

Revealing cell types *in vivo* via dimensionality reduction and graph clustering of spike waveforms

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Background

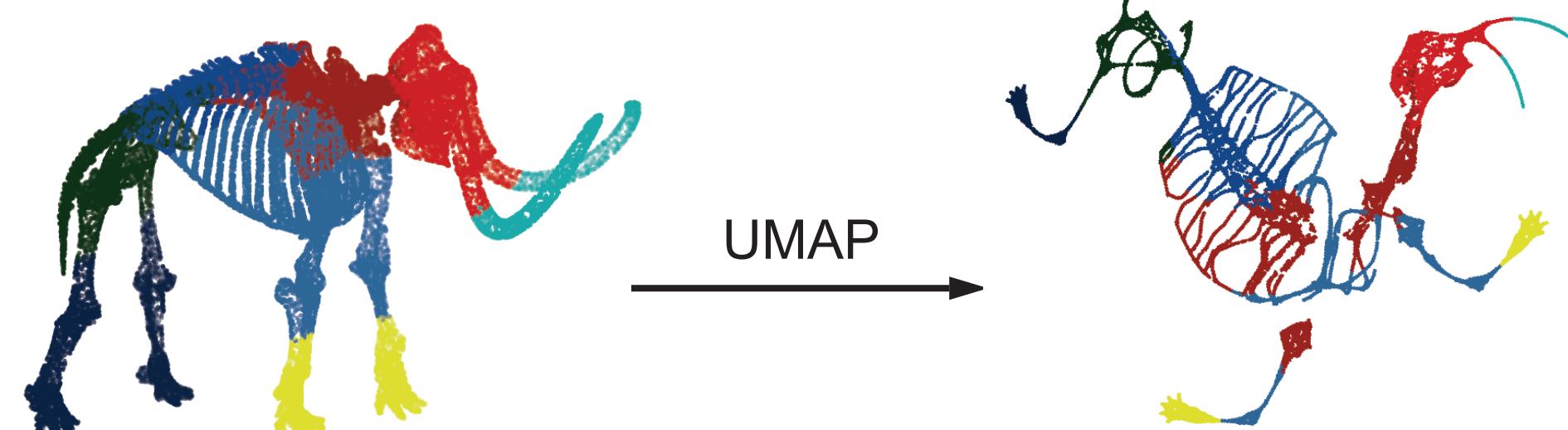
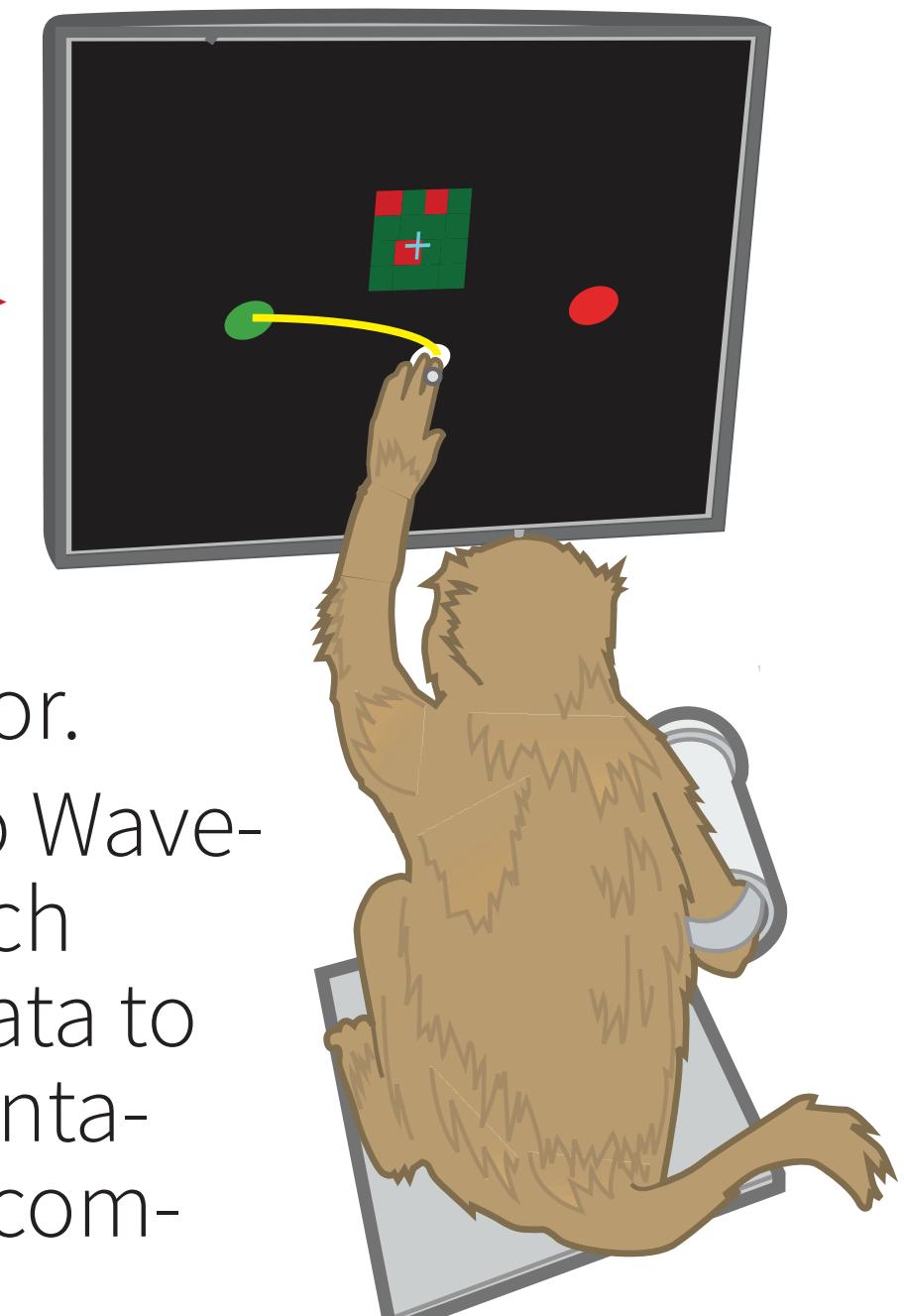
Anatomical, physiological, and transcriptomic studies suggest a diverse range of neuronal cell types. However, current *in vivo* methods—such as clustering on waveform features—only differentiate between broad- (**BS**) and narrow-spiking (**NS**) neurons. This gap between “known” and “observable” diversity limits our understanding of how cell types shape behavior.

