

**Primary Afferent Depolarization of Low-Threshold Afferents
Does Not Require Trisynaptic Pathways**

Jacob Shreckengost¹, Jorge Quevedo², & Shawn Hochman¹

¹ *Department of Physiology, Emory University, Atlanta, GA 30312.*

² *Cinvestav del IPN, AV Inst Politecnico Nacional, Mexico City, Mexico.*

A principle mechanism for control of primary afferent transmission is via depolarization of their terminals, termed primary afferent depolarization (**PAD**) which can be measured following its antidromic electrotonic spread as a dorsal root potential (**DRP**). In low-threshold afferents, PAD is thought to occur via a trisynaptic pathway involving last-order GABAergic interneurons.

We tested if PAD of low threshold afferents occurs through more direct synaptic mechanisms. In the *in vitro*, hemisected, young rat cord, we stimulated lumbar roots at 100 μ A/100 μ s (~10T) or peripheral nerves from 4T-10T to maximally activate low-threshold afferents and recorded the DRPs generated in both the stimulated (homonymous) and adjacent (heteronymous) lumbar dorsal roots. This DRP investigated was blocked by GABA_A receptor antagonists and low dose tetrodotoxin.

Mephenesin or high divalent cation solutions were used to greatly reduce synaptic transmission. Whole-cell recordings of synaptic currents (EPSC), recordings of ventral root potentials (VRP), and evoked field potentials (EFP) provided evidence that only monosynaptic transmission remained. We observed that a significant portion of the DRP persisted and that DRP onset remained constant, demonstrating that classical trisynaptic and probably even disynaptic pathways were not required to generate the DRP. Monosynaptic EPSCs and EFPs were either unaffected or only marginally depressed in both mephenesin and a high divalent cation solution, whereas longer latency components were depressed. VRPs further confirmed a preferential depression of polysynaptic potentials and a block of polysynaptic spiking.

In nominally Ca²⁺-free solution or in the presence of EGTA to chelate Ca²⁺, the DRP was completely blocked confirming that Ca²⁺ entry-mediated synaptic transmission is required for DRP generation. Following near complete block of synaptic transmission with a high Mg²⁺/low Ca²⁺ solution, the homonymous evoked DRP was reduced but remained while intracellular synaptic events and the heteronymous DRP were virtually abolished. A train of stimuli (5 pulses at 20 Hz) caused facilitation, generating a clear heteronymous DRP and synaptic currents. This demonstrates that the DRP can be generated under conditions of severely restricted synaptic transmission.

Overall these results demonstrate that presynaptic inhibition of low-threshold primary afferents can occur by more direct synaptic mechanisms, independent of trisynaptic pathways.

Supported by: The Christopher and Dana Reeve Foundation