



Title	Ca2+ imaging of cricket protocerebrum responses to air current stimulation
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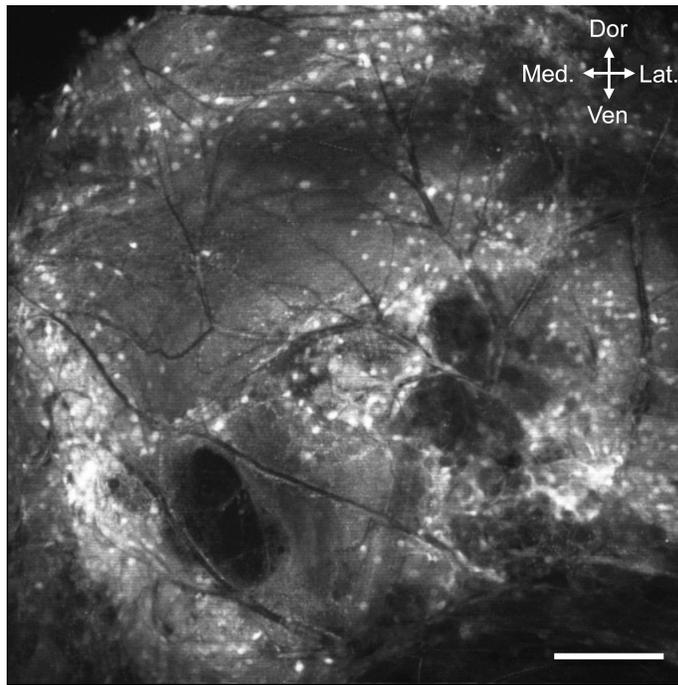


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## Supplementary Methods

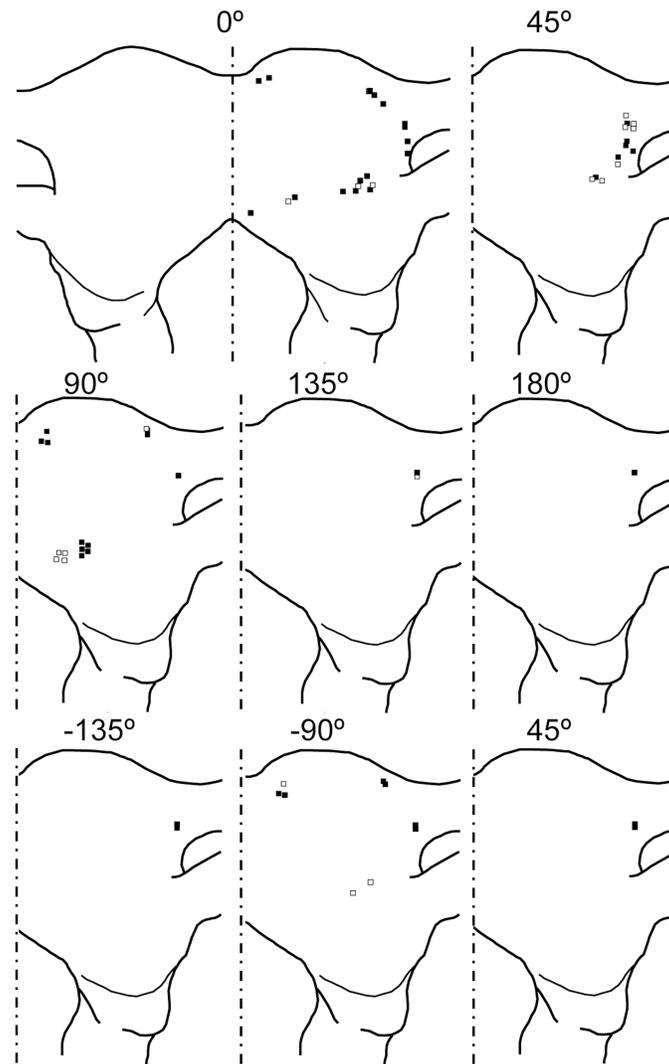
### *Electrophysiology*

Extracellular recordings of VLNP neurons were made using a tungsten-wire ( $\text{\O} = 0.06 \text{ mm}$ ) electrode that was electropolished and coated with polystyrene. The wire electrode was penetrated into VLNP using a micromanipulator. Signals were digitized as 20 kHz through an A-D converter (Powerlab 4/s, ADInstruments, Dunedin, New Zealand) and filtered with 150–3000 Hz band pass. Individual spikes were detected using the commercially available spike sorting software (Spike Taro, Chinou Jouhou Shisutemu, Kyoto, Japan), but not sorted to different units in this experiment.