Here, we developed a new method (WaveMAP) combining non-linear dimensionality reduction (UMAP) with graph clustering (Louvain community detection) on spike waveforms and show that it better reveals candidate cell classes *in vivo*.

Introduction

We collected single neuron extracellular action potentials (EAP) premotor cortex while a monkey performed a decision-making task [1]. ►

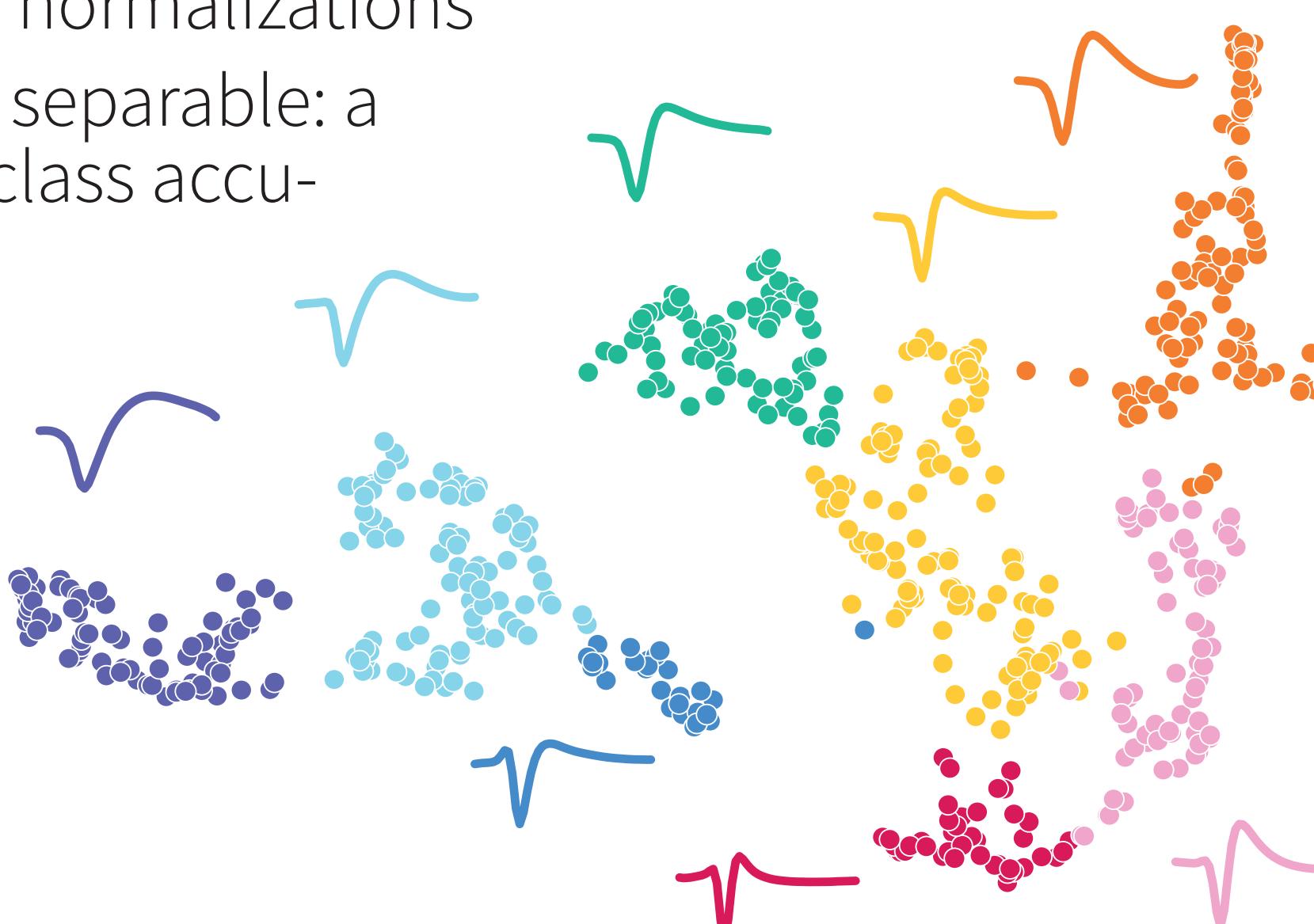
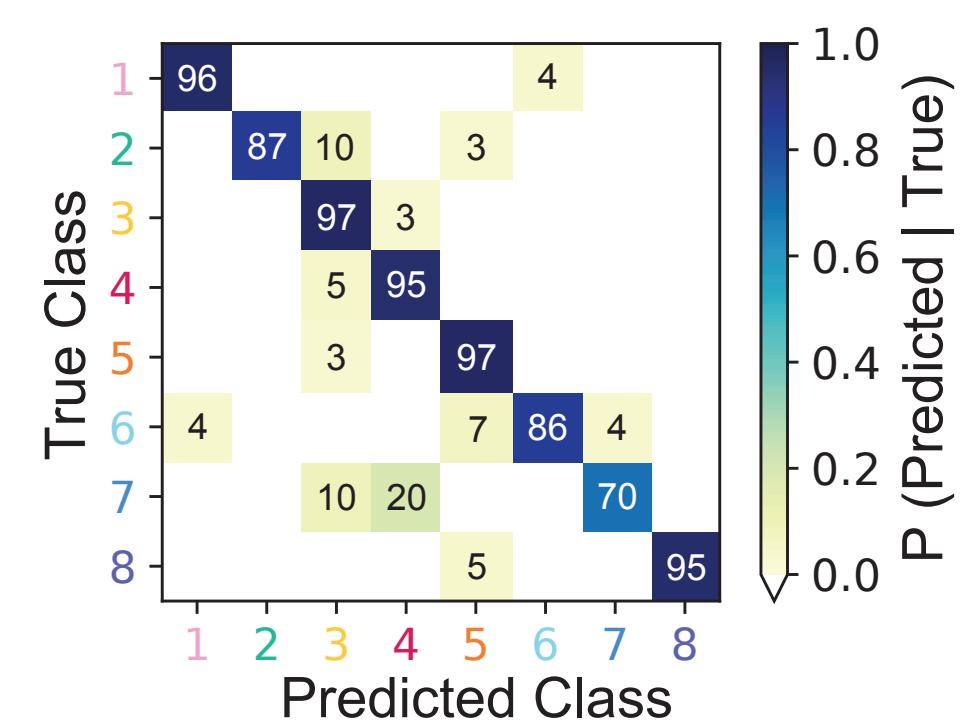
Trial difficulty was modulated by **coherence**: 50% means an even red-green mix; 100% means all one color. Average EAPs were passed to WaveMAP. This uses UMAP [2] which projects high-dimensional data to a lower-dimensional representation; it then applies Louvain community detection [3] to find clusters. ▼



