ANALYSIS OF EFFECTIVE CONNECTIVITY BETWEEN DORSAL HORN NEURONS AND PRIMARY AFFERENTS FROM ADULT MICE

S06-598

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Introduction

Primary afferent depolarization (PAD) is the mechanism underlying presynaptic inhibition as first described in the ventral horn of the spinal cord. Primary afferents have abnormally high concentrations of Cl^- ions, presumably due to the activity of the chloride transporter NKCC1. Activation of $GABA_A$ receptors causes an outflow of Cl^-, which leads to PAD. PAD occurs in the dorsal horn as well, where it may serve as a mechanism of sensory modulation. Under certain conditions PAD may be enhanced reaching action potential threshold to produce dorsal root reflexes (DRR) or backfiring in primary afferents.

Here we set out to study the dorsal horn neurons and circuits that generate backfiring in primary afferents. To this purpose we obtained simultaneous recordings from sets of dorsal horn neurons and primary afferents and used an AI algorithm to analyze the obtained spike trains.

Methods

Experiments were performed *in vitro* on spinal cord longitudinal slices obtained from adult CD1 mice (36-50 days old). Details of slice preparation and maintenance in Lucas-Romero et al., 2018. Sets of dorsal horn neurons were obtained with microelectrode arrays (MEA, Buzsaki 32-A32, NeuroNexus). Signals from the MEA were amplified, band pass filtered between 200 Hz and 3 KHz and digitized using RHS2116 amplifier chips and an RHS2000 stimulation /recording controller (Intan Technologies, US). Data was then stored for offline analysis using Spike-2 software from CED.

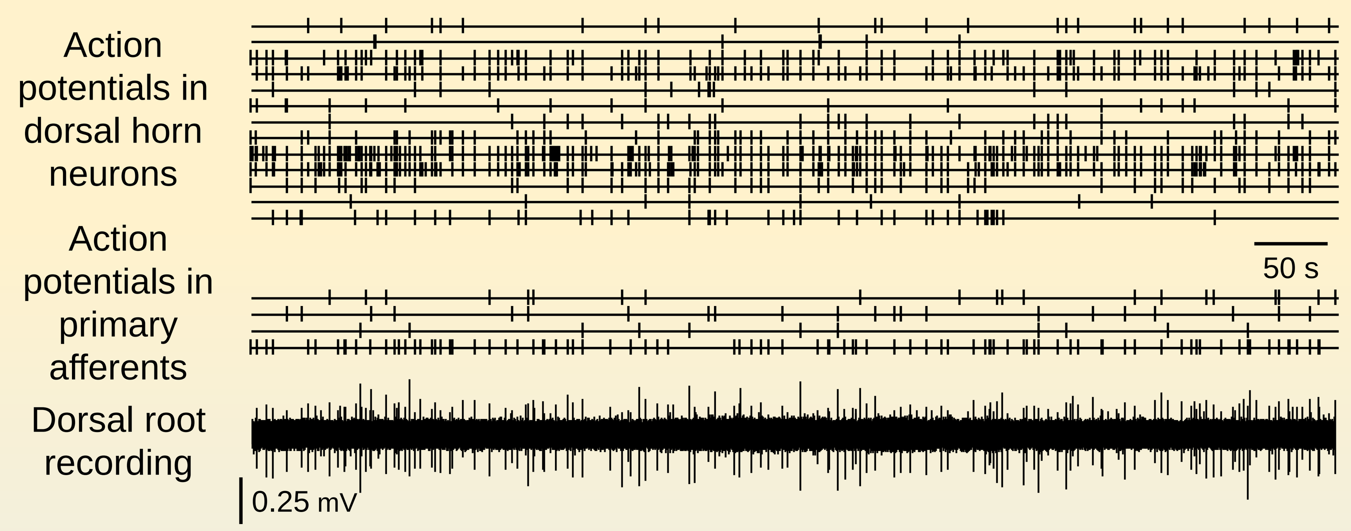
Primary afferents were recorded by introducing teased dorsal rootlets into micro-suction electrodes. Signals were amplified (AxoClamp 2B, Axon instruments), AC and DC filtered (Digitimer Ltd., NeuroLog Systems), digitized (RHS2000 stimulation/recording controller, Intan Technologies) and stored for offline analysis.

