ROLE OF CENTRAL TERMINALS OF PRIMARY AFFERENTS IN THE MAINTENANCE OF COORDINATED SPONTANEOUS ACTIVITY OF DORSAL HORN NEURONS.

J. Lucas-Romero, J. Lopez-Garcia, I. Rivera-Arconada
Department of Systems Biology (Physiology), Universidad de Alcala, Madrid (Spain)

Introduction

The excitability of the central terminals of primary afferents (PA) is critical in regulating neurotransmitter release to second-order neurons located in the spinal cord. Primary afferent depolarisation (PAD) is an important mechanism of presynaptic inhibition that regulates somatosensory inputs to the spinal cord, and primary afferent hyperpolarisation (PAH) can produce opposite effects (1,2). In addition, activity in primary afferents may serve as a mechanism for spreading excitation to spinal cord neurons, thereby maintaining the excitability of spinal cord circuits (3,4).

Our aim was to investigate how changes in the excitability at PA terminals modify activity in spinal circuits. By expressing opto- and chemogenetic tools under the control of the advillin promoter, the excitability of central terminals of PA was manipulated to test the influence of these manoeuvres on the rhythmic spontaneous and induced activity recorded from dorsal roots and dorsal horn neurons.

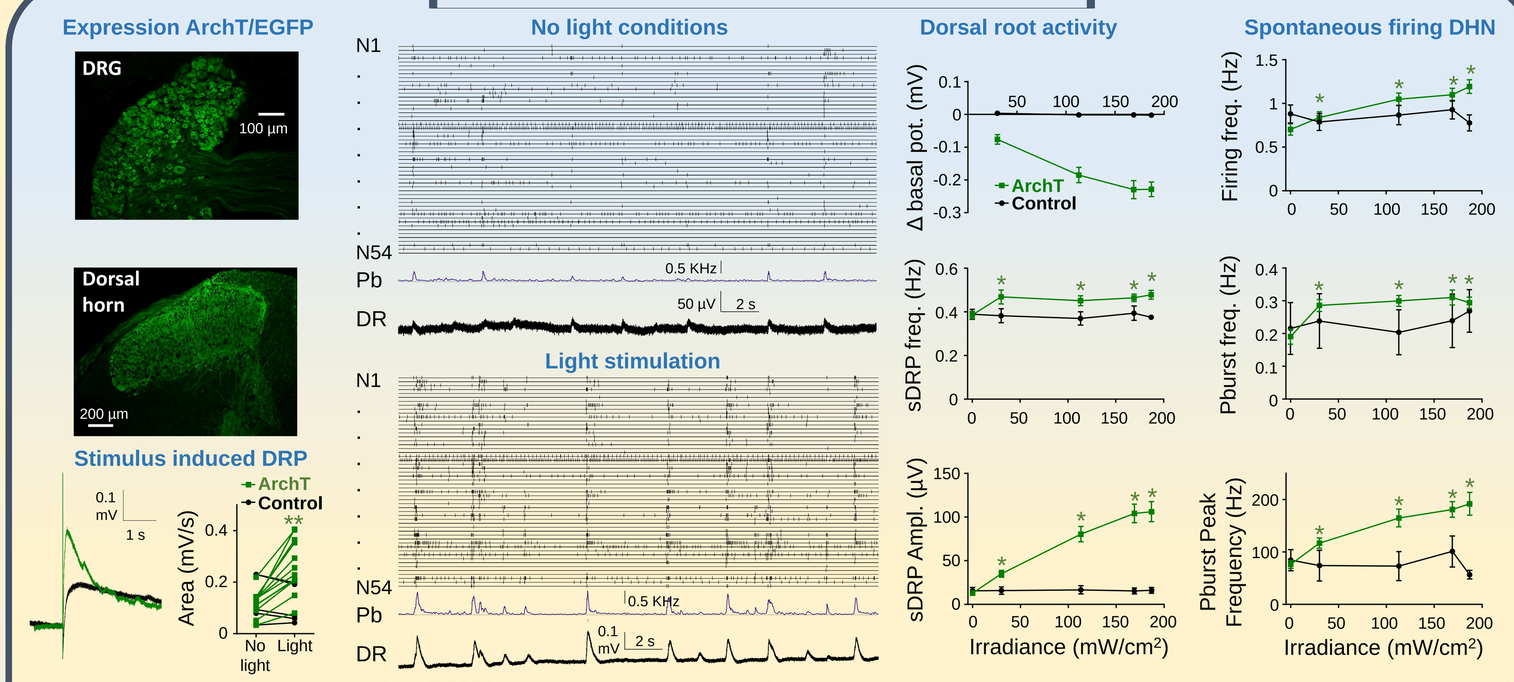
Methods

Spinal cords were extracted from C57BL/6 mice pups (6-13 days old) under urethane anaesthesia. A single 500 µm horizontal slice containing the dorsal horn and attached dorsal roots was obtained with a vibratome and maintained *in vitro* superfused with oxygenated artificial cerebrospinal fluid at 22 ± 1 ºC. Male