

# Ageing at a snail's pace

*Greg Scutt BPharm  
MRPharmS  
DipClinPharmPrac  
Clinical Lecturer,  
Care of the Elderly*

*Mark Yeoman Ph.D  
Senior Lecturer in  
Pharmacology  
University of Brighton*

## **Introduction**

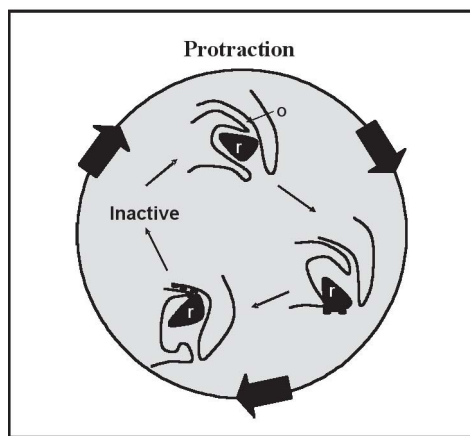
You may think that attempting to record electrophysiological data from the central nervous system (CNS) of an elderly snail would be a particularly uneventful task given that the snail is one of the slowest creatures to crawl the earth, and being elderly is even more lethargic than its younger relations. Yet the CNS of the common pond snail, *Lymnaea stagnalis*, proves to be a highly intriguing organ, and a useful model for the investigating the basic biology of neuronal ageing.

The practical advantages of using *Lymnaea stagnalis* for ageing research are many. The snails are cheap and easy to obtain and maintain, and being invertebrates require no ethical approval for study. They have a short lifespan (= 12 months) that allows laboratories to set up rolling batches of young and elderly snails in a relatively short period of time. The CNS, which is comprised of large orange neurones, is extremely easy to dissect. Individual neurones in the CNS can be reproducibly identified from one preparation to the next by means of their characteristic colour, position and electrophysiological properties. The ability to make electrophysiological recordings from identical neurones in many different preparations has made it possible to map small neural networks that control defined behaviours. Quantifying behaviour is made easy by the fact that the *Lymnaea* performs only a limited number of daily activities, each one relatively simple and of course accomplished at a 'snails pace'. Such advantages allow us to perform a unique top-down approach where age-related changes in behaviour can be linked to changes in the functioning of defined neurones and the genes expressed by those neurones.

But is *Lymnaea* a good model to study the basic biology of neuronal ageing? At the organismal level, survival curves of populations of laboratory bred *Lymnaea* fit well to a Weibull function and manipulations such as calorie restriction which have been shown to extend lifespan in organisms as diverse as worms and mammals, have similar effects in *Lymnaea*<sup>1</sup>. At the network/cellular level the main changes that are seen with increasing age in a wide range of organisms are changes in the strengths and numbers of the synaptic connections within the CNS. Molluscs, such as *Lymnaea*,<sup>2,3</sup> have contributed enormously to our current understanding of synaptic plasticity suggesting they may prove useful in unravelling the basic biology of neuronal ageing.

### History of Research into *Lymnaea stagnalis*

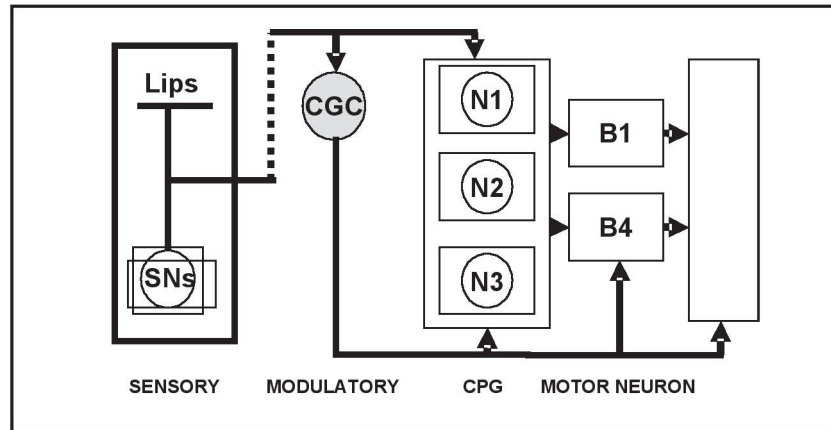
The intricate workings of the *Lymnaea* CNS were first described by Rose and Benjamin in the 1960s and 70s. The group identified a number of the neurones that comprise the *Lymnaea* CNS and related their firing patterns to many physical functions. One such function, which appears at the top of the list of *Lymnaea*'s daily activities, is that of feeding<sup>4</sup>. The process of feeding in *Lymnaea* is rhythmical, with each repetitive cycle consisting of four distinct phases (protraction, rasp, swallow, and the rest or inactive phase; see Fig 1). The muscles that are activated during the feeding process belong mainly to the buccal mass which lies posterior to the mouth opening. The motor neurones which stimulate contraction of these muscles have been identified, and they in turn are driven by a special collection of three types of inter-neurones called the N1-N3