Spike trains from dorsal horn neurons and primary afferents were then analyzed with an AI algorithm to infer effective connectivity between them. The algorithm was adapted from the C5.0 and detailed description is available at Pozo-Jimenez et al., 2021. The algorithm uses spike trains from one or several neurons as attributes to predict the behavior of a class (neuron or afferent). The algorithm is trained in a data subset by analyzing the activity in the attribute during the 50 ms preceding each spike of the target. During training, the algorithm builds rules to take decisions and then the predictive value of these rules and decisions are tested in a different data subset. The overall predictive value of the rules created for a target was assessed with the Matthew's correlation coefficient (MCC) calculated from the results of the confusion matrix obtained when the predictions made by the algorithm are compared with actual data during the testing phase. MCC values ≥ 0.18 for single neurons as attributes were considered as putative monosynaptic connections.

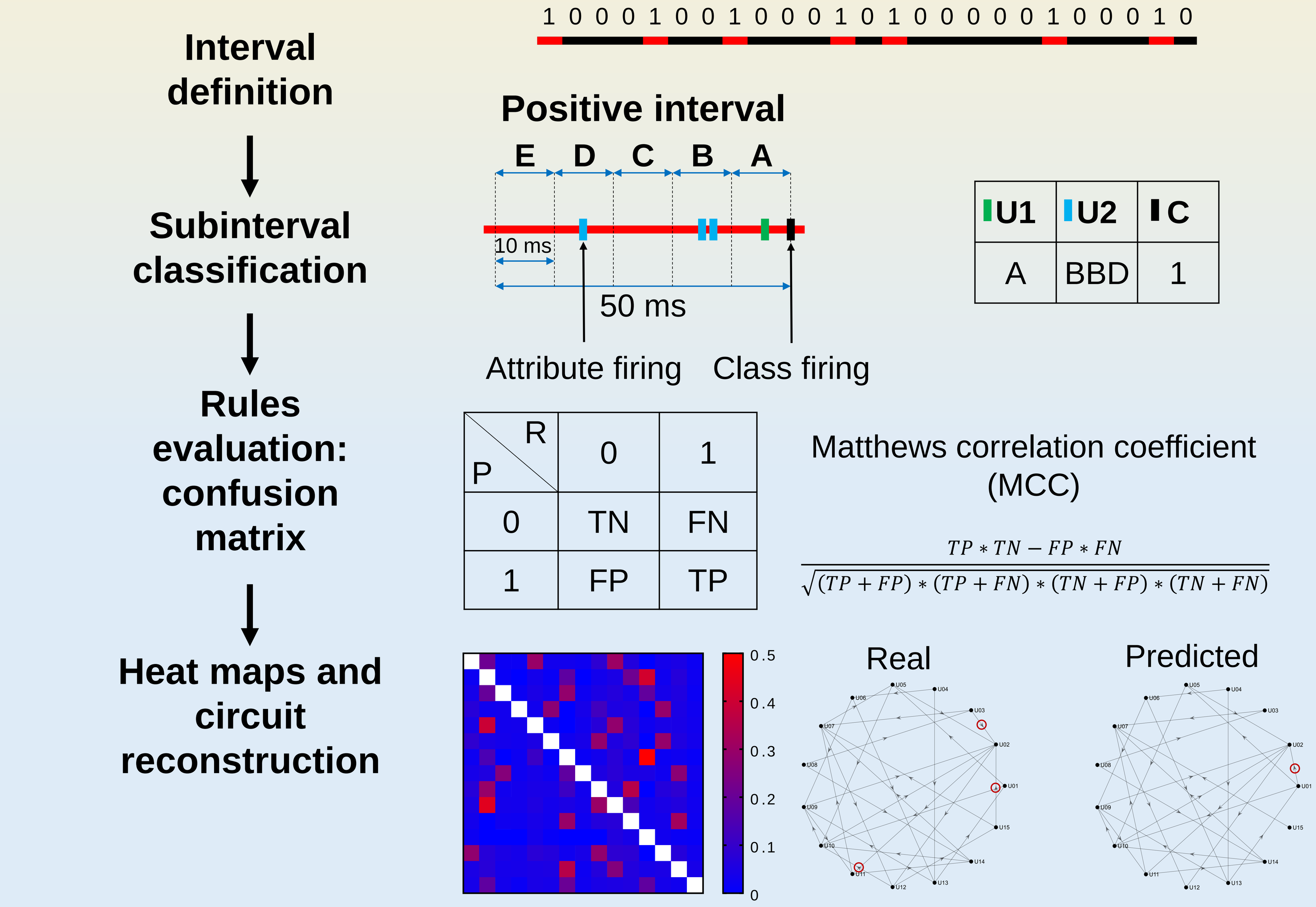
Results

SLICE AND RECORDING SETUP

SPONTANEOUS ACTIVITY RECORDED

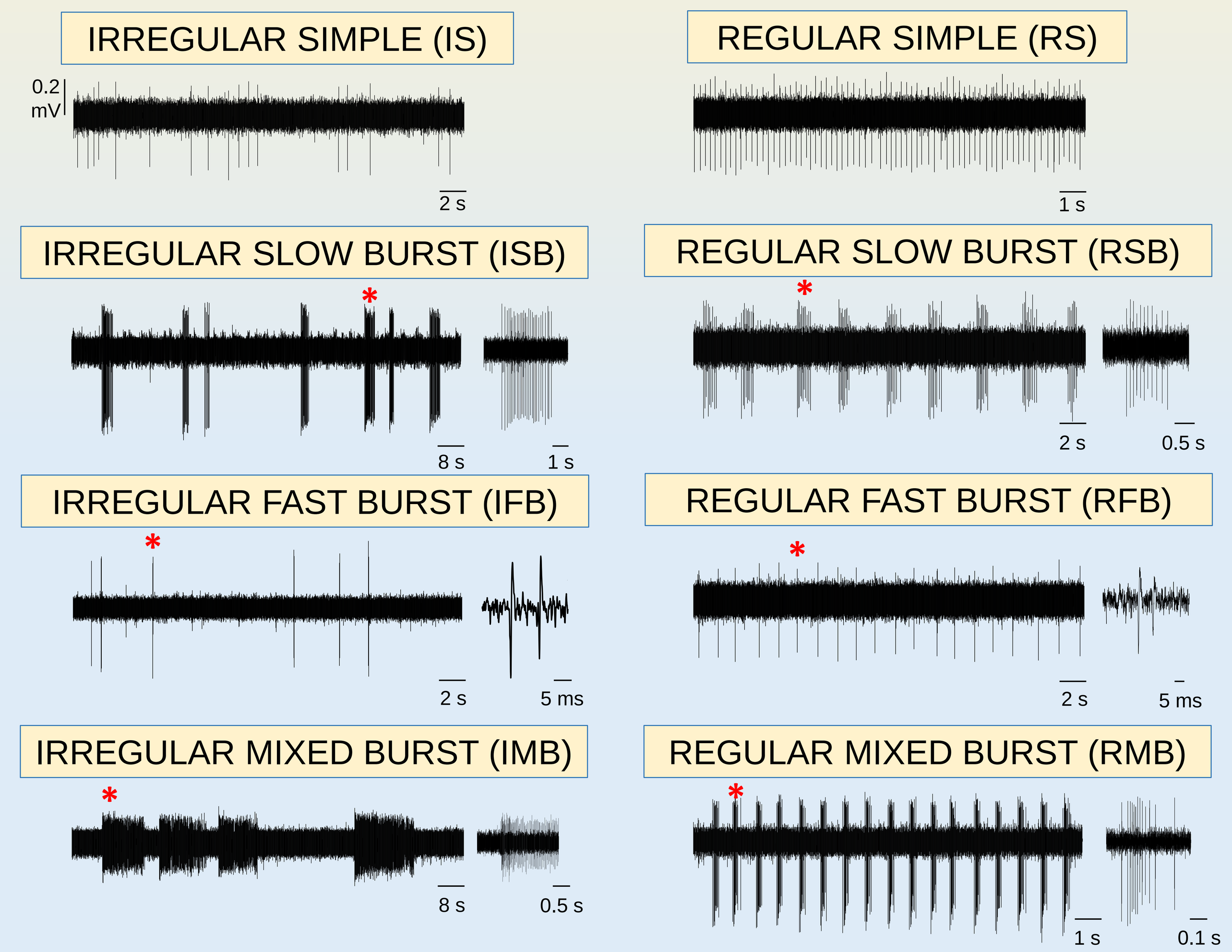


ALGORITHM WORKFLOW



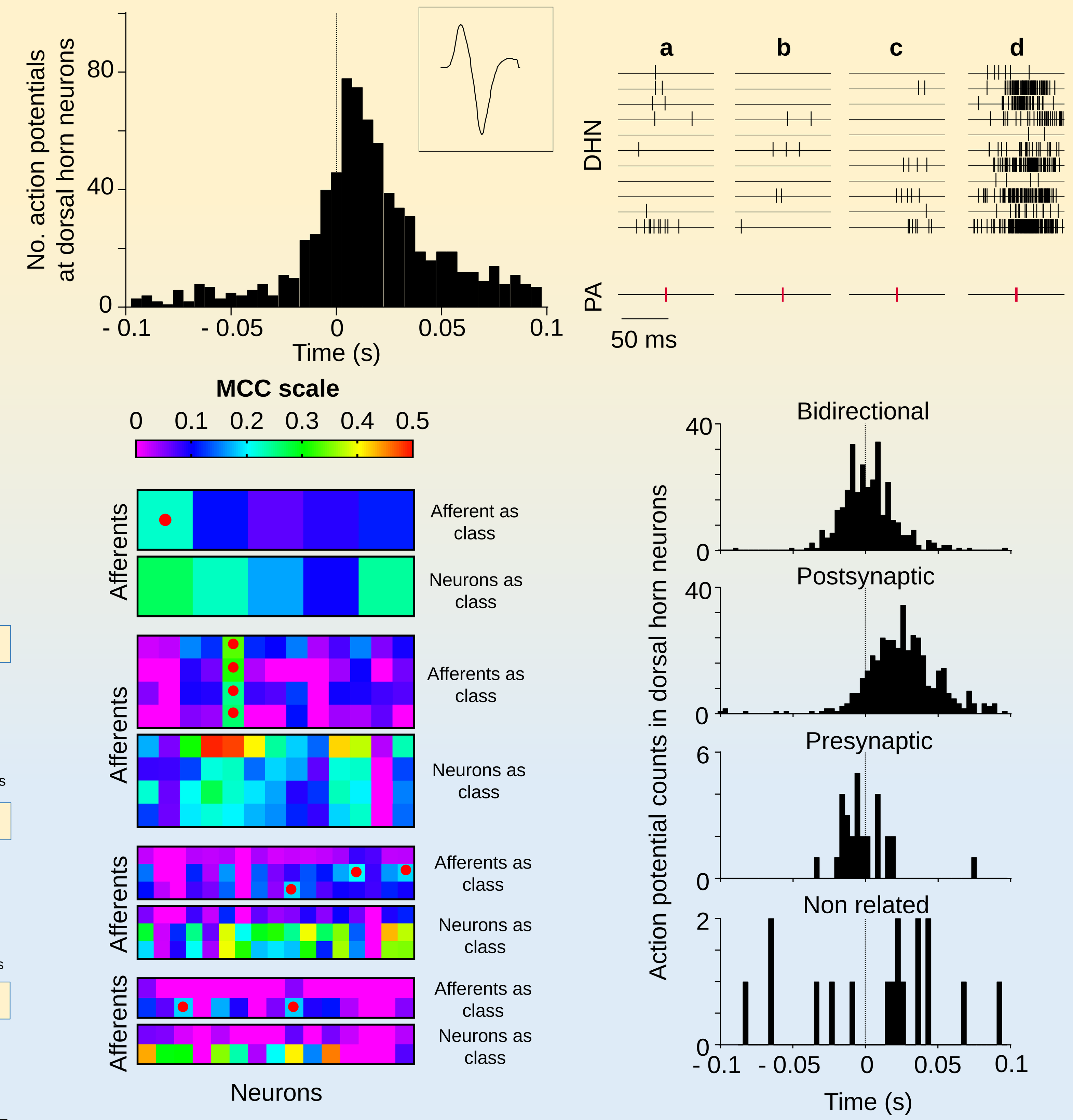
Pre-processing and analysis procedures included in the machine learning tool used to define functional connectivity in electrophysiological multiunit recordings .

DETECTED FIRING PATTERNS



Different firing patterns were defined according to regularity and burst firing. We classified them using an in-house developed algorithm publicly available (see Lucas-Romero et al. 2018).

FUNCTIONAL CONNECTIVITY ANALYSIS



Analysis of functional connectivity. Top left: cross-correlogram showing the dorsal horn neurons spikes around primary afferent firings, as shown in the top right graph. Bottom left: heat maps with the potential connections. Bottom right: Different connectivity relationships found between dorsal horn neurons and primary afferents.

Conclusions

The algorithm detects the neurons that hold strong effective connectivity with the primary afferents. Some neurons appear to be presynaptic to the afferents and may trigger backfiring. Others are likely postsynaptic to the afferents and a third category seems to have complex pre-and post-synaptic relations. Many of the neurons presynaptic to the afferent are of the irregular fast burst type, an electrophysiological signature that is easy to identify.

References

1.- Lucas-Romero J, Rivera-Arconada I, Roza C, Lopez-Garcia JA (2018) Origin and classification of spontaneous discharges in mouse superficial dorsal horn neurons. Sci Rep 8:9735

2.- Pozo-Jimenez P, Lucas-Romero J, Lopez-Garcia JA (2021) Discovering Effective Connectivity in Neural Circuits: Analysis Based on Machine Learning Methodology. Front Neuroinform 15:561012.

Acknowledgements

Financial support was obtained from the University of Alcala, grants CCG2018/BIO-028 and PIUAH21/CCS-039.