

Stimulus-induced modulations of oscillatory neural activity in olfactory areas of snail brains recorded with a 64 electrode planar microarray

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We have developed the first invertebrate adaptation of multi-electrode recording with the MED64 planar microarray. In many molluscan brains, it is possible to record oscillations in the local field potential (LFP) from the procerebrum of the cerebral ganglia, and the frequency and amplitude of the oscillations are modulated by olfactory learning and olfactory discrimination. We used MED probe dense arrays with 100 μm inter-electrode distance to record LFP oscillations from the brains of two species of snail. *Helix aspersa*, the garden snail is a well-studied molluscan model of olfactory processing, and *Euglandina rosea*, the rosy wolfsnail, is a predatory snail native to the southeastern US. *Euglandina* that uses slime trails to track both prey snails and conspecifics. It follows slime trails using a specialized lip extensions and a chemosensory system that is separate from olfactory sensing. We investigated changes in spatial and temporal patterns of oscillatory activity in response to application of two neurotransmitters reported to affect LFP oscillations in mollusks, as well as stimulation of sensory epithelia with odorants and snail slime extracts. The neurotransmitters investigated were Nitric oxide (NO) which has been shown to play a role in controlling procerebral oscillations in a number of molluscan species, and serotonin which has been linked to increases in the amplitude and frequency of LFP oscillations.

Interestingly, in most of our experiments recording procerebral LFP from *Helix* and *Euglandina* preparations with the olfactory nerves and sensory epithelia intact, the activity is in a highly synchronized mode in which the oscillations occur simultaneously across widely spaced electrodes instead of propagating across the procerebrum with a phase delay. In the slug, *Limax*, the propagating oscillatory gradient of the procerebrum has been reported to “collapse” with olfactory stimulation resulting in widespread synchronization of activity across the PC lobe. In many of our experiments most of the electrodes in contact with the ganglia record synchronized activity with little phase delay, even in unstimulated conditions. Addition of serotonin to the cerebral ganglion appears to increase the amplitude and frequency of field potential oscillations, and also increases the number of electrodes with synchronous activity. While other investigators using voltage sensitive dyes have reported on oscillatory LFP activity only in the procerebral region, when the entire cerebral ganglion is covering the electrode grid, the oscillatory activity we record can sometimes spread across the entire cerebral ganglion. Serotonin increases the frequency of the oscillatory LFP activity, as well as recruiting more cerebral ganglion cells into synchronized activity. NO tended to increase the number of active electrodes and the frequency of LFP oscillations but did not have a consistent effect on the number of synchronized electrodes.

The effect of stimulating sensory epithelia with natural stimuli such as odorants and snail slime was also investigated. *Euglandina* snails use specialized epithelia on a unique set of lip extensions to detect and follow slime trails of prey snails and conspecifics. Application of slime to the lip tentacle increased oscillatory LFP activity and increased the number of electrodes recording synchronized activity.

MED64 recordings share many features with EEG recordings of human brains. EEG signals measured from the scalp are produced by the partial synchronization of neuronal field potentials across areas of cortex, and this synchronization is thought to optimize communication within and between brain areas. Our MED64 data also show varying amounts of synchronization across the snail cerebral ganglia that appear to be related to the state of the ganglion and the stimuli applied to it. The technique of independent component analysis (ICA) has been applied to EEG data, and we are adapting this technique to identify separate independent sources that contribute to the ensemble local field potential signals recorded by the MED64.