Neuroscience Letters 671 (2018) 103–107

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Suction electrode recording in locus coeruleus of newborn rat brain slices reveals network bursting comprising summated non-synchronous spiking

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ARTICLE INFO

Keywords:
Rhythmic field potentials
Locus ceruleus
Rhythm generation

ABSTRACT

The brainstem locus coeruleus (LC) controling behaviors like arousal, sleep, breathing, pain or opioid withdrawal is an established model for spontaneous action potential synchronization. Such synchronous 'spiking' might produce an extracellular field potential (FP) which is a crucial tool for neural network analyses. We found using ≥10 µm tip diameter suction electrodes in newborn rat brainstem slices that the LC generates at ~1 Hz a robust rhythmic FP (rFP). During distinct rFP phases, LC neurons discharge with a jitter of ± 33 ms single spikes that summate to a ~200 ms-lasting population burst. The rFP is abolished by blocking voltage-gated Na^+ channels with tetrodotoxin (TTX, 50 nM) or gap junctions with mefloquine (100 µM) and activating µ-opioid receptors with [D-Ala2,N-Me-Phe4,Gly5-ol]-Enkephalin (DAMGO, 1 µM). Raising superfusate K^+ from 3 to 7 mM either increases rFP rate or transforms its pattern to slower and longer multipeak bursts similar to those during early recovery from DAMGO. The results show that electrical coupling of neonatal LC neurons does not synchronize their spiking as previously proposed. They also indicate that both increased excitability (by elevated K^+) and recovery from inhibition (by opioids) can enhance spike desynchronization to transform the population burst pattern. Both observations show that this gap junction-coupled neural network has a more complex connectivity than currently assumed. These new findings along with the inhibitory drug effects that are in line with previous reports based on single neuron recording point out that field potential analysis is pivotal to further the understanding of this brain circuit.

1. Introduction

The locus coeruleus (LC) provides noradrenergic innervation to various brain structures and controls multiple behaviors including the sleep-wake cycle, arousal, memory, breathing, pain modulation and opioid withdrawal [1–5]. In vitro studies mostly using newborn rodent brain slices established the LC as a model for analysing action potential ('spike') discharge patterning in neural networks. Such research presented evidence that gap junction-coupled neonatal LC neurons show synchronous subthreshold membrane potential oscillations that cause discharge of a spike at their peak [6–9]. If such rhythmic single spike discharge occurs synchronously, this might generate an extracellular field potential (FP) which is a pivotal measure to analyse neural network properties in other brain regions [10,11]. Yet, no study reported a rhythmic FP (rFP) reflecting normal repetitive neonatal LC activity. In newborn rodent brainstem slices, a rFP in the spontaneously active breathing center is not seen with sharp or patch microelectrodes often used for FP recording whereas a robust signal is revealed with suction electrodes originally designed for nerve recording [12,13]. Accordingly, it was the aim of our study to identify in horizontal newborn rat brain slices with suction electrode rFP recording novel features of the LC neural network.

2. Materials and methods

2.1. Preparation and solutions

All procedures were approved by the University of Alberta Animal Care and Use Committee and carried out in compliance with guidelines of the Canadian Council for Animal Care and the Society for Neuroscience's Policies on the Use of Animals and Humans in Neuroscience Research. Experiments were performed on horizontal LC-containing brain slices from 0 to 4 days-old Sprague-Dawley rats (Charles River Laboratory Inc) as previously described [14]. In brief, rats were anesthetized with 2–3% isoflurane until the paw withdrawal reflex disappeared. They were then decerebrated and the neuraxis isolated at 18–20 °C in saline containing (in mM): 120 NaCl, 3 KCl, 1.2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 D-glucose (pH

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https://doi.org/10.1016/j.neulet.2018.02.020
Received 31 October 2017; Received in revised form 25 January 2018; Accepted 9 February 2018
Available online 12 February 2018
0304-3940/

