Snail Model System
Intro to snails as a model system in neurobiology

Our lab snail

Fig. 1. The herbivorous gastropods studied include the marine opisthobranchs *Aplysia* (10 cm long) and *Pleurobranchaea* (5 cm long, eating a nudibranch, *Flabellina* which is coloured purple and orange) and the pulmonates *Helix* (terrestrial, 2 cm long) and three freshwater genera (*Planorbis*, *Helisoma* and *Lymnaea*, all 1 cm long). The pictures are not to scale. We are grateful to Rhanor Gillette and Andy Bulloch for the pictures of *Pleurobranchaea* and *Helisoma*. 
**Figure 5.1. Snail brain.** The gut runs through the space between cerebral and subesophageal ganglia. Note the pedal nerves attached to the subesophageal ganglia; they indicate the ventral surface. The aorta is a hollow tube running through the ganglia.

**Figure 5.6. Circumesophageal ganglia.** A. The snail brain consists of nine ganglia surrounding the gut. You will record from the visceral and right parietal ganglia. B. Some of the cells of the visceral and right parietal ganglia, as numbered by Kerkut et al. (1975).
Figure 5.4. Dissected brain. A. The entire ring of circumsophageal ganglia (dorsal view). B. The desheathed subesophageal ganglia (dorsal view). Go down a layer to remove the labels.
Pond snail brains
Identified neurons in pond snail brains

dorsal

ventral
Snail Buccal Ganglia
Buccal ganglia neurons
Smail Week 1. Firing properties of snail neurons

Figure 5.7. Action potential measurements. **A.** If the cell is spontaneously active or has been stimulated, count the number of spikes in an interval. Spike rate is number of spikes/period. **B.** With a single spike, measure the threshold, AP height, duration at half-height, and depth of afterhyperpolarization.
Responses to current injection

Figure 5.A: Silent cell stimulation. A cell without spontaneous activity was stimulated with depolarizing current for 5 s. The plot shows spike rate during the stimulus vs. the amount of current injected. Insets show activity traces for two of the stimuli.

Figure 5.D: Tonically firing cell. A cell with regular spontaneous activity was stimulated with 4 s of hyperpolarizing or depolarizing current. The top inset shows activity without stimulation; the lower inset shows activity with ~2 nA of current. Note the period of reduced activity after the stimulus in this inset.
Firing Accommodation

**Figure 5.C. Spike amplitude decrease.** A Ca$^{2+}$ cell without spontaneous activity was stimulated with depolarizing current. The amplitude of APs after the first one decreases due to inactivation of voltage-gated Na$^+$ and/or Ca$^{2+}$ channels. After hyperpolarization amplitude decreases due to inactivation of the K$^+$ current responsible for repolarization. Spike failure (note the depolarization after the last AP) occurs when the membrane reaches AP threshold but there are too few active Na$^+$ channels to trigger a full AP.

**Figure 5.B. Spike broadening and accommodation.** A Ca$^{2+}$ cell without spontaneous activity was stimulated with depolarizing current. Each spike is wider than the previous one (spike broadening) due to increasing inactivation of the voltage-activated channels that carry the repolarizing K$^+$ current. The interval between spikes increases (accommodation) due to a slowly developing outward K$^+$ current that counters the depolarizing stimulus. This current is also makes the membrane potential hyperpolarize slightly after the end of the stimulus.
Post-inhibitory rebound

**Figure 5.5. Tonic cell hyperpolarization.** During hyperpolarization, the amplitude of postsynaptic potentials increases due to greater difference between the membrane potential and the Nernst potential of the ion responsible for the PSP. Also note the brief increase in the spike rate following the stimulus. This is due to deactivation of inactivated voltage-gated Na⁺ channels.

**Figure 5.9. Postinhibitory rebound.** This cell is normally silent. When released from period of hyperpolarization, it fires an action potential. This form of excitability is due to a hyperpolarization-activated channel, which carries the h₉ current.
Burster neurons

Figure 5.7. Burster stimulation. Constant current was injected into a bursting cell, changing the number of spikes per burst and the rate of bursting. The center inset shows the activity of the cell without current injection. Note the increase in burst rate with current.

