Published in final edited form as:

Cold Spring Harb Protoc. ; 2017(5): . doi:10.1101/pdb.top095281.

Cellular and Synaptic Properties of Local Inhibitory Circuits

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Abstract

Inhibitory interneurons play a key role in sculpting the information processed by neural circuits. Despite the wide range of physiologically and morphologically distinct types of interneurons that have been identified, common principles have emerged that have shed light on how synaptic inhibition operates, both mechanistically and functionally, across cell types and circuits. Here I will summarize how electrophysiological approaches have been used to illuminate these key principles, including basic interneuron circuit motifs, the functional properties of inhibitory synapses, and the main roles for synaptic inhibition in regulating neural circuit function. I will also highlight how some key electrophysiological methods and experiments have advanced our understanding of inhibitory synapse function.

Main Text

Background

The concept of inhibition was not widely recognized by many early pioneers of neuroscience who relied on anatomical and behavioral approaches. Ramon y Cajal, for example, despite his tremendous contributions to neuronal theory and circuit organization, never acknowledged a role for inhibition in his writings. In large part, the modern concept of inhibition arose from the work of Charles Scott Sherrington and his seminal experiments on spinal reflexes. Sherrington was the first to formally describe the inhibition of decerebrate muscle rigidity that results from a variety of tactile stimuli (Sherrington 1898), as well as the concept reciprocal inhibitory innervation that produces relaxation of antagonist muscle groups (Sherrington 1906). However, it was only through the advent of new electrophysiological approaches that the key principles of synaptic inhibition were ultimately elucidated.

Inspired by the findings of Sherrington and the emerging concept of inhibition in the central nervous system, several investigators utilized *in vivo extracellular electrophysiology* to provide key experimental evidence describing both the mechanism and role of inhibitory synaptic transmission in spinal circuits during the mid 20th century. In particular, the work of Lloyd (Lloyd 1941), Renshaw (Renshaw 1941) and others (for review, see Callister and Graham 2010) demonstrated that evoked excitatory potentials in ventral horn motor neurons could be inhibited by anterograde electrical stimulation of sensory neurons in the dorsal root, or retrograde stimulation of motor neuron axons themselves. In each case, the authors

noted a brief 2–3 ms delay between the onset of evoked excitation and inhibition in the recorded potentials. Such delayed latency measurements between excitation and inhibition suggested the presence of intermediate interneurons in the circuit. Even today in the era of optogenetics, latency measurements are a valuable tool to dissect circuit connectivity, and can provide essential information about whether optically or electrically evoked inhibition results from direct interneuron activation or a multisynaptic pathway.

The results of Renshaw and others also established the basis for our understanding of the two fundamental inhibitory circuit motifs that have since been documented across the nervous system: **feedforward and feedback inhibitory circuits**. Feedforward inhibition occurs when the same excitatory pathway that impinges on a principal neuron also activates inhibitory neurons that synapse onto the same principal cell population (Figure 1a). In the spinal cord, feedforward inhibition results from dorsal root stimulation because these sensory neurons both directly excite motor neurons, and excite inhibitory interneurons that synapse onto the same motor neuron population. Conversely, feedback inhibition results when the activity of a given principal neuron recruits inhibitory interneurons that feed back onto the same target population (Figure 1b). In the spinal cord example, feedback inhibition results from axon collaterals from motor neurons that recruit inhibitory neurons synapsing onto the very same motor neurons.

Despite the growing recognition of inhibitory circuit function in the mid 20th century, the mechanism of neural inhibition remained highly controversial. While Otto Loewi's famous experiments had already established the idea that neuronal communication was a chemical process, many early investigators including Eccles believed that inhibition was a distinct, non-chemical process mediated by electrical transmission (Hultborn 2006). The key experiment demonstrating that synaptic inhibition was indeed a chemical process required the advent of crucial new technique; **intracellular recording**. By impaling motor neurons with a glass electrode, Eccles and colleagues were, for the first time, able to measure the neuronal membrane potentials evoked by stimulation different synaptic pathways. In doing so, they demonstrated the first intracellularly recorded **inhibitory postsynaptic potential, or IPSP** (Brock et al. 1952). The central observation in these recordings was that evoked inhibitory potentials had the opposite polarity of excitatory potentials. That is, rather than depolarizing the neuronal membrane potential, IPSPs hyperpolarized the motor neurons, taking them further from spike threshold. Because there was no possible mechanism for electrical transmission to invert the sign of a signal and convert an excitatory impulse to a hyperpolarizing potential, Eccles correctly concluded that synaptic inhibition was a chemical process requiring a distinct neurotransmitter.

