Operant Conditioning using self-stimulation in *Aplysia*

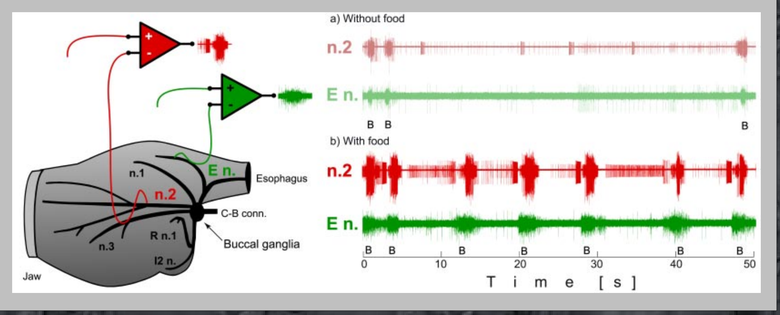
Björn Brembs, Fred Lorenzetti, Elizabeth Wilkinson, Fredy Reyes, Douglas A. Baxter and John H. Byrne
University of Texas – Houston Medical School
Department of Neurobiology and Anatomy
6431 Fannin, Houston, Texas, USA
http://nba.uth.tmc.edu/homepage/jbyrne/

I. Introduction

For most of the 20th century there has been a debate over the equivalence of classical and operant learning processes. 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. If such a preparation existed, the old psychological problem could be developed into a biological experiment.
Ideally, both operant and classical processes should be studied in the same model system. As part of our effort to develop operant and classical paradigms in *Aplysia* feeding behaviour, we developed an *in vivo* operant conditioning procedure, complementing an already established classical protocol (Lechner at al. 2000a, b). Unlike the classical procedure that used food reinforcement, we replaced the food by virtual stimuli. Virtual reality encompasses the replacement of physical stimuli with neural stimulation. Thus, we started by monitoring putative afferent activity in the esophageal nerve (E n.) in the feeding animal (see II). The recorded activity was then mimicked by electrical stimulation of the E n. as reinforcement. The effectiveness of the reinforcement was determined by pairing it with biting behaviour in an operant conditioning experiment. The animals controlled the electrical stimulation of the E n. by their own behaviour (i.e. self-stimulation; see. III). Neuronal correlates of the operant memory after reinforcement with virtual stimuli were obtained from an interneuron in the buccal ganglion of *Aplysia* (see IV).

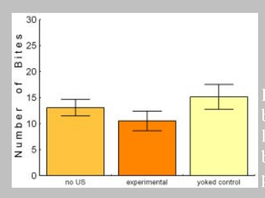
II. In vivo recordings

Chronic extracellular recordings were performed by surgically implanting hook electrodes on intact *Aplysia* nerves. One set of electrodes was placed on buccal nerve 2 (n.2) and served as a behavioural monitor for biting and swallowing behaviour. The other was attached to the anterior branch of the esophageal nerve (E n.). Reference electrodes were floating freely in the hemocoel. The recordings were performed one day after the surgery. Prolonged activity (3s/30Hz) in the E n. is generated when food is present in the buccal cavity (b), versus during spontaneous biting behaviour without food (a).

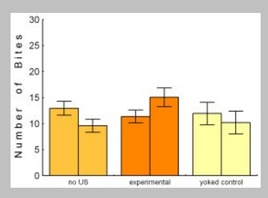


III. Operant conditioning

Operant conditioning of feeding behaviour was carried out in blocks of five minutes. A five minute baseline period was followed by either two or three 5min training blocks. The animals were then either tested immediately after training or 24h after the start of the experiment. Before each experiment, the animals were randomly assigned to one of three groups: an 'experimental' group in which each bite during training was followed by 3 seconds of 30Hz (8V) stimulation of the E n., a 'yoked control' that received the same sequence of reinforcements as the experimental group, but independent of their behaviour and finally a 'no US' group that did not receive any stimulation at all. During test phases, none of the animals received any stimulation.

