Communicative & Integrative Biology 6:3, e23545; May/June 2013; © 2013 Landes Bioscience

Layer-specific regulation of cortical neurons by interhemispheric inhibition

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Processing of sensory information from both sides of the body requires coordination of sensory input between the two hemispheres. This coordination is achieved by transcallosal (interhemispheric) fibers that course though the upper corticals layers. In a recent study by Palmer et al. (2012), we investigated the role of this interhemispheric input on the dendritic and somatic activity of cortical pyramidal neurons. This study showed that interhemispheric input evokes $GABA_B$-mediated inhibition in the distal dendrites of layer 5 pyramidal neurons, decreasing the action potential output when paired with contralateral sensory stimulation. In contrast, layer 2/3 pyramidal neurons were not inhibited by interhemispheric input possibly due to transcallosal fibers evoking more excitation in these neurons than layer 5 neurons.. These results highlight both the precise nature of the microcircuitry of interhemispheric inhibition and how the balance between excitation and inhibition is different in the different layers of the cortex. Identifying the cellular and molecular elements involved in interhemipsheric inhibition is crucial not only for understanding higher brain function and but also dysfunction in the diseased brain.

Keywords: interhemispheric inhibition, $GABA_B$, corpus callosum, dendrite, cortical pyramidal neurons

Submitted: 01/05/13

Accepted: 01/08/13

http://dx.doi.org/10.4161/cib.23545

Citation: Palmer LM, Schulz JM, Larkum ME. Layer-specific regulation of cortical neurons by interhemispheric inhibition. Commun Integr Biol 2013; 6:e23545

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Addendum to: Palmer LM, Schulz JM, Murphy SC, Ledergerber D, Murayama M, Larkum ME. The cellular basis of GABA(B)-mediated interhemispheric inhibition. Science 2012; 335:989–93; PMID:22363012; http://dx.doi.org/10.1126/science.1217276.

One of the complexities of sensory processing is the coordination of information across both hemispheres of the cerebral cortex. This is achieved mostly via a huge bundle of fibers called the corpus callosum. It has long been known that an important action of these transcallosal fibers is to mediate interhemispheric inhibition[1,2] which influences fine motor control,[3,4] visuospatial attention[5,6] and somatosensory processing[7,8] and might contribute to or even underlie behavioral laterality.[9] Furthermore, transcallosal fibers have been shown to regulate the efficacy, or gain, of sensory input, for example, during sensory perception.[10] Gain modulation can be measured at the level of single neurons[11] and may play a fundamental role in the control of numerous behaviors (for a review see Salinas and Sejnowski, 2001).[12] In a recent study by Palmer et al. (2012), we identified the cellular basis of slow interhemispheric inhibition that may be principally involved in regulating the gain in the principle output neurons of the cortex.

In this study,[13] sensory stimulation of the contralateral hindpaw increased the firing rate in layer 5 (L5) pyramidal neurons of the primary somatosensory cortex by approximately 3-fold, while stimulation of the hindpaw on the ipsilateral side had little influence on the firing rate. However, an inhibitory influence on evoked firing was revealed when the ipsilateral hindpaw was stimulated just before (200–400 ms) the contralateral hindpaw (**Fig. 1A–C**). This observation was surprising in two ways. First, ipsilateral stimulation had no apparent effect on the postsynaptic membrane potential of the L5 neuron. Hence, inhibition was "silent" in the absence of action potential output (**Figs. 1 and 2**). This suggests that the decrease in action potentials during

