HOW TO IDENTIFY A NEURAL CIRCUIT RESPONSIBLE FOR A BEHAVIOR

What follows is a rubric for identifying neural circuits that control a stimulus-triggered behavior of interest.

Note: cell types other than neurons can function in the circuit for behavior (e.g. intestine, hypoderm), but we'll assume neurons for now.

Note: When we use the word "synapse", we're referring to both chemical synapses and electrical synapses (aka gap junctions).

Step 1: Find at least one neuron involved in the circuit

A) Use systematic inactivation or activation
    a. Use a laser to ablate neurons (e.g. pharynx in *C. elegans* for pumping behavior)
    b. Use a transgene to activate or inactivate neurons (optogenetics, genetic ablation, electrical silencing, synaptic silencing, electrical activation)
        i. Mosaic analysis
        ii. Random driver crosses
        iii. Promoters targeting specific neurons

B) Use neural activity to identify candidate neurons
    a. Use a transgene or dye or electrode that reports on neural activity and find neurons that are active during the behavior of interest
    b. Then identify the neurons (see C3 below)
    c. This approach has an important caveat: It is <u>correlational</u> rather than causal. Once a neuron has been correlated its functional relevance still needs to be determined with neural activation or inactivation.

C) Use genetics to dissect a functional neural circuit
    1) Find mutant defective in your behavior of interest
        a. Test existing pool of available mutants
        b. Generate new mutants using a mutagen to screen for defective animals
    2) Generate expression pattern
        a. Generate transgenic with gene's promoter fused to a fluorescent protein (transcriptional fusion)
        b. Use FISH (fluorescent in situ hybridization) to find where RNA is expressed
    3) Identify cells that express the gene of interest
        a. Learn to identify cells by microscopy (e.g. *C. elegans*)
        b. Learn to identify neurons by location and process morphology (e.g. *C. elegans, Drosophila*)
        c. Cross strains with RFP in neuron X and look for overlap with transcriptional fusion GFP
    4) Restore expression of the gene in an identified cell
        a. Generate transgenic with ectopic promoter driving expression in the identified cell

b. Use mosaic analysis, where random loss of rescuing transgene can be used to identify cells in which expression is necessary and sufficient for rescue

Step 2: With a neuron as a beachhead, identify additional functional neurons

A) Repeat doing step 1 to identify additional neurons
B) Identify connectivity of the neuron of interest and activate or inactivate to show function
    1) Use serial section electron microscopy to trace cell processes and observe synapses
    2) Use transgene to map connections (PA-GFP, transynaptic virus)
    3) Note that this method may not work as the functional connection may not act through an anatomical synapse, but may instead rely on neuropeptides or hormones that are secreted and diffuse to the cells that they affect

Step 3: Once one or more neurons have been identified, confirm acute and instructive role in signal propagation

A) Use transgene or dye or electrophysiology to report on neural activity in response to stimulus
    1) If no activity observed, sensor may not be sensitive enough or may not be sensing the relevant molecular event
B) Acutely activate or inhibit the neuron to show instructive rather than permissive function
C) Acutely restore gene of interest to show instructive rather than permissive function

Step 4: Once several neurons have been identified, organize them into a circuit using <u>epistasis analysis</u>

- If the stimulus depolarizes the neuron, “activate” and “inactivate” below mean depolarize and hyperpolarize, respectively
- If the stimulus hyperpolarizes the neuron, “activate” and “inactivate” below mean hyperpolarize and depolarize, respectively (it’s inverted).

A) Inactivate a pair of neurons together and observe whether the behavioral defect is enhanced
    1) If enhanced, neurons likely function in parallel
    2) If not enhanced, neurons likely function in series
B) Activate pairs of neurons and observe whether the behavior is enhanced over single activation
    1) If enhanced, neurons likely function in parallel
    2) If not enhanced, neurons likely function in series
C) Activate neuron X and observe whether neuron Y responds
    1) If it does, neuron Y is downstream of X
    2) If it does not, neuron Y is upstream of or in parallel with X
D) Inactivate neuron X while providing the stimulus and observe whether neuron Y responds
    1) If it does, neuron Y is upstream of or in parallel with X
    2) If it does not, neuron Y is downstream of X

Step 5: Draw the circuit and you’re done!