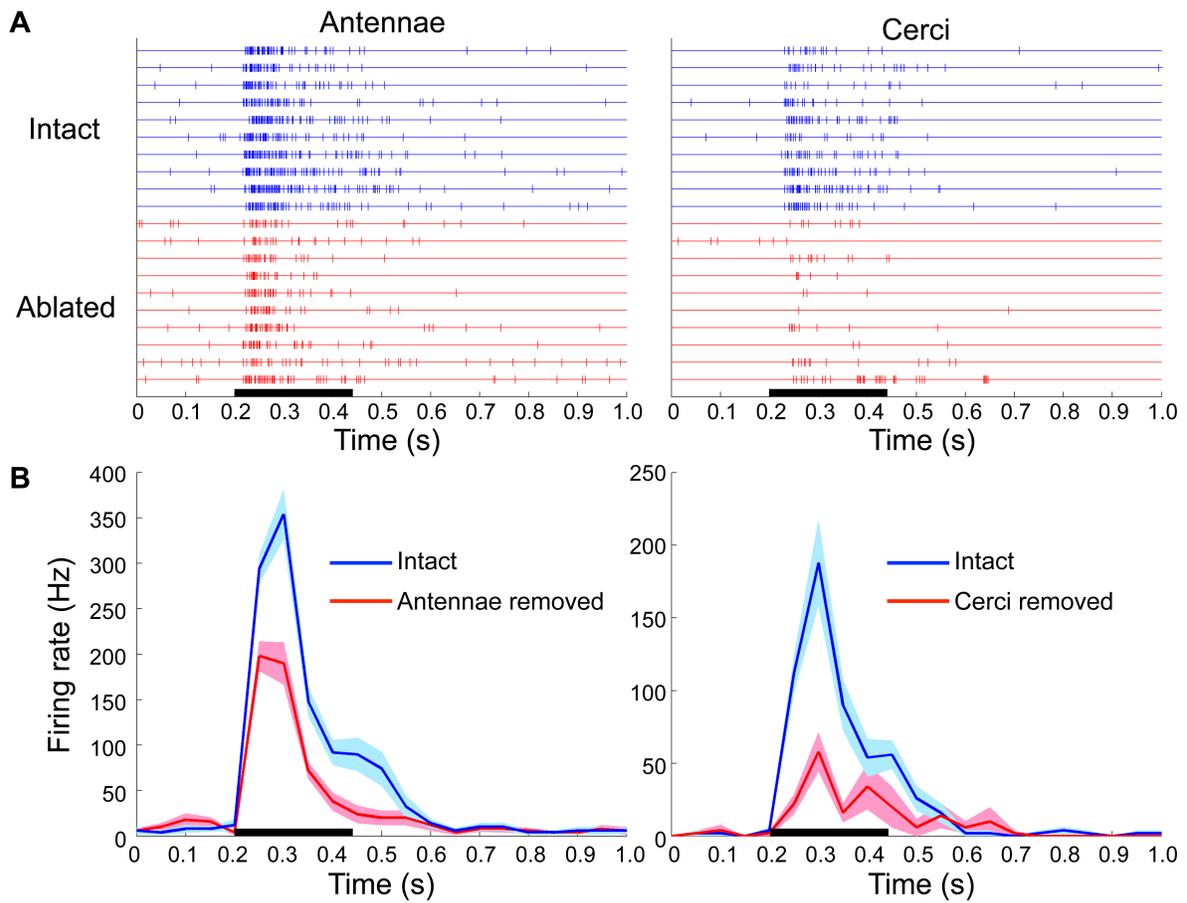
Supplementary Fig. 1

A merged confocal image of the VLNP region in left half hemisphere of brain labeled with OGB-1 acetoxy-methyl ester. This image was built from 11 images acquired at different focal planes with each 3.46  $\mu\text{m}$  of depth. Scale bar = 100  $\mu\text{m}$ .



Supplementary Fig. 2

Spatial location of cells responding to air current stimuli from different directions. Solid and open squares indicate the location of ROIs in which excitatory (positive fluorescence change) or inhibitory (negative fluorescence change) responses, respectively, were measured. ROIs from five different animals are superimposed on the outline of a representative brain ganglion sample after the images were aligned to the ganglion contour.



Supplementary Fig. 3

Electrophysiological recording of air-current-evoked responses in VLNP. (A) Raster plots of the evoked spikes before (blue traces) and after (red traces) the ablation of antennae (left) or cerci (right). The air currents were delivered from front ( $0^\circ$ ). (B) Temporal profiles of the firing rate in all evoked spikes during the air current stimuli in intact and antennae- (left panel) or cerci-removed condition. Data indicated by left and right panels were acquired from different animals.