

GAP-FREE NEURAL CIRCUITS – CLASS #3: *C. elegans* touch-induced locomotion

OUTLINE:

- ***C. elegans* background**
 - General background
 - Neural signaling: no action potentials
- **Excitatory circuits: Touch-induced locomotion**
 - Gentle touch
 - Harsh touch
- **Disinhibitory circuit: Nose touch-induced reversal**
 - Nose touch

KEY: For circuit drawings, I'll annotate neurons with these letters to indicate the evidence present

- **I -> inactivation evidence (ablation or halorhodopsin)**
- **A -> activation evidence (channelrhodopsin)**
- **C -> connection from anatomical evidence (EM)**
- **-> Excitatory chemical synapse**
- **-| Inhibitory chemical synapse**
- **-o Gap junction**

***C. elegans* BACKGROUND:**

- **SLIDES:** Video and description of nervous system and connectome
 - A worm swims on its side, NOT on its belly
 - Neural network browser for finding paths between neurons: <http://wormweb.org>
- **No action potentials (WHITEBOARD)**
 - Genetic evidence: Genome sequenced -> No voltage-gated sodium channels
 - Electrophysiological evidence: Difficult to get
 - Worm neurons: ~5 microns in diameter vs. 100 microns for LG axon
 - Worm is pressurized -> pops
 - Action potential – DRAW (not in the worm)
 - Plateau potential – DRAW (in the worm)
 - Graded potential – DRAW (in the worm)
- **Other notes**
 - No electrophysiology has been done of motor neurons, but calcium imaging is consistent with lack of action potentials there as well.
 - *C. elegans* neurons are often bilaterally symmetric, so I call something a neuron but what I mean is that it is a class of neurons, from 1 to more.
- **SLIDES:** Electrophysiology examples

EXCITATORY CIRCUITS: Touch-induced locomotion

- **Types of touch avoidance**

1. Gentle touch (with eyebrow hair)
 2. Harsh touch (with metal prod)
 3. Nose touch
- SLIDES: Video of reversal in response to anterior gentle touch
 - Laser ablation process in *C. elegans*
 - **Circuit for gentle touch**
 - Anterior touch causes reversals
 - Posterior touch causes accelerations
 - DRAW sensory/behavior frame
 - DRAW worm with neurons in different colors
 - 1. Sensory neurons: (Chalfie & Sulston 1981)
 - Suggested by anatomy (EM) due to microtubules, minimal synaptic input, location in hypoderm and spread across animal body (1979)
 - Anterior: ALMs, AVM
 - Posterior: PLMs
 - Necessity shown by laser ablation (1981)
 - 2. Motor neurons [16 total] (shown by ablation, 1985)
 - Line the ventral cord and makes synapses onto body wall muscles
 - Reversals: DAs
 - Forward locomotion: DBs
 - 3. Command interneurons (shown by ablation, 1985)
 - Only 4 pairs of neurons synapse onto motor neurons along full length of cord, and these also receive synapses from touch cells
 - Anterior touch: AVDs
 - Posterior touch: PVCs
 - Backward locomotion: AVDs and AVAs
 - Forward locomotion: PVCs and AVBs
 - AVDs required for anterior gentle touch sensitivity, but other reversals intact
 - PVCs required for posterior gentle touch sensitivity, but forward motion intact
 - 4. Body muscle
 - 5. Behavior
 - SLIDES:
 - Anatomy of touch neurons
 - ALMs and PLMs respond to gentle touch
 - Anatomical structure of touch circuit
 - SUMMARY:
 - Excitatory chain likely through gap junctions and chemical synapses
 - NOTES:

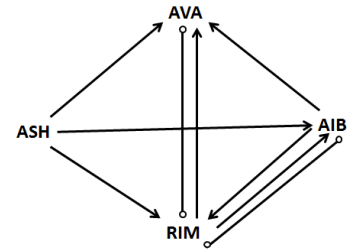
- MEC-4 & MEC-10 form a DEG/ENaC ion channel thought to be the mechanosensory protein in the gentle touch sensory neurons
- MEC-2 & MEC-6 are accessory proteins required for MEC-4/10 localization and function
- SLIDES: Video of harsh touch
- **Circuit for harsh touch**
 - Candidate neurons selected based on connectome!
 1. Sensory neurons
 - Anterior touch: BDUs, SDQR, FLPs, ADEs, AQR (upstream of AVAs, AVDs, AVEs)
 - Posterior touch: PDEs, PVDs (upstream of PVCs)
 - Anus touch: PHAs, PHBs (ciliated neurons)
 - Sensory neurons shown to be **sufficient** by **optogenetic** activation
 2. Command interneurons
 - Anterior touch: AVAs, AVDs, and AVEs
 - Posterior touch: PVCs
 - Anus touch: DVA and PVCs
 3. Motor neurons (same as above)
- SLIDES:
 - Differential response to gentle vs harsh touch in PVC
- **SUMMARY:**
 - The circuits are **excitatory** via chemical synapses and gap junctions
 - Sensory neurons carrying different signals **converge** on the command interneurons