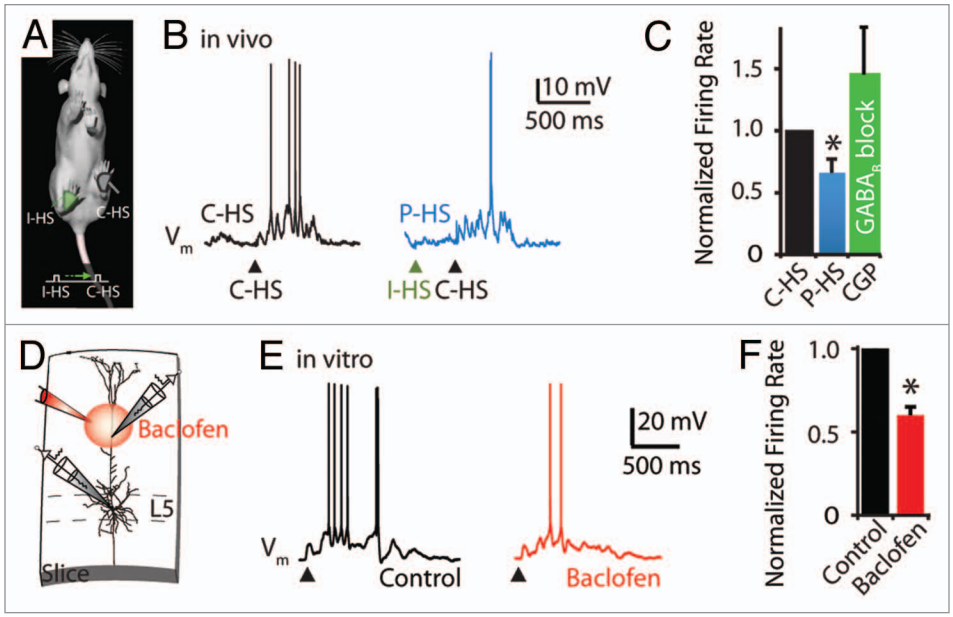

Figure 1. Dendritic $GABA_B$ activation decreases somatic output in layer 5 pyramidal neurons. (**A**) Schematic of in vivo experimental design. Layer 5 (L5) pyramidal neurons were patched with a whole-cell recording pipette and the voltage response to contralateral (C-HS) and paired (P-HS; ipsilateral 400 ms before contralateral) hindpaw stimulation was recorded. (**B**) Stimulation of the C-HS (black) evokes a large voltage response in neurons which is decreased when both hindpaws are stimulated (P-HS; blue). (**C**) The decrease in APs during P-HS was blocked by cortical application of the $GABA_B$ channel blocker CGP. (**D**) Schematic of in vitro experimental design. Layer 5 (L5) pyramidal neurons were patched with dual whole-cell recording pipettes at the soma and dendrite and the voltage response to current injections were recorded. (**E**) Example somatic traces during suprathreshold current injection alone (black) and during dendritic application of the $GABA_B$ agonist baclofen (red). (**F**) Dendritic baclofen application significantly decreased the firing rate during current injection.

paired hindpaw stimulation was not simply due to the typical action of inhibitory inputs that counterbalance excitatory synaptic inputs. Second, this effective downregulation of activity was mediated by slow-acting $GABA_B$ receptors (since $GABA_B$-receptor antagonist CGP52432 to the cortical surface abolished the inhibition; **Fig. 1C**), and not via the faster and stronger action of $GABA_A$ receptors. This suggests an unusual specificity of GABA receptor targeting during the activation of a specific microcircuit.

$GABA_B$ receptors in the apical dendrites of L5 pyramidal neurons are known to directly downregulate the active properties of dendrites, principally by blocking the underlying calcium conductances[14] and activating G-protein coupled inwardly rectifying potassium channels (GIRK; for a review see Bettler et al., 2004).[15] We tested in a slice preparation whether these postsynaptic mechanisms were in principle sufficient to account for the decrease of firing observed during interhemispheric inhibition (**Fig. 1D**). Local application of the $GABA_B$ agonist, baclofen, to the apical dendrite during dual current injections of in vivo waveforms into the dendrite and soma had little effect on somatic depolarization but dramatically reduced the spiking output (**Fig. 1E and F**). Direct block of dendritic calcium channels by local application of the calcium channel blocker Cadmium/Nickel accounted for the majority of the decrease in firing induced previously by $GABA_B$ receptor activation. The effects on L5 pyramidal neuron activity by direct dendritic $GABA_B$ receptor activation was similar to interhemispheric inhibition and suggests that dendritic depolarization during hindpaw stimulation increases the gain of the conversion of synaptic inputs into action potential output[11] and that $GABA_B$ receptor activation counteracts this gain increase.

The specific activation of $GABA_B$ receptors was puzzling on two levels. First, the vast majority of fibers crossing the corpus callosum are glutamatergic,[16] and second, it is a priori difficult to understand why the influence of the release of the neurotransmitter GABA was mainly confined to one inhibitory receptor type. To investigate this we used an optogenetic approach using light to specifically activate axons from the opposite cerebral hemisphere. This was achieved by prior injection of a virus expressing channelrhodopsin (a light-activatable protein channel)[17] in the opposite hemisphere which allowed us to investigate interhemispheric information transfer very precisely in vivo and in vitro.