Basic properties of inhibitory synapses

We now recognize that the primary inhibitory neurotransmitter in the spinal cord is **glycine**, while gamma-Aminobutyric acid, or **GABA**, is the most common inhibitory transmitter throughout the rest of the central nervous system. Both GABA and glycine can bind to ionotropic ligand gated receptors (GABA_A and glycine receptors) that mediate a **chloride conductance**, and it is the influx of chloride ions across the neuronal membrane that produces the hyperpolarizing potentials that underlie IPSPs at most physiological membrane

potentials. GABA can also bind metabotropic GABA_B receptors that couple to G-proteins, and these receptors typically act to either open a potassium conductance or inhibit calcium channels involved in vesicular release. Hence, GABA_B receptors are also inhibitory, but can act on slower time scales than GABA_A receptors. Effects mediated by each these receptors can be dissociated pharmacologically, as GABA_A receptors are blocked by the selective antagonist gabazine (SR95531), while GABA_B receptors can be blocked by CGP54626.

Importantly, GABA receptors can be tailored to suit specialized circuit functions. For example, by expressing different subunit combinations, GABA_A receptors can have different kinetics, open probability, desensitization properties, and sensitivity to modulators such as benzodiazepines (Mody and Pearce 2004; Farrant and Nusser 2005; Jacob et al. 2008). One such example of specialized GABA_A receptors can be found in the cerebellum, where granule cells contain GABA_A receptors that express the $\alpha 6$ subunit (Brickley et al. 1996; Wall and Usowicz 1997; Rossi and Hamann 1998). These GABA_A receptors do not desensitize in the continuous presence of GABA, allowing them to mediate a tonic inhibitory current that is distinct from the typical transient currents mediated by desensitizing GABA_A receptors. These, and certain other GABA_A receptor subtypes can be distinguished pharmacologically. For example, $\alpha 6$ subunit containing GABA_A receptors are sensitive to furosemide, but not to benzodiazepine modulation by diazepam (Hamann et al. 2002). One useful approach that has often been used to test the properties of different GABA receptors is to pull outside-out patches of membrane from a neuron of interest using standard glass capillary recording electrodes (Jones and Westbrook 1995). With such patches, it is possible to rapidly apply agonists and antagonists by using a gravity fed flow pipette attached piezoelectric translator. This setup allows for microsecond switching of solutions, and thus provides the ability to record receptor mediated currents that are shaped predominately by the kinetics of the receptors themselves, without the influence of synaptic release dynamics, diffusion, transmitter uptake, or other factors that can influence the time-course of synaptic currents.

Interneuron identification and function in neural circuits

In the central nervous system, inhibitory interneurons exhibit a wide diversity of morphologies, biophysical properties, molecular markers, and functional roles (Markram et al. 2004; Monyer and Markram 2004). Hence, the most common way of classifying inhibitory cells involves a combination of these parameters, and whole-cell recording techniques along with transgenic approaches provide a valuable way to identify and manipulate individual interneuron subtypes. For example, interneurons typically classified as “basket cells” are so called because they target the somatic compartment of their postsynaptic partners (Hu et al. 2014). By adding Biocytin to a whole-cell recording electrode, it is possible to recover the morphology of a recorded neuron by performing post-hoc histology to visualize this characteristic axonal arborization (Sik et al. 1995). Basket cells also typically express the calcium binding protein parvalbumin, and hence are often referred to as “PV cells”. This marker has proved a valuable tool for establishing transgenic lines that, for example, express either GFP or channelrhodopsin (ChR2) allowing targeted recordings or optical manipulation of these interneurons (Cardin 2012). In addition, many basket cells exhibit high firing frequencies in response to depolarization, and little adaptation

of their spike rates over time. Hence, they are also often classified as “fast-spiking” cells, and current-clamp recordings can be used to provide such physiological characterization for initial identification during recordings. In contrast, interneurons called “Martinotti cells” target the dendrites of their postsynaptic targets, express the peptide hormone somatostatin, and exhibit slower, adapting spike rates in response to depolarization (Wang et al. 2004). However, it is important to recognize that these parameters often do not clearly divide interneuron types into unique categories, and interneuron classification remains a hotly debated topic (DeFelipe et al. 2013).

The role of inhibitory interneurons in synaptic processing can depend both on the circuit motif they are engaged in and the unique functional and anatomical properties of the interneurons in question. For example, feedforward inhibitory circuits often regulate spike timing by establishing a narrow period of excitability referred to as an “integration time window” (Pouille and Scanziani 2001). This window is established by the latency between the arrival of direct synaptic excitation and disynaptic inhibition engaged by the same feedforward pathway. In contrast, feedback inhibitory circuits are typically involved in regulating population level spike synchrony (Cobb et al. 1995), and regulating global levels of principal cell activity by normalizing inhibition according to the spiking of the principal cells themselves (Isaacson and Scanziani 2011). Such actions of inhibitory interneurons can serve to regulate the strength of excitatory synaptic transmission in both an **additive and divisive** manner by respectively moving the membrane potential away from spike threshold and increasing the conductance of the neuronal membrane according to Ohm’s law.

