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Barrington's nucleus CRH neurons conditionally drive bladder contraction: A multi-unit optogenetic recording study in mice

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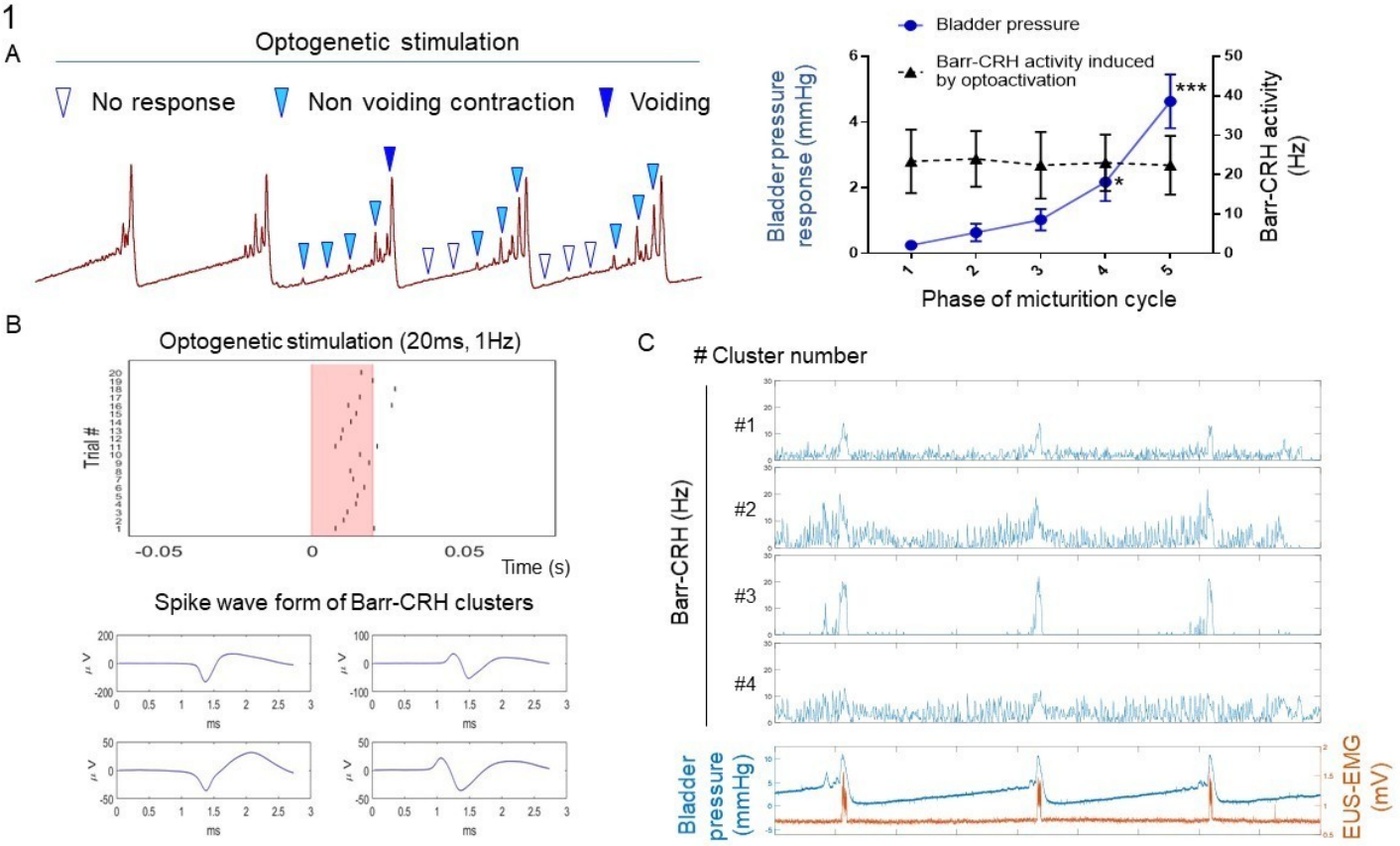
Introduction & Objectives: More than half of Barrington's neurons express corticotropin-releasing hormone (CRH) and recent studies using CRH-ires-CRE knock-in mice has enabled optogenetic manipulation of their activity (*Cell 2016*, *Nature Neuro. 2018*). Those studies indicate that the Barr-CRH neurons caused bladder contraction and an increase in the probability of co-ordinated micturition but they do not regulate external urethral sphincter (EUS) activity. We hypothesised that the Barr-CRH neurons are not high-fidelity controllers of bladder pressure but they do influence timing of voids. To address our hypothesis, we recorded the activity of optogenetically identified Barr-CRH neurons.

Materials & Methods: Stereotaxic injections of Cre-inducible vector (AAV-EF1a-DIO-hChR2-mCherry) in CRH-ires-CRE mice allowed to express Channelrhodopsin2 and opto-activation. Subsequently under urethane anaesthesia an optic fibre was placed above Barrington's for optogenetic stimulation. Recordings from the Barr-CRH neurons used a 32 channel silicon probe (NeuroNexus) and an open-ephys system. Spike waveforms were clustered in Kilosort and verified in Phy followed by analysis in MATLAB. Bladder pressure and EUS-electromyography (EMG) were recorded.

Results: The bladder contractions were induced by optogenetic activation (473 nm, 20ms, 20Hz for 5sec) of Barr-CRH during filling phase and the amplitude of these bladder contractions significantly increased during the phase of micturition cycle, in turn, eventually caused voiding ($P<0.001$, Figure 1A). The Barr-CRH neurons were optoidentified by reliable short latency spike entrainment (Figure 1B). In total 128 neurons were recorded in isolation in 4 mice and 27 units were identified as Barr-CRH neuron. They showed a characteristic pattern of activity during the micturition cycle with bursting (20.5 ± 4.1 Hz of bursting) just before micturition (Figure 1C). The optogenetic stimulation evoked Barr-CRH

activity which was independent of micturition phase (ranging from 22.4 ± 7.7 to 24.0 ± 6.5 Hz across 5 different micturition phases, Figure 1A).

Figure 1



Conclusions: This is first study to investigate Barr-CRH firing activity from optogenetically identified neurons. The bladder pressure change induced by optoactivation of CRH neurons is dependent upon the phase of the micturition cycle indicating that the response to stimulation reflects the state of the downstream parasympathetic circuit.