Advillin$^{Cre/-}$ heterozygous mice were mated with homozygous females of the Ai40(RCL-ArchT/EGFP), Ai32(RCL-ChR2(H134R)/EYFP) and R26-LSL-hM4Di-DREADD strains to express archaerhodopsin-3 (ArchT), channelrhodopsin-2 (ChR2) and hM4Di, respectively, in all types of primary afferents. Light sources at 567 nm (Luxeon Star LEDs, irradiance from 30 to 180 mW/cm2) and at 455nm (Thorlabs, irradiance from 1 to 40 mW/cm2) were used for stimulation of ArchT and ChR2. The hM4Di receptor was activated by bath application of clozapine-N-oxide (CNO, Sigma) for 30 minutes at 10 µM.

Dorsal horn neuron (DHN) recordings were obtained using microelectrode arrays (MEA, NeuroNexus). Signals from the MEA were amplified, band-pass filtered between 200 Hz and 3 KHz, and digitised using RHS2116 amplifier chips and an RHS2000 stimulation/recording controller (Intan Technologies, US). Data were then stored for offline analysis using Kilosort, Spike-2 software from CED and in-house developed algorithms written in MATLAB. Neurons were classified according to their firing patterns of spontaneous activity as previously reported (5). Synchronous activity was analysed as population burst activity (4). Spontaneous and evoked activity in dorsal roots was recorded using glass suction electrodes with an AxoClamp 2B, amplified and filtered, digitised using the RHS2000 and stored for offline analysis. Electrical stimulation of an adjacent dorsal root was used to elicit synaptic responses. Frequency and amplitude of spontaneous dorsal root potentials (DRPs) and depolarised area (1s) of the responses to dorsal root electrical stimulation were measured.

