ELECTROPHYSIOLOGICAL CLASSIFICATION OF INTERNEURONS

An end goal of interneuron research is to determine how different types of neurons contribute *in vivo* to the complex emergent dynamics of the brain and behaviors of the organism. It would be ideal, for example, to record from identified interneurons in the motor cortex to determine how different cell classes affect the timing of spikes of pyramidal neurons encoding motion perception or reach direction. While this is not possible at the present time, studies in hippocampal interneurons, for example, show that specific IN subtypes uniquely participate in distinct rhythms, demonstrating that it is, in principle, possible to identify the anatomical identity of the different INs on the basis of their *in vivo* behavior. A concerted effort to establish a classification scheme for INs based on *in vivo* behavior is, however, lacking.

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Interneurons can exhibit a number of different electrophysiological responses when stimulated by current injection. For an isolated neuron, in for example, a brain slice, this response diversity is mostly due to the passive and active properties of the neuron, which are determined by the repertoire of 1-2 dozen ion channels expressed by neurons. However, for a neuron embedded in a functional network *in vivo*, the response diversity is influenced by a host of conditional factors including the synaptic bombardment from the network, neuromodulation, and the general brain state which act on the constraints imposed by the passive and active properties. There are important and different advantages to being able to separate the different response types *in vivo* and *in vitro*, correlate these with the biophysical properties of neurons and ultimately also with the anatomical and molecular properties.

The *in vitro* classification has evolved spontaneously as researchers have encountered different discharge responses when a step current pulse is injected into the soma. While this method is a very fast method of establishing the electrophysiological identity of neurons, debate rages as to whether this is a meaningful approach. In vivo, the action potential discharge to a step current pulse is strongly influenced by the synaptic bombardment to such an extent that virtual any type of behavior can be recorded. *In vitro*, however, the synaptic bombardment is removed and the discharge response reflects a combination of the neurons passive membrane properties and repertoire of ion channels. The advantage *in vitro* is therefore that neurons with different passive and active membrane properties can be isolated and classified. It is however important to establish uniformly agreed upon classification schemes for both *in vivo* and *in vitro* and eventually establish the correspondence between the two schemes.

The in vitro classification scheme is in a severe crisis because of lab-to-lab variability in the complexity of the stimulation protocols, the intracellular and extracellular solutions used, the recording temperature, age, species and brain regions studied, the method of slice preparation and incubation (eg submerged vs interface chambers) and the method of recording (whole cell vs sharp). While we can be encouraged by surprisingly similar behaviors reported in the literature despite such immense potential sources of variability, a uniformly agreed upon stimulus set and resulting classification is crucial to facilitate correspondence between labs and to ultimately understand the full spectrum of electrophysiological diversity of INs.

Proposed Schemes for Subjective Electrophysiological Classification in vitro and in vivo

IN VITRO CLASSIFICATION

Contributing factors to response diversity *In vitro*

1. No-little synaptic bombardment
2. Passive properties
3. Ion channel repertoire
4. Morphologically incomplete
5. Variable extracellular and intracellular? solutions
6. Loosening of the extracellular matrix

Advantages of Classifying *in vitro*

1. Determine the number of different electrical phenotypes,
2. Determine the relationships with differential ion channel composition and calcium binding protein gene expression,
3. Determine the number of different electrical subtypes in distinct anatomical classes,
4. Determine INs development/cell lineage – the tree of diversification,
5. Determine the correspondence with synaptic determination,
6. Determine differential functional contribution to circuit dynamics
7. Determine the function of ion channel diversity for circuit behavior.

Sources of *In Vitro* Variability

1. Differences in stimulation protocols,
2. Intracellular and extracellular solutions,
1. Method of slice preparation (perfusion, anesthesia, etc)
2. Methods of slice incubation (submerged vs interface chambers)
3. Methods of Recording (whole cell, perforated, sharp).
4. Recording temperature,
5. Age,
6. Species,
7. Brain regions.

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Proposed Classification

1. **Principles of the Classification**
   a. Based on step current pulses injected into the somata
   b. Based on the prior established subjective, visually obvious features of interneurons
      i. can be supported with measurements if measurement criteria are defined for the features.
   c. Based on the steady state spike pattern

    d. Based on the initial response
    e. Definitions to add to "Features"
        i. Two types of bursts:
        ii. Intrinsic bursting: Defined as a burst followed by a sAHP
        iii. Bursting: Defined as a burst not followed by a sAHP
        iv. Continuous: Definition: Initial == Steady-state spike pattern

2. Classifying Spike Patterns

Numbers need to be added, perhaps in the features document

1. *Fast Spiking (FS)*
    a. Definition: neurons with non-adapting spiking at steady-state, brief APs and large fastAHPs.
    b. Subtypes
        i. Continuous (eg Continuous Fast Spiking Cell - cFS)
        ii. Delayed (eg delayed fast spiking cell - dFS)
        iii. Stuttering (eg Stuttering Fast spiking Cell (sFS), Continuous Stuttering Fast Spiking Cells (csFS)

2. *Non-Adapting, Non-Fast Spiking (NA-NFS)*
    a. Definition: neurons that display no visually obvious increase in ISI's at steady-state, expandable definition
    b. Subtypes
        i. Continuous (eg Continuous NA-NFS cell)
        ii. Burst (eg Burst NA-NFS cell)

