area.

Once we identified a site at which glutamate uncaging elicited synaptic responses in the LSO neuron (Fig. 1B, top), we placed a glutamate containing pipette and pressure injected glutamate (1–2 mM) within the area defined by uncaging (Fig. 1B, bottom). This second step at a finer spatial scale further narrowed down the candidate area. In a last step, we used a patch pipette to scan individual MNTB neurons, eliciting spikes by applying brief current pulses (4–5 ms) in a loose seal configuration (Petersen and Sakmann, 2000). An example of pre- and postsynaptic traces is shown in Fig. 1C.

Previous recordings using minimal stimulation techniques have shown that single fiber MNTB inputs to immature LSO neurons gen-