Figure 5.8. Burster measurements. A. With a long recording, measure the amplitude of the oscillating membrane potential, count the number of spikes per burst, measure the burst duration, and measure the burst period. Burst rate is 1/period; spike rate within a burst is number of spikes/burst duration. B. With a single spike, measure the threshold, AP height, duration at half-height, and depth of afterhyperpolarization.
Snail week 2. Ionic mechanisms of excitability

Reduced Na

Reduced Ca

Figure 6.A. Reduced calcium. The red trace shows a Ca\(^{2+}\)-based AP in normal saline. The blue trace shows an AP from this cell after 15 min in low Ca\(^{2+}\) saline. AP amplitude decreases due to the decreased Nernst potential for Ca\(^{2+}\). The AP is broader because less Ca\(^{2+}\) enters the cell to activate Ca\(^{2+}\)-activated K\(^+\) channels involved in repolarization.
K+ Channel block with TEA
K+ Channel block with TEA/Cs

Figure 6.C. Effect of TEA/Cs. The red trace shows a Ca\(^{2+}\)-based AP. The blue trace shows an AP from this cell 5 min after injection with a mixture of TEA and Cs\(^+\). This mixture blocks the voltage-gated K\(^+\) channels and the Ca\(^{2+}\)-activated K\(^+\) current, thus broadening the AP.
TEA/CS block progressing over time (5 minutes)
K+ Channel block with barium

**Figure 6.B. Effect of barium.** The red trace shows a Ca\(^{2+}\)-based AP in normal saline. The blue trace shows an AP from this cell after 15 min in saline with Ba\(^{2+}\) substituted for Ca\(^{2+}\). Although Ba\(^{2+}\) depolarizes the cell by entering through the Ca\(^{2+}\) channels, it does not activate the Ca\(^{2+}\)-activated K\(^{+}\) channels involved in repolarization.
Excitability mechanisms: Voltage Clamp
Describing the ionic conductances underlying the AP

**Figure 5.C. Spike amplitude decrease.** A Ca^{2+} cell without spontaneous activity was stimulated with depolarizing current. The amplitude of APs after the first one decreases due to inactivation of voltage-gated Na^{+} and/or Ca^{2+} channels. Afterhyperpolarization amplitude decreases due to inactivation of the K^{+} current responsible for repolarization. Spike failure (note the depolarization after the last AP) occurs when the membrane reaches AP threshold but there are too few active Na^{+} channels to trigger a full AP.
Voltage clamp technique to examine ionic currents
Problems analyzing conductances underlying AP

1. $I_{Cm}$ obscured $I_{Rm}$
2. Conductances changed during AP

Current across membrane

$$I_m = I_{Rm} + I_{Cm}$$
Ohm's law for V-Clamp

\[ g_{ion} = \frac{I_{ion}}{E_{m} - E_{ion}} \]

- \( E_{m} \): recorded continuously, constant...clamped at a known level.
- \( E_{ion} \): constant...calculated from Nernst.
Ionic driving forces at rest

\[ g_{ion} = \frac{I_{ion}}{E_m - E_{ion}} \]

- \( E_m \): constant, clamped at a known level.
- \( E_{ion} \): constant, calculated from Nernst.

Diagram:
- Inside: 60mV
- Outside: 60mV
- Electrical gradient (60mV)
- Net gradient (15mV)
- Concentration gradient (\( \equiv 75mV \))
** Ionic driving forces at rest **

$$ g_{ion} = \frac{I_{ion}}{E_m - E_{ion}} $$

- Recorded continuously...
- Constant...clamped at a known level.
- Constant...calculated from Nernst.

** INSIDE **

- 60mV
  - Electrical gradient (60mV)

** OUTSIDE **

- Na⁺
  - Net gradient (110mV)
  - Concentration gradient (≈50mV)
Current across membrane with hyperpolarizations
\[ I_m = I_{Rm} + I_{Cm} \]
Current across membrane with weak depolarizations
Current across membrane with strong depolarizations
Measure I

Membrane potential (mV)

Membrane current density ($\mu$A/cm$^2$)

INa

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