PARALLEL EXCITATORY & DISINHIBITORY CIRCUITS: Nose touch

- **Circuit for nose touch avoidance**
 - ALM and AVM not required for this response (by ablation)
 - Started by testing mutants of ciliated neurons (specialized sensory structures at neuron process tips)
 - Ablations of neurons in the amphid indicate that ASH is partially required (Kaplan & Horvitz 1993)
 - Ablation of FLP, a non-amphid neuron that expresses genes known to be important for gentle touch mechanosensation, is also partially required
 - **How do you show that a neuron involved in a behavior is a sensory neuron?**
 - To show sensory status of ASH, killed all neurons that synapse onto ASH, and worms still responded normally
 - ASH (known from previous work to be required for nose touch avoidance)
 - P: Nose touch depolarizes ASH (P = physiological evidence)
 - A: Optogenetic activation induces reversals
 - AVA (try to identify command interneurons required)

- I: AVA, AVD, and AVE are partially required for nose touch avoidance, but will focus on AVA
- P: Nose touch depolarizes AVA
- A: Not yet been done (as far as I could find)
- AIB (try to identify additional neurons, since AVA/D/E ablated animals still respond sometimes)
 - I: AIB ablation leads to partial loss of nose touch avoidance
 - P: Nose touch also depolarizes AIB
 - A: Depolarization induces reversals
- RIM (try to identify additional neurons)
 - I: RIM ablation leads to partial loss of nose touch avoidance
 - P: Nose touch hyperpolarizes RIM
 - A: Hyperpolarizing RIM induces reversal

- So how do we order these neurons in a network? The connectome is virtually useless.



- C: AVA, AVD and AVE receive synapses from ASH
- C: AIB receives synapses from ASH
- C: RIM receives synapses from ASH
- C: AVA and RIM share a gap junction
- C: AIB and RIM share a gap junction
- And more – see right
- How do AIB and RIM relate?
 - Upstream: nose touch -> AIB -> RIM -> reversal
 - Downstream: nose touch -> RIM -> AIB -> reversal
 - Parallel: nose touch -> AIB -> reversal
nose touch -> RIM -> reversal
 - Ablate AIB, give nose touch and observe response in RIM -> Gone
 - Ablate RIM, give nose touch and observe behavioral response -> Gone
 - Activate AIB and observe response in RIM (without nose touch) -> Hyperpolarizes
 - If AIB is upstream of RIM, AIB must inhibit RIM
- How do RIM and AVA/D/E relate?
 1. Upstream: RIM -> AVA -> reversal
 2. Downstream: AVA -> RIM -> reversal
 3. Parallel: RIM -> reversal
AVA -> reversal
 - Hyperpolarize RIM, and see if AVA is required for reversal -> AVA/D/E is not
- **Let's go deeper: molecular mechanisms within the circuit**
 - Mutant analysis: mutations usually break genes
 - Can test mutants to see if they're defective in your behavior
 - If they are, then you can attempt to activate candidate neurons by expressing a working copy of the broken gene only in a specific neuron

- If this restores the behavioral response, we call that “rescue”
- *eat-4*, a gene that enables glutamate to function as a neurotransmitter, is required for nose touch avoidance
- Where is *eat-4* functioning?
 - *eat-4* mutants lack signaling in AVA and ASH and RIM, so good guess would be ASH -> that does restore signaling in AVA and AIB
 - cell-specific removal via RNAi shows function in AIB for RIM response
- *glr-1*, a gene that makes an excitatory glutamate receptor, is also required for nose touch avoidance
 - *glr-1* mutants lack signaling in AVA and ASH -> expression there restores response of AVA and AIB
- *avr-14*, a gene that makes an inhibitory glutamate receptor, is also important
 - *avr-14* mutants lack signaling in RIM -> expression there restores response
- **Unexpected results that don’t fit with the model**
 - Except osmotic shock induces an increase in calcium in RIM (expected decrease)
 - Interpret as excitatory pathway from AVA, as ablation produces inhibitory response in RIM in response to osmotic touch
- **SLIDES:**
 - Data for functions of AVA, RIM and ASH
- **SUMMARY:**
 - Nose touch-induced reversals rely on **parallel** circuits, one which is purely excitatory and another which is disinhibitory
 - There is some signaling between the two circuits which remains unexplained