WaveMAP outperforms traditional clustering

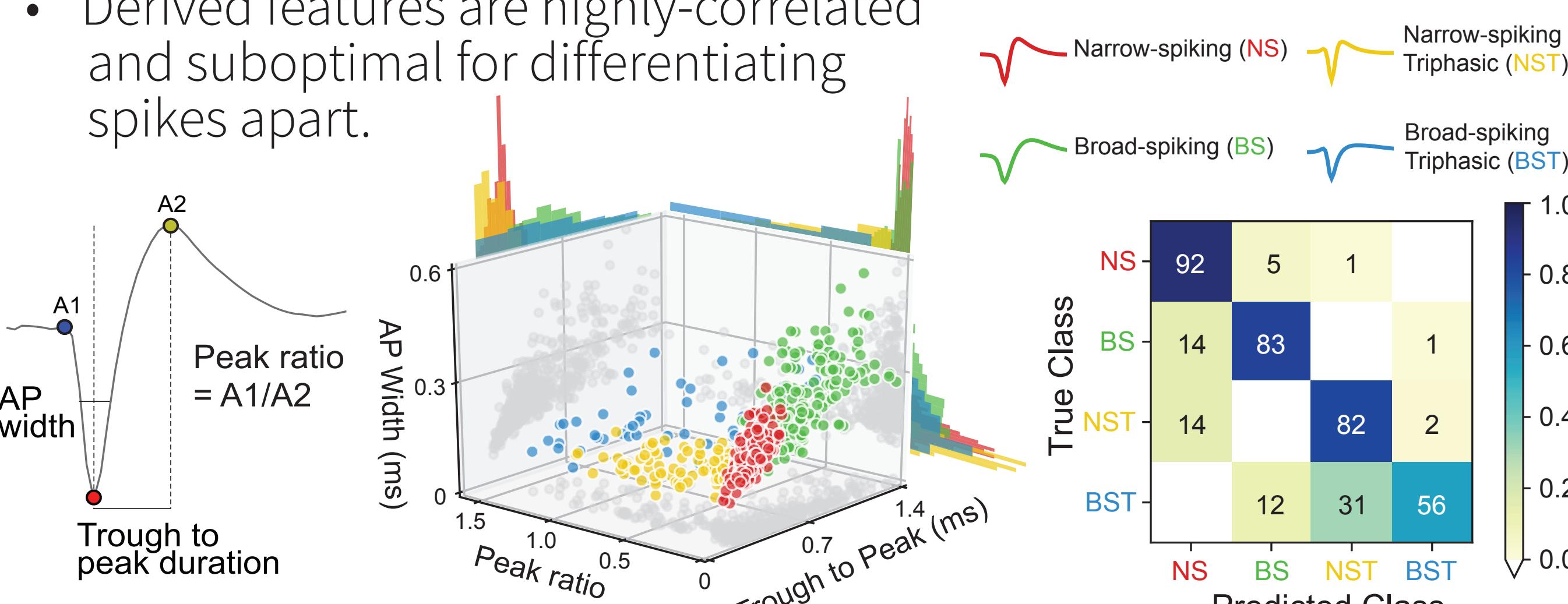
WaveMAP stably yielded **eight** clusters separated into broad- (**BS**) and narrow-spiking shapes (**NS**; cool and warm colors, respectively). ▼