In vivo, optogenetic activation of callosal fibers evoked the same effect on contralateral hindpaw stimulation as ipsilateral hindpaw stimulation, i.e., it decreased the evoked firing. In vitro, activation of callosal fibers indicated that interneurons in the upper cortical layers received much more excitatory inputs from the contralateral hemisphere than L5 neurons. Thus, inhibitory interneurons in the upper cortical layers were the most likely candidates to mediate the observed interhemispheric inhibition. Having narrowed the focus to glutamatergic activation of upper-layer interneurons, we injected small amounts of the glutamate channel-blocker, CNQX, layer 1 (L1) and layer 2/3 (L2/3) separately which revealed that the source of interhemispheric inhibition

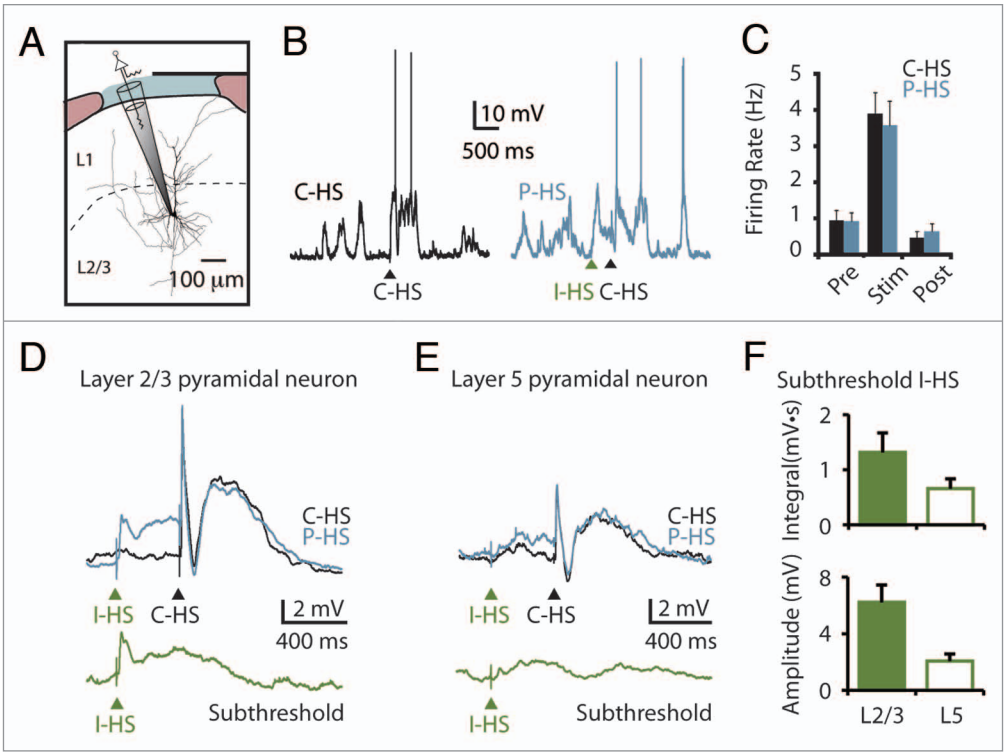

Figure 2. Ipsilateral hindlimb stimulation does not inhibit L2/3 neurons. (**A**) Schematic of in vivo experimental design. Layer 2/3 (L2/3) pyramidal neurons were patched with a whole-cell recording pipette and the voltage response to hindpaw stimulation was recorded. (**B**) Somatic recordings from a L2/3 pyramidal neuron during contralateral (C-HS; black) and paired (P-HS; ipsilateral-HS (I-HS) 400ms before C-HS; blue) hindpaw stimulation. (**C**) There is no significant difference in the evoked firing response due to C-HS (black) and P-HS (blue; n = 9). (**D**) Superaverage subthreshold voltage response to C-HS (black) and P-HS (blue) and I-HS (green) in L2/3 pyramidal neurons (n = 13). (**E**) Superaverage subthreshold voltage response to C-HS (black) and P-HS (blue) and I-HS (green) in L5 pyramidal neurons (n = 22). (**F**) Both the intergral (top) and amplitude (bottom) of the subthreshold I-HS response was larger in L2/3 than L5 pyramidal neurons. For subthreshold responses, all action potentials were truncated and averaged.

was from interneurons specifically in L1. Among L1 interneurons neurogliaform cells have recently received much attention because they generate slow inhibition via volume transmission and are known to activate $GABA_B$ receptors.[18-20] The activation of neurogliaform cells therefore neatly explains the mechanism of slow inhibition recruited by callosal activation. It also suggests that stimulation of body parts on the ipsilateral side activates only a subset of callosal fibers (since broad activation by light of the same fiber tract had excitatory effects that we didn't observe with natural stimuli).

L1 cells have also been implicated in the regulation of learning.[21] During fear conditioning, cholinergic fibers most likely from the nucleus basalis directly activate L1 neurons via nicotinic receptors in response to a foot-shock. In turn, L1 neurons inhibit other inhibitory interneurons in layer 2/3 (L2/3) which enables memory formation.[21] The cortical projection pattern of the nucleus basalis indicates that this disinhibition is not restricted to a specific cortical area, but is a non-specific neuromodulatory phenomenon. In contrast, the interhemispheric inhibition that we observed was highly specific to the stimulation of matching body parts, i.e., stimulation of other areas on the ipsilateral hindlimb did not result in inhibition.[13] Furthermore, interhemispheric inhibition of neuronal activity was only found in pyramidal neurons from deep cortical L5, the principal output neurons of the cortex, but not in pyramidal neurons in L2/3 (**Fig. 2A–C**). This may be simply due to these neurons receiving more excitation from interhemispheric input than L5 pyramidal neurons (**Fig. 2D–F**). These results highlight the possibility that, besides global modulatory signals, L1 neurons convey specific information from the contralateral hemisphere to a particular layer of the cortical microcircuitry.

