Towards field neurobiology:

developing an autonomous implantable computer to record from the brain of *Tritonia diomedea*

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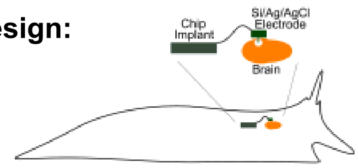
Tritonia diomedea in its natural habitat, with its prey, sea pens, in the background.

Design:



Schematic diagram of the implant inside *Tritonia*

Our vision of the implant:
A silicon based Ag/AgCl intracellular electrode is attached to the brain and connected by a short lead to a chip implant with:

- microcircuitry amplifiers, filters, and compressors
- memory
- battery
- download connection

Intracellular signals recorded by a single or multiple electrodes and stored in the implant will be downloaded after several hours or days, and analyzed alonside simultaneous video records of behavior.

Goal:

Intracellular recordings from the brain of the sea slug, *Tritonia diomedea*, in its natural habitat, with a simultaneous behavioral video record.

Steps accomplished:

1. Preliminary work on a silicon based intracellular electrode
2. Surgical procedure for implantation
3. Wire tether appuratus to conduct signal to or from a behaving animal
4. Baseline behavioral observation and analysis

Rationale:

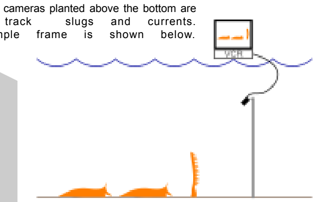
This technology would open new avenues for neuroethology:

- verification of lab results
- avoid negative impacts of lab apparatus or dissection
- sensory integration: responses to multiple stimuli; decision making
- neuroethology of mating
- detailed motor analysis during real behaviors

Field Behavior

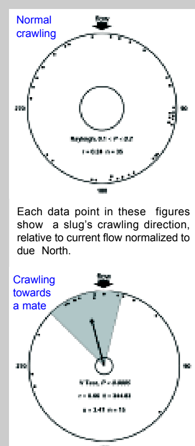
Underwater video recordings of *T. diomedea* are giving us detailed information of behaviors in its natural habitat.

Video from 4 cameras planted above the bottom are used to track slugs and currents. A sample frame is shown below.



Video provides information on crawling directions, current flow, and specific behaviors including feeding,mating, and escape swims.

In addition to accumulating baseline behavioral data to assess implant effects on animals, we are discovering new aspects to the field habits of *T. diomedea*.

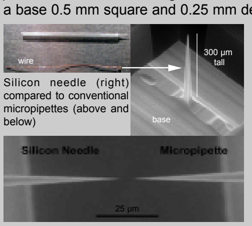


Each data point in these figures show a slug's crawling direction, relative to current flow normalized to due North.

By comparing the direction slugs crawl (relative to current flow), we are finding evidence that *T. diomedea* uses rheotaxis in only certain situations. The slugs in the lower figure were crawling just prior to encountering a mate, and are actively crawling upstream. The animals in the upper figure did not go on to mate. This suggest the possibilities that the upstream slug is releasing a chemical into the currents which a downstream slug senses and crawls upstream to find a mate, and/or the downstream slug enters a premating behavioral state, and crawls upstream. The direction of crawling at other times does not necessarily involve heading directly into the currents. What are the other factors which contribute to slug headings, and how do they use their magnetoreceptive capabilities?

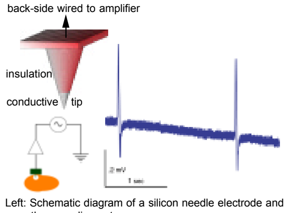
Silicon based intracellular electrodes

We designed and built silicon needles to use as intracellular electrodes. Using Micro Electro Mechanical Systems techniques, we etched very sharp needles from doped (conductive) silicon. This creates a high aspect ratio needle extending from the center of a base 0.5 mm square and 0.25 mm deep.



Silicon needle (right) compared to conventional micropipettes (above and below)

The needles are coated with a fine layer of gold or silver, and then insulated with a fine layer of silicon nitride. This outer insulation was etched away at the very tip of the needles, giving a small conductive cone a few microns tall, with sub-micron sharpness. After wiring and insulation of the base, we were able, in 2 instances, to record what may be intracellular potentials the brain of *Tritonia*.

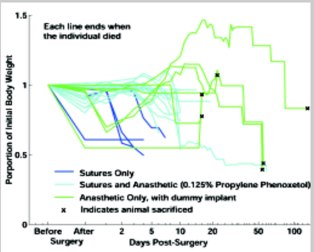


Left: Schematic diagram of a silicon needle electrode and the recording setup.
Right: Potentials recorded from neuron Pd5??? in an isolated *T. diomedea* brain, using a needle electrode.

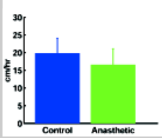
Notice the very small potentials recorded (1 mV full range). Are these truly intracellular? Why are they so small, and will we be able to detect synaptic activity?

Implantation surgery

We have developed an anasthesia and surgical method which allow us to glue inert silicon implants onto the brain of *T. diomedea*.



Surgery survivorship was greatly improved by eliminating the use of sutures and using an anaesthetic to control blood loss. Survivorship is now a month or more with implantation.



Average nightly crawling activity was similar between two groups of animals, 4 controls and 4 treated with anaesthetic (dose & duration similar to implant procedure) over 5 days following treatment.

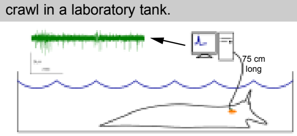
The anasthetic wears off after 3-4 hours, does not have any longterm behavioural effects, and we have pilot results suggesting it has fully reversible effects on neurons. Animals with dummy implants recover from surgery, crawl, feed, mate, swim, all similar to untreated animals held in captivity.

2 days post-implantation

We feel confident animals can survive surgery and behave normally afterwards. Will patients survive functional implants?

Tethered Recordings

Computer chip hardware design requires testing of different modules in isolation. For example, we would like to be able to test amplifiers without the limitations of storing the data in a memory module implant.
Accordingly we have developed a method that allows us to transfer electrical signals through a fine wire 'tether' to or from *Tritonia* free to crawl in a laboratory tank.



Schematic diagram (not to scale) of the wire tether recording setup. The slug is free to crawl, with an extracellular electrode glued to the brain with a wire connection to digitization hardware. An example of a multiunit recorded during crawling is shown.

We will use this method in the future to refine and test electrodes, amplifiers, compression algorithms, filtering, and other circuitry and firmware independently, as we progress towards a full-fledged implant design.

Next Steps:

1. Silicon electrode refinement:
   Dual recordings with conventional and silicon needle electrodes in the same cell.
   Characterization of the electrical properties.
2. Glue and implant effects:
   What are the acute and longterm effects of glues and silicon objects on the neurons of the brain?
3. Microcircuitry design:
   Use the tether apparatus as a test bed for the different implant modules, with both silicon intracellular and extracellular electrodes.
4. Field work:
   More!... and then generate a full ethogram.
   Add exact current flow data to examine possible directional cues (mates, prey, and predators).

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