3. *Adapting (AD)*
    a. Definition: neurons that display visually obvious *increase* in ISI's at steady-state — Formatted: Font: Italic
    b. Subtypes:
        i. Continuous (eg. Continuous AD, cAD)-LTS — Deleted:
        ii. Burst (eg Burst AD cell, bAD)
        iii. Delayed (eg Delayed AD, dAD)

4. *Accelerating (AC)*
    a. Definition: neurons that display visually obvious *decrease* in ISI's at steady-state — Formatted: Font: Italic
    b. Subtypes:
        i. Continuous (eg Continuous AC cell, cAC)
        ii. Delayed (eg Delayed AC cell, dAC)

5. *Irregular Spiking (IS)*
    a. Definition: neurons that display the feature "irregular spiking"
    b. Subtypes:
        i. Continuous (eg Continuous IS cell, cIS)
        ii. Burst (eg Burst IS cell, bIS)

6. ***Intrinsic Burst Firing (IB)***
    a. Definition: neurons that produce a burst of 2 or more spikes riding on a depolarization envelope followed by a slow AHP
    b. Subtypes:
        i. Rhythmic (eg Rythmic Intrinsic Bursting cell, rIB)
        ii. Initial bursting (eg Initial Intrinsic Burst Firing cell, iIB)

In vivo Classification

Contributing factors to response diversity *In vivo* vs *In vitro*

1. Synaptic Bombardment
2. Passive properties
3. Ion channel repertoire
4. Ongoing modulation
5. Morphologically complete
6. Extracellular solution????
7. Intact extracellular matrix
8. Emergent properties of network behavior

Advantages of Classifying interneurons *in vivo*?

1. Understand one of the potential sources of response diversity *in vivo*,
2. Determine the different functional contribution to *system* dynamics,
3. Relate in vivo findings to *in vitro* cell types where much is known about the ion channels composition of the neurons, the synaptic embedding and the general position and role of the interneuron in the microcircuit.
4. Ultimately allow understanding of the contribution of the different interneurons, their individual ion channels, synaptic properties and positions in the microcircuit to behavior.

Sources of *In Vivo* Variability

1. Input statistics of the stimulation protocols,
2. Methods of recording (whole cell, sharp, extracellular, type of extracellular).
3. Context/brain state determinants,
4. Anesthesia
5. Task determination,
6. Age
7. Species
8. Brain regions.

3. Potential Bases of Classifying Spike Patterns *in vivo*

Species, age, etc needs to be defined

Separation of principal cells and interneurons in extracellular recording experiments

a. In the cross-correlogram of cell pairs, decreased spike activity after the reference is indicative of the inhibitory nature of the reference cell.
b. In the cross-correlogram of cell pairs, short latency increase of spike activity after reference neuron is indicative of the inhibitory nature target neuron. (Henry; THE STATEMENTS MAKE SENSE ONLY IF WE SHOW EXAMPLES AS WELL.

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<#>Principles of the Classification Scheme¶
In vivo response diversity is strongly dominated by the network environment the response to step current injections into the somata may not reflect the biophysical distiinctions between neuronas. While a standardized stimulus set is still recommended, different principles are required in an attempt to identify the different types of interneurons *in vivo* that correspond to the types identified *in vitro*. The in vivo environment is also the one where the interneuron anatomical, electrical molecular and synaptic properties are finally put into action to impact the network and identifying this impact may therefore be the means by which interneurons can be separated *in vivo*.¶
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The *concept* of opposing forces is fundamental to all systems and the opposing inhibitory action of GABAergic interneurons in vivo is an important potencial jeans of identifying the different functional impact of different interneurons on the emergent dynamics and behaviors. However, the *mechanisms* of competing forces have different realization in different substrates (push and pull, positive and negative, excitation and inhibition). Neuronal networks with excitation only can only recruit more excitation. Inhibition is what provides autonomy of neurons and neuronal group ... [1]

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Definition: measured from cross-correlograms

STATING VALUES OF LATENCIES AND BIN WIDTH IS NOT MEANINGFUL BECAUSE THEY VARY WITH DISTANCE BETWEEN THE PAIRS).

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Definition: measured from cross-correlograms

Anatomical identification of extracellularly recorded interneurons require at least two steps.

Step 1. Network-guided feature of interneuron types

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c. Discharge properties during distinct network patterns

d. Phase relationship of spikes related to distinct oscillatory pattern

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2. Stimulus Probing, using standardized input statistics

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a. Identifying the conditions for response

b. Identifying how context modulate responses

c. Spontaneous discharge statistics

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Step 2. Replication (if feasible) of the network pattern under anesthesia or head-fixed preparations, which allow for juxtacellular or intracellular recording and labeling of the neurons in vivo after network and/or stimulus characterization.

Complementary method of classification
In intracellular in vivo experiments, the in vitro-derived standardized stimulus set can be used.

These schemes are offered here as examples and potential guidance for future experiments to facilitate inter-laboratory experiments and to allow comparison between various levels of analysis (anatomical, physiological and molecular). The schemes should be viewed as a progress report rather than a set of recommendations that should be enforced by reviewers or funding agencies. Whereas classification schemes have historically proven power and utility, we are aware that, at times, they are in the way of progress. The recommendations above were compiled to faciliate interlevel comparisons rather than restrictive means.

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Proposed Classification

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