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

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
    - <u>Action potential</u> – DRAW (not in the worm)
    - <u>Plateau potential</u> – DRAW (in the worm)
    - <u>Graded potential</u> – 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)
        - <u>Anterior</u>: ALMs, AVM
        - <u>Posterior</u>: 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
        - <u>Reversals</u>: DAs
        - <u>Forward locomotion</u>: 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
        - <u>Anterior touch</u>: AVDs
        - <u>Posterior touch</u>: PVCs
        - <u>Backward locomotion</u>: AVDs and AVAs
        - <u>Forward locomotion</u>: 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

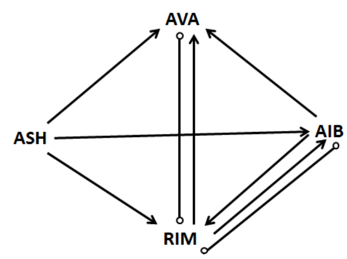
- SLIDES: Video of harsh touch
- **Circuit for harsh touch**
  - Candidate neurons selected based on connectome!
  1. Sensory neurons
     - <u>Anterior touch</u>: BDUs, SDQR, FLPs, ADEs, AQR (upstream of AVAs, AVDs, AVEs)
     - <u>Posterior touch</u>: PDEs, PVDs (upstream of PVCs)
     - <u>Anus touch</u>: PHAs, PHBs (ciliated neurons)
     - Sensory neurons shown to be **sufficient** by **optogenetic** activation
  2. Command interneurons
     - <u>Anterior touch</u>: AVAs, AVDs, and AVEs
     - <u>Posterior touch</u>: PVCs
     - <u>Anus touch</u>: 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 <u>ciliated neurons</u> (specialized sensory structures at neuron process tips)
  - Ablations of neurons in the <u>amphid</u> indicate that ASH is partially required (Kaplan & Horvitz 1993)
  - Ablation of FLP, a non-amphid neuron that <u>expresses genes known to be important for gentle touch mechanosensation</u>, is also partially required
  - **How do you show that a neuron involved in a behavior is a sensory neuron?**
    - To show <u>sensory status</u> 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?
    i. Upstream: nose touch -> AIB -> RIM -> reversal
    ii. Downstream: nose touch -> RIM -> AIB -> reversal
    iii. 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