Results

Light stimulation of Archaerhodopsin-3



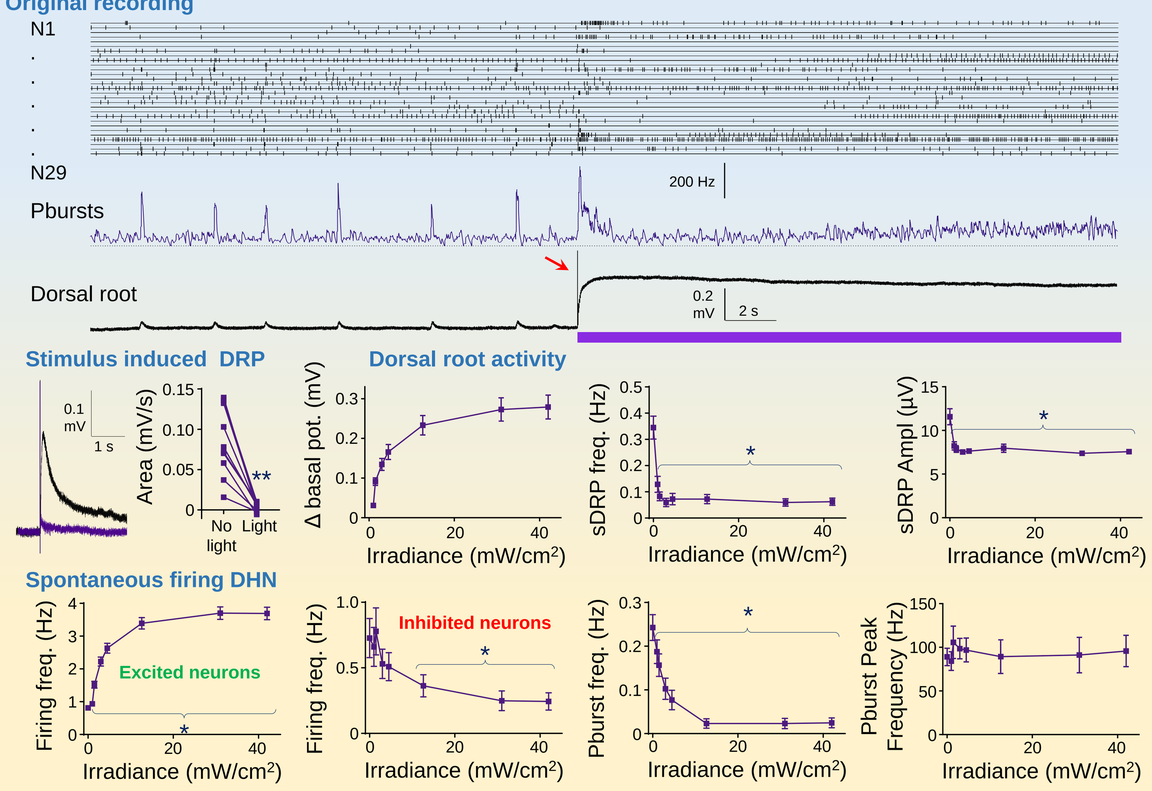
Advillin promoter directed the expression of ArchT/EGFP to dorsal root ganglion neurons of all sizes and their central terminals in the spinal cord. High intensity electrical stimulation of a dorsal root elicited a response in the adjacent root consisting of short latency peaks and a depolarising wave or dorsal root potential (DRP). During ArchT activation, the stimulated DRP increased in all preparations tested (** p > 0.01 paired t-test). Original recordings of the spontaneous activity recorded from the dorsal root (DR line) and 54 dorsal horn neurons (lines N1 to N54, below coordinated activity in Pbursts (Pb)) in control and during light stimulation (169 mW/cm²). Light activation of ArchT produced hyperpolarisation of the dorsal root basal potential and increased the frequency and amplitude of spontaneous dorsal root potentials (sDRP). The mean firing frequency of dorsal horn neurons (DHN) was also increased, with an augmentation in the occurrence of coordinated firing events (population bursts, Pburst) and their size. * p < 0.05 compared to zero light; Dunnett's test after One-way ANOVA.

Summary of the effects on DHN

Treatment / Firing pattern	ArchT (12/6)			ChR2 (13/7)			hM4Di (6/6)		
Irregular simple firing (IS)	166	4	145	324	85	242	11	79	34
Irregular Fast Burst (IFB)	40	0	8	17	15	8	0	44	2
Irregular Slow Burst (ISB)	12	2	12	11	13	7	0	4	5
Irregular Mixed Burst (IMB)	0	0	0	1	0	1	0	1	0
Regular simple firing (RS)	2	0	11	15	0	4	0	2	1
Regular Fast Burst (RFB)	0	0	0	0	1	0	0	2	0
Regular Slow Burst (RSB)	0	0	7	11	3	5	0	2	0
Regular Mixed Burst (RMB)	1	0	0	0	0	1	0	0	0
Total	**223**	**6**	**184**	**385**	**117**	**281**	**11**	**134**	**42**