- WaveMAP was stable over random sample subsets and seeds
- Robust to different data normalizations
- WaveMAP clusters were separable: a classifier averaged per class accuracy was **91%**. ▼



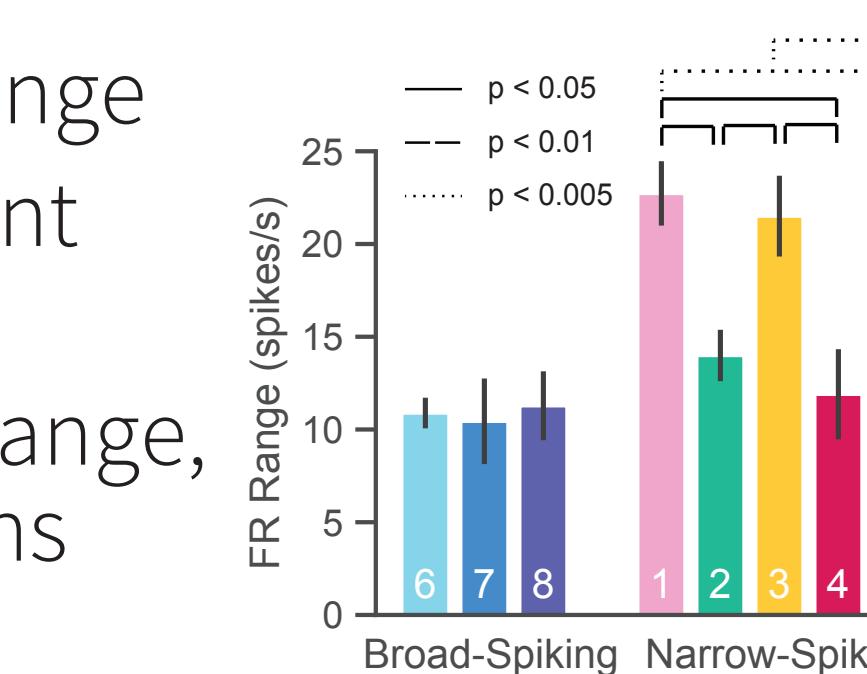
Traditional methods of classifying neurons by their waveform shape apply clustering methods, such as Gaussian mixture models (GMMs), to derived feature spaces. Excitatory neurons are commonly understood to be **BS** while inhibitory neurons are **NS**.

- Traditional methods yielded **four** clusters on our averaged EAPs.
- These were less differentiated: accuracy was on average **78%** per class on *half* the number of classes.
- Derived features are highly-correlated and suboptimal for differentiating spikes apart.