**Fig 1: Diagrammatic representation of a typical feeding cycle of the pond snail, *Lymnaea*. Diagram shows the radula (r) and oesophagus (o). Adapted from<sup>5</sup>.**

cells which fire in the order  $N1 \rightarrow N2 \rightarrow N3$  (collectively termed the Central Pattern Generator, CPG). The N1 cells can fire spontaneously to initiate a complex pattern of inhibition and excitation amongst the N cells and the motor neurones involved in feeding, which ultimately maintains the precise order of motor neurone firing needed to execute one complete feeding cycle. The firing of the N1 cells is primarily associated with protraction, the N2 cells with rasp, and the N3 cells with swallow.

Other neurones in the CNS are able to regulate the frequency and duration of the different phases of the feeding cycle. One of these modulatory cell types are the serotonergic Cerebral Giant Cells (CGCs). The CGCs, cannot initiate a feeding cycle themselves, but are necessary to allow the feeding system to respond to food and to regulate the frequency of feeding<sup>6,7</sup> (Fig 2).



**Fig 2. Diagrammatic representation of the neural circuit that controls the feeding behaviour of the pond snail, *Lymnaea stagnalis*. Shown are the sensory neurones (SNs), cerebral giant cells (CGCs), the “N” cells of the central pattern generating circuit and a pair of motor neurones, B1 and B4. Adapted from<sup>8</sup>**

### Age related changes in *Lymnaea stagnalis*

So how can our knowledge of the complex neuronal circuitry of the *Lymnaea* feeding system be exploited to investigate the effects of age? Well, one key question concerning that still remains unanswered is “Why are all neurones not affected equally by the increasing age?” and it seems to me that this question and others could be answered by studying this defined neural network. Firstly, to get an idea of which neurones are affected by age, let’s look at the bigger picture, the feeding habits of the intact *Lymnaea stagnalis*. Once we know how this is affected by age we can relate the observed changes to the known properties of key neurones.

In experiments conducted to assess the ability of snails of varying ages to initiate a feeding cycle following the application of sucrose solution (a stimulus for feeding) to their lips, it was found that it was possible to evoke feeding responses in 100% of young snails, but only 88% of older snails<sup>9</sup>. Furthermore, of those old animals that did respond there was a slowing of the frequency of the evoked rhythm that was due specifically to an increase in the duration of the swallow phase of the feeding cycle<sup>9</sup>.

The question therefore arose: “What is happening to the neurophysiology of the *Lymnaea* CNS as they age?” We have recently shown that there is no measurable age-related change in the sensory information entering the CNS but that there were changes in the way in which the CNS processed the incoming signal<sup>7</sup>. As the CGC’s have the ability to allow the feeding system to respond to food and to regulate its frequency, it was proposed that the functioning of these modulatory neurones might alter over the lifetime of the *Lymnaea*. Experiments to detect changes in CGC function with increasing age have yielded interesting results. The basal firing rates of the CGCs was reduced with increasing age as was their excitability following the application of a food stimulus to the lips<sup>10</sup>. The reason for this reduced excitability is currently unclear although there are clear increases in the amplitude and duration of afterhyperpolarisation in the older snails which would act to reduce the firing rate of the CGCs in the old animals.

Although the frequency of CGC action potentials is reduced with increasing age, the amount of neurotransmitter released per action potential appears to increase, inferring a level of compensation within the CNS. Electrochemical studies have shown that the majority of this compensation is due to an age-related decrease in the

efficacy of the transporter protein whose job is to remove serotonin from the synapse and pump it back into the neurone. Decreases in the functioning of this protein would allow concentrations of serotonin in the synapse to increase, increasing synaptic efficacy. However, although the nervous system is trying to compensate for a decrease in more sensitive to the effects of age than neuronal firing this level of compensation is not complete and hence we still observe a marked behavioural deficit.

The precise mechanisms by which ageing reduces CGC excitability and the underlying compensatory processes are currently unclear but are under investigation.

Our observations that an age-related change in feeding behaviour could be linked to changes in the activity of a single pair of serotonergic neurones are interesting. Does this mean that in molluscs only serotonergic neurones are sensitive to the effects of age or more likely that serotonergic neurones are among the first population of neurones to be affected by age? If the latter is true then what is it about these serotonergic neurones that make them more sensitive to the effects of age than other neurones in the network? Answering this question will be difficult but could hold the key to understanding how age affects the nervous system and may provide novel targets for drugs that may alleviate the rate of neuronal ageing.

### **Conclusion and Summary**

In an ageing population, where diseases and the physiological consequences of old age are becoming commonplace, having a robust model for ageing research such as *Lymnaea stagnalis* is becoming increasingly important. With its advantages as a model for ageing research and a huge potential for further studies, it proves to be a very exciting and interesting organism indeed.

But not very tasty!

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