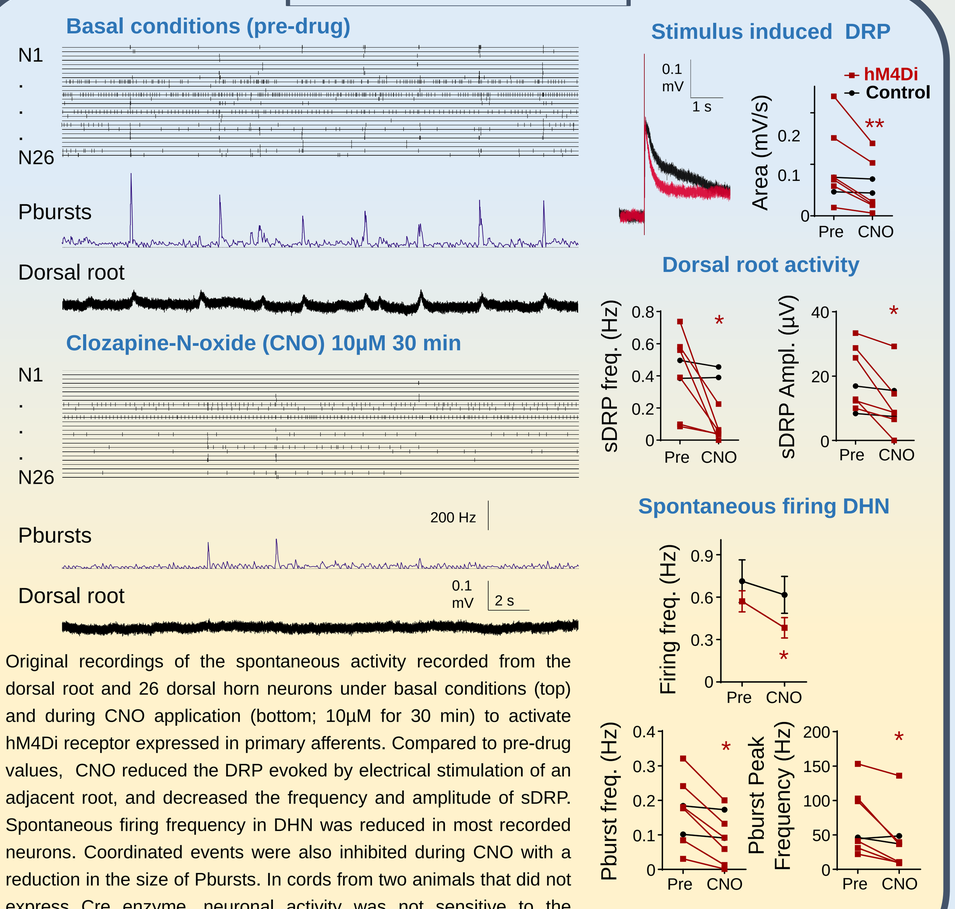
Table shows a summary of the changes in spontaneous firing frequency of dorsal horn neurons after stimulation of ArchT, ChR2 and hM4Di (number of assays/animals in brackets). Neurons were classified by spontaneous firing pattern as previously reported (5). Green represent excited neurons (change in firing frequency from control values >150%), red inhibited (<75% of control) and blue neurons that showed no change (75-150% of control).

Light stimulation of Rhodopsin-2



Original recording showing the spontaneous activity recorded from the dorsal root, 29 dorsal horn neurons (out of a total of 61 neurons in the track) and Pbursts in control and during light stimulation of ChR2 (purple line,12.6 mW/cm²) expressed in primary afferents. Light application depolarised the afferents and evoked a volley in the onset of the stimulus (red arrow), activating some DHN. Coordinated activity and sDRP were almost abolished during the depolarisation. Some neurons increased (385) while other decreased (117) their spontaneous firing as can be seen from the recording and the graphs. The dorsal root potential elicited by electrical stimulation of an adjacent dorsal root was abolished.

Activation of hM4Di



Original recordings of the spontaneous activity recorded from the dorsal root and 26 dorsal horn neurons under basal conditions (top) and during CNO application (bottom; 10µM for 30 min) to activate hM4Di receptor expressed in primary afferents. Compared to pre-drug values, CNO reduced the DRP evoked by electrical stimulation of an adjacent root, and decreased the frequency and amplitude of sDRP. Spontaneous firing frequency in DHN was reduced in most recorded neurons. Coordinated events were also inhibited during CNO with a reduction in the size of Pbursts. In cords from two animals that did not express Cre enzyme, neuronal activity was not sensitive to the application of CNO under equivalent conditions.

Conclusions

- Hyperpolarisation of primary afferents (PA) enhanced the coordinated activity of dorsal horn neurons (DHN) as well as spontaneous and evoked responses in the dorsal root.
- Depolarisation of PA excited many DHN but abolished the spontaneous and evoked activity in the dorsal root and impaired coordinated activity. Similar effects on PA were observed by activating the hM4Di receptor, but in contrast the activity of dorsal horn neurons was also reduced.
- Results demonstrate the role of primary afferents as a synchronising element in the rhythmic activity generated in spinal circuits.

References

1.- Rudomin et al, 1974. J. Neurophysiol. 37(3):413-29.
2.- Rudomin, 2009. Exp. Brain Res. 196(1):139-51.
3.- Bos et al, 2011. J. Neurosci. 31(28):10184-8.
4.- Lucas-Romero et al, 2022. Front. Cell. Neurosci. 16:1004956.
5.- Lucas-Romero et al, 2018. Sci. Rep. 8(1):9735.

Acknowledgements

Financial support was obtained from the Spanish Ministry of Science and Innovation PID2021-126330OB-I00.