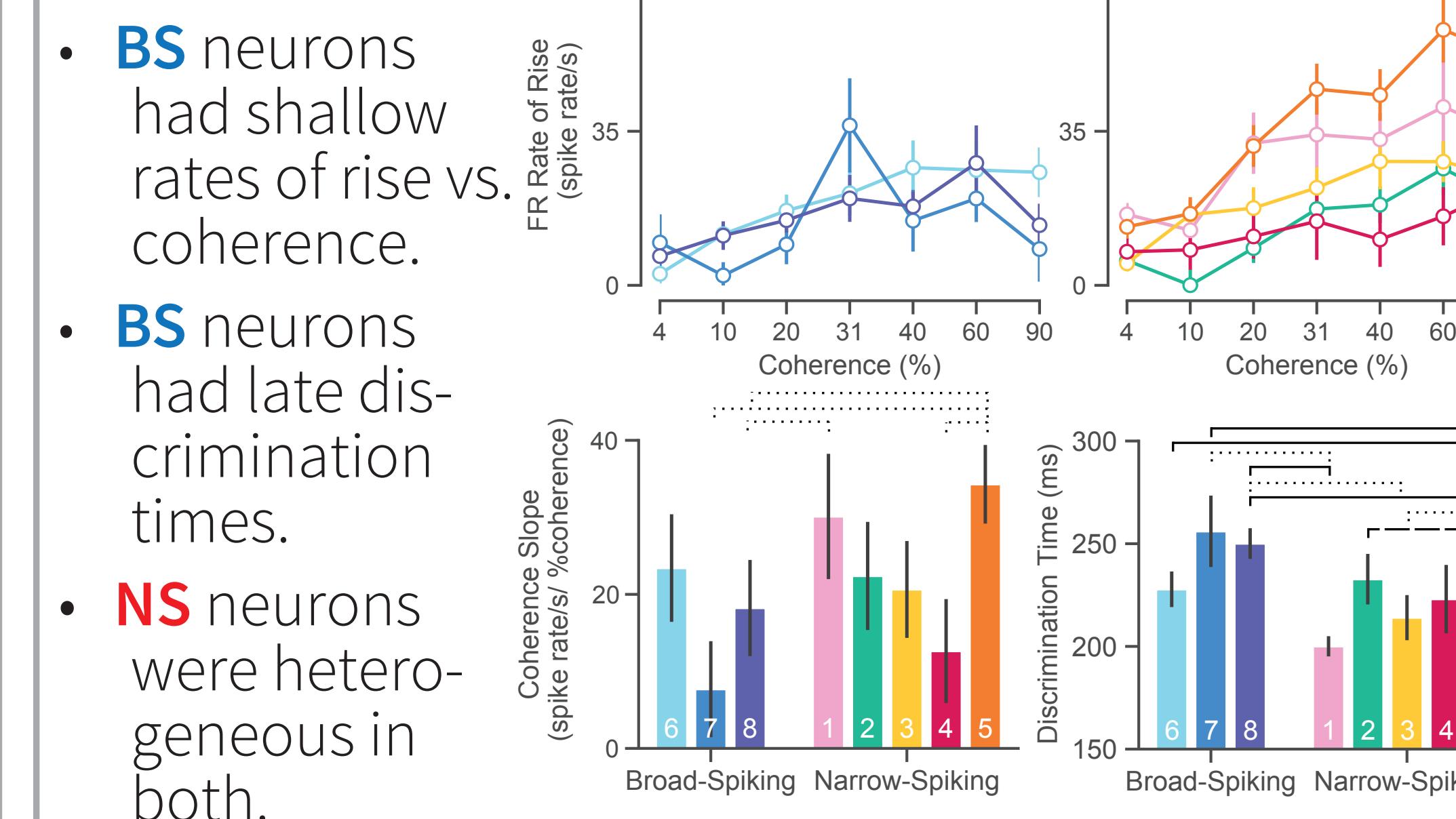


Clusters are distinct in physiology & function

- **BS** neurons were low in FR range
- **NS** neurons showed significant heterogeneity.
- Clusters **2** and **4** had low FR range, in line with excitatory neurons despite being **NS**.



