Operant conditioning using self-stimulation in *Aplysia*

Björn Brembs, Elizabeth Wilkinson, Fredy Reyes, Douglas Baxter and John Byrne

University of Texas – Houston Medical School, Department of Neurobiology and Anatomy, 4631 Fannin, Houston, Texas, USA.

Due in part to studies of learning in *Aplysia*, a great deal is known about the cellular basis of classical conditioning. In comparison, relatively little is known about the mechanisms underlying operant conditioning. This deficit results, in part, from the lack of a suitably traceable model system that manifests operant conditioning and that is amenable to cellular and molecular studies. To address this issue, we developed an *in vivo* conditioning procedure using the feeding behaviour of *Aplysia* and virtual stimuli.

Virtual reality encompasses the replacement of physical stimuli with neural stimulation. In *Aplysia*, sensory receptors in the buccal cavity transmit stimuli to the buccal ganglion. In pilot studies, we have performed *in vivo* recordings from the anterior branch of the oesophageal nerve in feeding *Aplysia*. These studies revealed presumably afferent activity (30Hz/3s) that coincided with biting and swallowing. In a novel operant conditioning paradigm, we have substituted food reinforcement (unconditioned stimulus, US) with extracellular stimulation of the anterior branch of the oesophageal nerve via surgically implanted electrodes. The pattern of stimulation was modelled after the recordings from feeding *Aplysia* (30Hz/3s). In an experimental group, nerve stimulation was made contingent on spontaneous biting behaviour. In other words, the animals could stimulate themselves by biting. In a yoked control group, the animals received the same pattern and amount of stimulation as the animals in the experimental group, but independent of their behaviour. A third group did not receive any stimulation at all (no-US group). Training lasted for 10 minutes. The animals were then tested for spontaneous biting behaviour either immediately after training (Fig. a) or 24 hours later (Fig. b). Each test session lasted 5 minutes. At both time points, contingent reinforcement lead to a significantly higher rate of biting in the experimental group compared to both controls. These results show that contingent *in vivo* stimulation of the anterior branch of the oesophageal nerve in *Aplysia* is sufficient to induce an operant memory that lasts for at least 24 hours. Further studies will reveal whether the neuronal correlates found in the *in vitro* analogue can be found in the CNSs of animals trained operantly with *in vivo* stimulation of the oesophageal nerve.