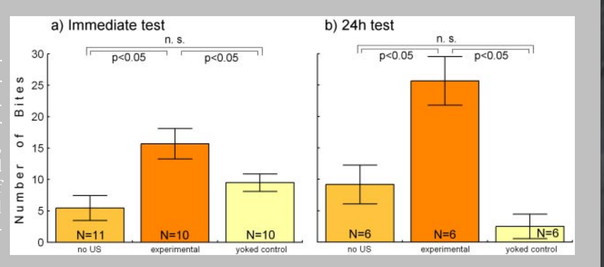


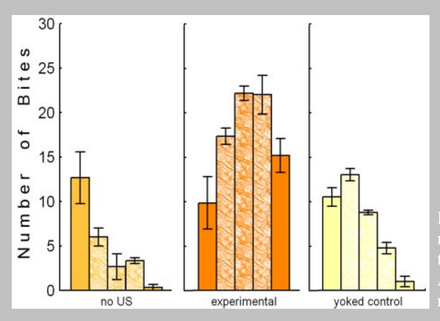
Left: 5 minutes of baseline test revealed no difference between the groups prior to training



Left: During training, a difference between the groups is starting to emerge.

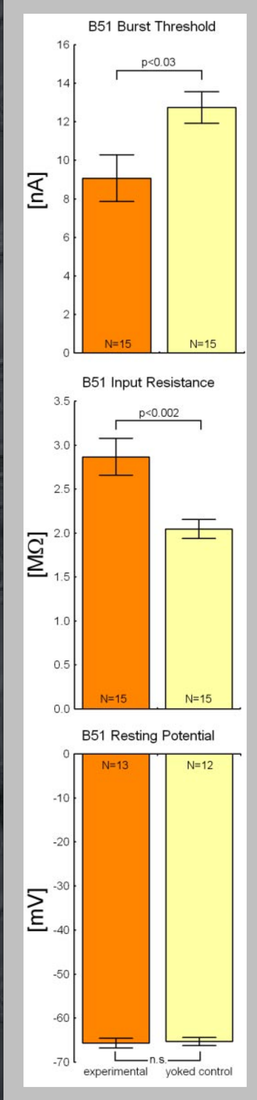
Right: Animals were tested for spontaneous biting behaviour either immediately after training (Fig. a) or 24 hours later (Fig. b). At both time points, contingent reinforcement lead to a significantly higher rate of biting in the experimental group compared to both controls.



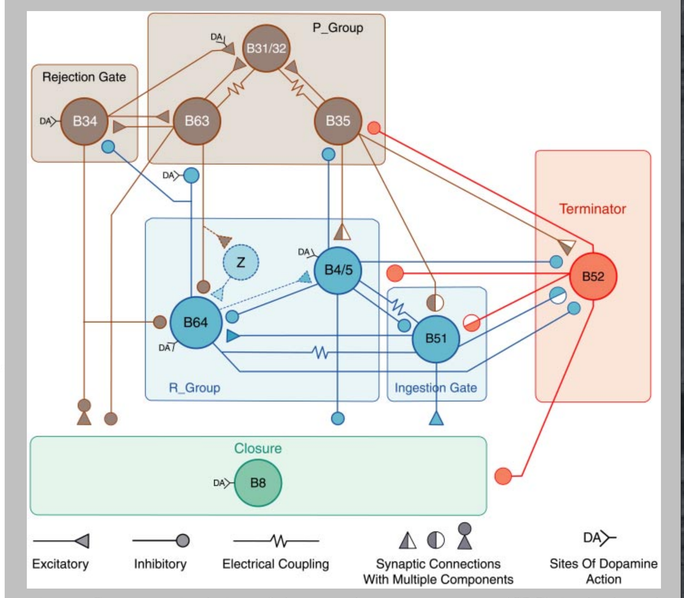


Left:In a pilot study for the search for neuronal correlates (see IV), we investi-gated the effects of three instead of two 5min training blocks.
Apparently, three training blocks increase the difference between the groups.