In summary, we have described a form of interhemispheric inhibition that specifically affects deep cortical output neurons and is only evident during periods of increased dendritic activity, e.g., after paired hindpaw stimulation (**Fig. 3**). This form of inhibition may mediate the competition between the two hemispheres and result in an interhemispheric balance in L5 pyramidal neurons. Identifying the cellular and molecular elements involved in interhemipsheric inhibition is crucial not only for understanding higher brain function and but reveals potential targets for direct therapeutic intervention in the diseased brain. In patients with a unilateral stroke for example, interhemispheric balance is thought to be disrupted and the affected hemisphere can become over-inhibited. The investigation of the roles of the upper layer of the cortex for network functions have just begun and this area of research likely remains a hot topic for years to come.

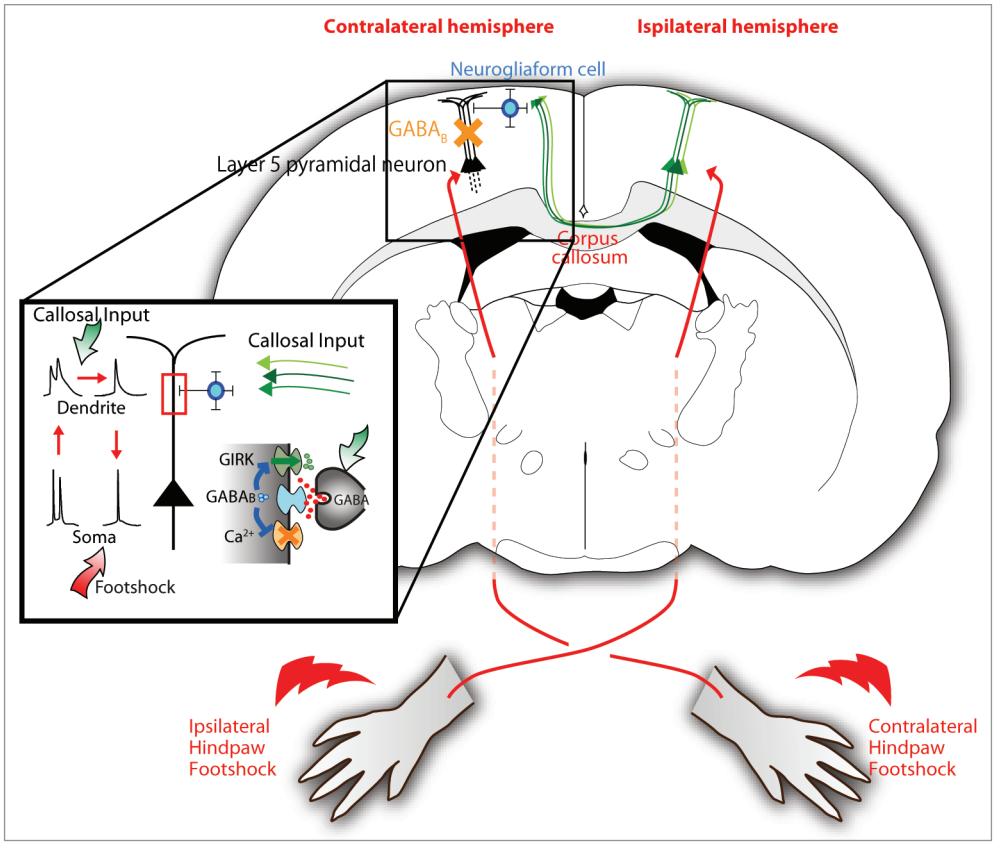

Figure 3. Proposed cellular mechanism of interhemispheric inhibition. Stimulation of the contralateral hindpaw (C-HS) generates dendritic input and backpropagating action potentials (APs) in somatosensory cortical layer 5 pyramidal neurons which activate dendritic voltage-sensitive channels leading to more APs. Activation of callosal input by ipsilateral hindpaw sitimulation (I-HS) activates GABA$_B$-mediating L1 interneurons (e.g., putatively neurogliaform cells) that open GIRK and block Ca^{2+} channels. This causes a silent inhibition (ie not seen in the absense of APs) which leads to a decrease in the firing response to C-HS.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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