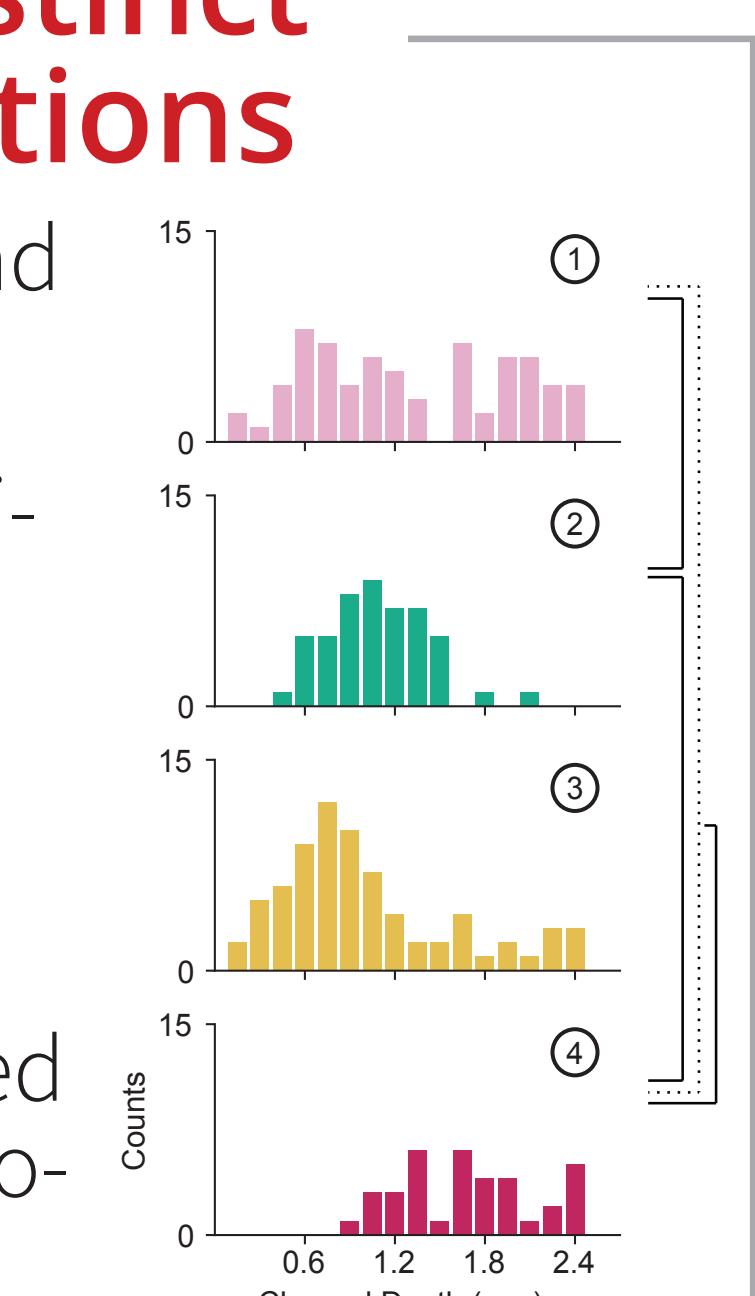
Discrimination time is the time point, within a trial, at which reach direction can be decoded from a given neuron.



Clusters have distinct laminar distributions

Focusing on four **NS** neurons (**BS** and **5** all had broad distributions), ►

- **NS** neurons each had unique laminar distributions
- No unique laminarities are found with traditional methods
- Cluster **3** was very superficial
- Clusters **2** and **4** were concentrated in Layer III and V/VI where excitatory neurons predominate.



Cluster laminarity matches cell types

- **CB⁺** and **CR⁺** cells are superficial like cluster **3**.
- **PV⁺** cells are more diffuse like cluster **1**.
- Depth with cluster ID explained **7.6%** of discrimination time variance vs. traditional methods and depth which explained **4.6%**.

Takeaways

- WaveMAP produced more clusters that were better separable than traditional methods.
- Clusters had significant differences in physiology, function, and laminarity not seen with traditional methods.
- WaveMAP explains more functional variance than traditional methods.
- We uncovered **NS** cells with properties matching excitatory neurons.