IV. Neuronal correlates of operant conditioning



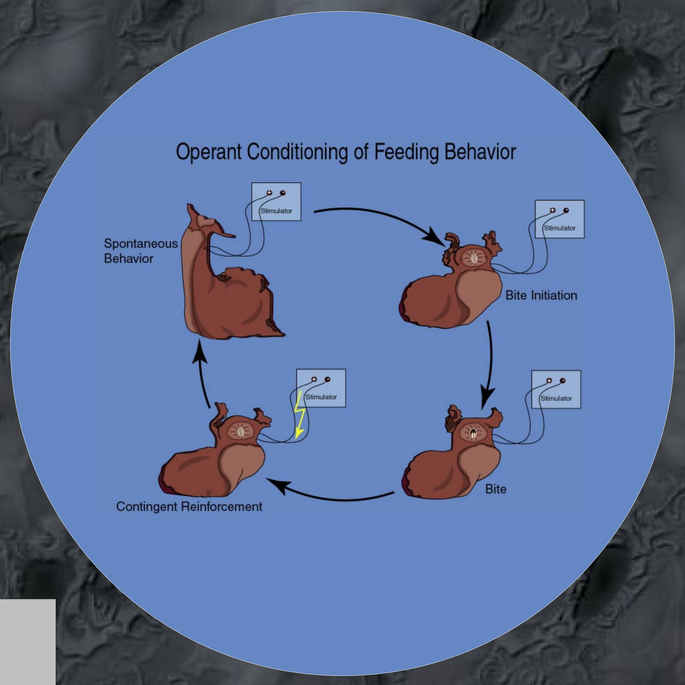
Aplysia buccal interneuron B51 is only active during ingestion-like activity in the buccal central pattern generator (CPG, below). It was found previously, that B51 exhibits lasting changes in its intrinsic properties after training in an *in vitro* analogue of operant conditioning (Nargeot et al. 1999a,b). In this analogue, the isolated buccal ganglion was reinforced via E n. whenever the CPG produced ingestion-like activity, a procedure very similar to our *in vivo* preparation (see III).
In the present study, we used animals after three 5min blocks of training (see III). Directly after training, the animals were anaesthetised and the buccal ganglia removed. Using standard electrophysiological techniques, the amount of depolarizing current needed to elicit a burst of action potentials ('burst threshold'), the magnitude of deflection of the membrane potential due to current injection ('input resistance'), as well as the cell's resting membrane potential was recorded. We found a significant increase in input resistance and a decrease in burst threshold in the experimental group vs. the yoked control (left). These results suggest that B51 should be more easily recruited during pattern generating activity in the buccal CPG. As B51 is only active during ingestion-like activity, the electrophysiological results are consistent with the behavioural observation of an increase in the number of bites after operant conditioning (see III).



Above: Computer simulation of the central pattern generator (CPG) in the buccal ganglia of *Aplysia*. The model incorporates the diverse physiological properties of the different identified cells as well as the multiple components and plasticity in most synaptic connections. Data from our physiological studies is constantly being added to upgrade the simulation.

V. Conclusion

We have recorded afferent activity in the E n. of feeding *Aplysia* that coincided with biting and swallowing (see II). When freely moving *Aplysia* can control the amount of E n. stimulation mimicking the activity during biting and swallowing food, they increase the behaviour controlling the stimulation compared to two control groups. The association between the behaviour and the stimulation can still be detected 24h after the experiment (see III). Comparing input resistance and burst threshold of buccal interneuron B51 in contingently reinforced animals versus yoked controls, a neuronal correlate of the operant memory can be detected (see IV).
These findings parallel the results of previous experiments where a similar change in the intrinsic properties of B51 was found in an *in vitro* analogue of operant conditioning (Nargeot et al. 1999a,b). Taken together, these experiments establish a direct line of evidence from a behavioural paradigm, via an operant analogue in the isolated nervous system to a single cell. This cell exhibits lasting modifications of its intrinsic properties after operant conditioning in both the whole animal and the *in vitro* analogue. A parallel set of *in vivo* and *in vitro* studies is currently being performed using classical training. Comparative studies of operant and classical conditioning in the same model system on a cellular and molecular level will finally be feasible.



References:
Lechner, H. A., Baxter, D. A. and Byrne, J. H. (2000a). Classical conditioning of feeding in Aplysia: I. Behavioral analysis. *J. Neurosci.* **20,** 3369-76.
Lechner, H. A., Baxter, D. A. and Byrne, J. H. (2000b). Classical conditioning of feeding in Aplysia: II. Neurophysiological Correlates. *J. Neurosci.* **20,** 3377-3386.
Nargeot, R., Baxter, D. A. and Byrne, J. H. (1999a). In vitro analog of operant conditioning in aplysia. I. Contingent reinforcement modifies the functional dynamics of an identified neuron. *J. Neurosci.* **19,** 2247-60.
Nargeot, R., Baxter, D. A. and Byrne, J. H. (1999b). In vitro analog of operant conditioning in aplysia. II. Modifications of the functional dynamics of an identified neuron contribute to motor pattern selection. *J. Neurosci.* **19,** 2261-72.