Whole cell electrophysiological approaches, particularly in combination with an *in vitro* brain slice preparation, have provided a key experimental platform for testing the role of distinct interneuron subtypes in neuronal circuit function. In the following protocol, I will describe methods for testing the effects of synaptic inhibition using cerebellar circuits as a model system.

References

- Brickley SG, Cull-Candy SG, Farrant M. 1996 Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. *J Physiol* 497 (Pt 3): 753–759. [PubMed: 9003560] ()
- Brock LG, Coombs JS, Eccles JC. 1952 The recording of potentials from motoneurons with an intracellular electrode. *J Physiol* 117: 431–460. [PubMed: 12991232]
- Callister RJ, Graham BA. 2010 Early history of glycine receptor biology in Mammalian spinal cord circuits. *Front Mol Neurosci* 3: 13. [PubMed: 20577630]
- Cardin JA. 2012 Dissecting local circuits in vivo: integrated optogenetic and electrophysiology approaches for exploring inhibitory regulation of cortical activity. *J Physiol Paris* 106: 104–111. [PubMed: 21958624]
- Cobb SR, Buhl EH, Halasy K, Paulson O, Somogyi P. 1995 Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378: 75–78. [PubMed: 7477292]
- DeFelipe J, Lopez-Cruz PL, Benavides-Piccione R, Bielza C, Larranaga P, Anderson S, Burkhalter A, Cauli B, Fairen A, Feldmeyer D et al. 2013 New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nat Rev Neurosci* 14: 202–216. [PubMed: 23385869]
- Farrant M, Nusser Z. 2005 Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. *Nat Rev Neurosci* 6: 215–229. [PubMed: 15738957]

- Hamann M, Rossi DJ, Attwell D. 2002 Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 33: 625–633. [PubMed: 11856535]
- Hu H, Gan J, Jonas P. 2014 Interneurons. Fast-spiking, parvalbumin(+) GABAergic interneurons: from cellular design to microcircuit function. *Science* 345: 1255263. [PubMed: 25082707]
- Hultborn H 2006 Spinal reflexes, mechanisms and concepts: from Eccles to Lundberg and beyond. *Prog Neurobiol* 78: 215–232. [PubMed: 16716488]
- Isaacson Jeffry S, Scanziani M. 2011 How Inhibition Shapes Cortical Activity. *Neuron* 72: 231–243. [PubMed: 22017986]
- Jacob TC, Moss SJ, Jurd R. 2008 GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci* 9: 331–343. [PubMed: 18382465]
- Jones MV, Westbrook GL. 1995 Desensitized states prolong GABAA channel responses to brief agonist pulses. *Neuron* 15: 181–191. [PubMed: 7542462]
- Lloyd DPC. 1941 A Direct Central Inhibitory Action of Dromically Conducted Impulses. *J Neurophysiol* 4: 184–190.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. 2004 Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* 5: 793–807. [PubMed: 15378039]
- Mody I, Pearce RA. 2004 Diversity of inhibitory neurotransmission through GABA(A) receptors. *Trends Neurosci* 27: 569–575. [PubMed: 15331240]
- Monyer H, Markram H. 2004 Interneuron Diversity series: Molecular and genetic tools to study GABAergic interneuron diversity and function. *Trends Neurosci* 27: 90–97. [PubMed: 15102488]
- Pouille F, Scanziani M. 2001 Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science (New York, NY)* 293: 1159–1163.
- Renshaw B 1941 Influence of discharge of motoneurons upon excitation of neighboring motoneurons *J Neurophysiol* 4: 167–183.
- Rossi DJ, Hamann M. 1998 Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit GABA(A) receptors and glomerular geometry. *Neuron* 20: 783–795. [PubMed: 9581769]
- Sherrington CS. 1898 Decerebrate Rigidity, and Reflex Coordination of Movements. *J Physiol* 22: 319–332.
- Sherrington CS. 1906 *The Integrative Action of the Nervous System* Yale Univ. Press, New Haven and London.
- Sik A, Penttonen M, Ylinen A, Buzsaki G. 1995 Hippocampal CA1 interneurons: an in vivo intracellular labeling study. *J Neurosci* 15: 6651–6665. [PubMed: 7472426]
- Wall MJ, Usowicz MM. 1997 Development of action potential-dependent and independent spontaneous GABAA receptor-mediated currents in granule cells of postnatal rat cerebellum. *Eur J Neurosci* 9: 533–548. [PubMed: 9104595]
- Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, Luo J, Markram H. 2004 Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *J Physiol* 561: 65–90. [PubMed: 15331670]

Figure 1.

Schematic of feedforward and feedback inhibitory circuits. *Left*, a feedforward interneuron (dark gray filled circle) is recruited by the same axons that excite a principal cell (light gray pyramid), while a feedback interneuron (right) is recruited by the activity of the principal cell itself. Positive signs (black) denote synaptic excitation, and negative signs (red) denote synaptic inhibition.