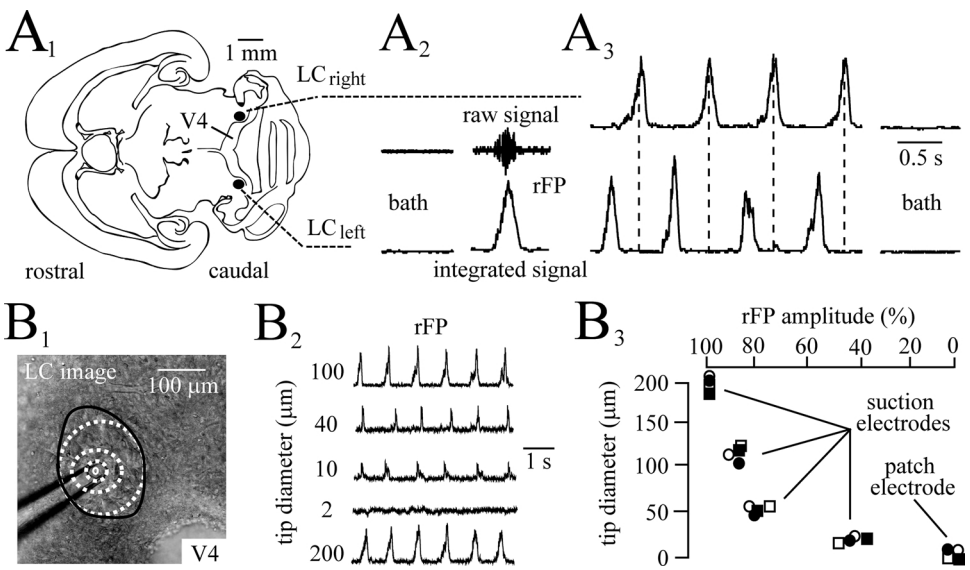

Fig. 1. Spontaneous rhythmic field potential (rFP) in locus coeruleus (LC) of newborn rat brain slices. A, schema represents the horizontal slice with the bilateral LC aspects (A_1). A_2 shows both the raw and integrated signal 24 min after a superfusate-filled suction electrode was repositioned from the superfusate in the recording chamber ('bath') to the surface of the left LC. After positioning a further suction electrode on the right LC, a non-synchronous rFP of similar amplitude and rate was seen (A_3). B, rFP recording in a different slice with (suction) electrodes of different diameter indicated by dashed lines (B_1). B_2, B_3 show that rFP amplitude was maximal using a 200 µm tip electrode and decreased with smaller tip sizes whereas no rFP was seen with a 2 µm tip superfusate-filled patch electrode (but compare Fig. 2). V4 in A_1 and B_1 stands for 4th ventricle and different symbols in B_3 for individial experiments in 4 slices.

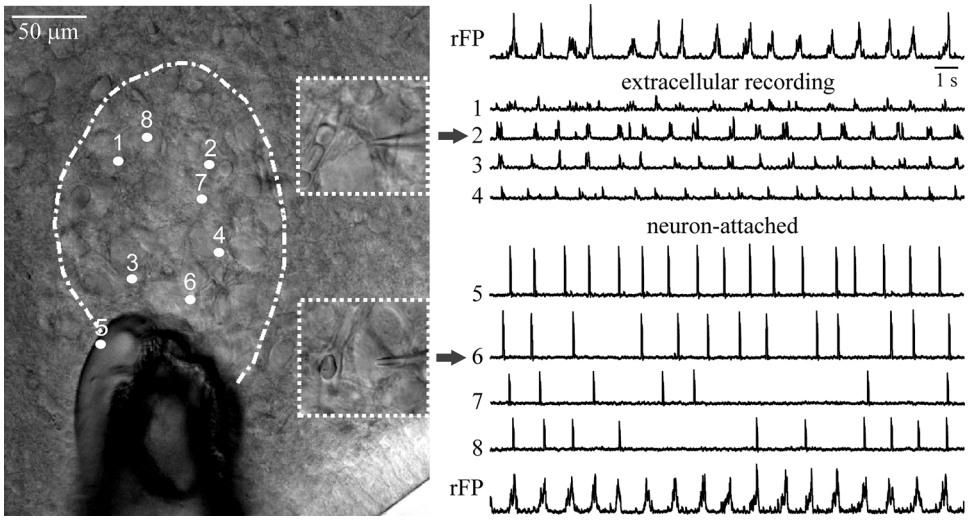

Fig. 2. Simultaneous rFP and single neuron action potential ('spike') recording. Image shows a suction electrode positioned close to caudal rim of LC whose neuronal somata area is outlined by dashed line. White circles and numbers indicate positions of a superfusate-filled patch electrode that was placed extracellularly firstly between cells (positions 1–4) and was then slightly pushed against 4 neuron somata (positions 5–8). In positions 1–4, a small amplitude rFP was only seen with 20 mmHg positive pressure applied to the electrode during positioning while afterwards 20–30 mmHg negative pressure was applied at the recording spot. In positions 5–8, single neuron spiking was seen independent on whether pressure was applied or not. Uppermost and lowermost traces show rFP recorded simultaneously with single neuron activity shown next to trace.

adjusted to 7.4 by gassing with carbogen, i.e. 95% O_2 plus 5% CO_2). The brain was glued ventral surface down to a metal plate to be inserted into a vibratome (Leica VT1000S; Leica Microsystems). Horizontal sectioning was done until the bilaterally-organized LC appeared and then one 400 µm thick slice was cut and fixed with a platinum 'harp' in an acrylic recording chamber with glass bottom (volume ~1 ml) (Fig. 1A). The LC and its neurons were visualized via a 20× objective with transmitted light of an Olympus 'multiphoton exclusive' microscope or video images from an Olympus-150 IR-DIC camera. Superfusate was applied at 28 °C in the chamber at 5 ml/min flow rate using a peristaltic pump (Watson-Marlow) and waste was removed via vacuum applied to a hypodermic needle. Agents were added to the superfusate from stock solutions: [D-Ala2,N-Me-Phe4,Gly5-ol]-Enkephalin (DAMGO, 1 µM, 1 mM in H_2O); mefloquine hydrochloride (100 µM, 100 mM in dimethyl sulfoxide); tetrodotoxin (TTX, 50 nM, 1 mM in H_2O). Drugs were obtained from Sigma-Aldrich except TTX (Alomone).

2.2. Electrophysiological recording

Patch pipettes were pulled from glass capillaries (GC-150TF-10, Harvard Apparatus) to an outer tip Ø of ~2 µm using a vertical puller (PC-10, Narishige). For suction electrode recording, patch pipettes were broken and subsequently manually beveled with sand paper (Ultra Fine 600 Grit, Norton-Saint Gobain) at an angle of 45° to an oval-shaped tip opening ranging from 10 to 200 µm. The mean dc resistances of superfusate-filled suction electrodes were 605 ± 26 kΩ, 218 ± 13 kΩ, 160 ± 6.2 kΩ and 108 ± 6.3 kΩ for approximately 10, 50, 100 and 200 µm tip sizes, respectively. Electrodes were positioned at a ~30° angle on the slice surface and their signal was amplified (x10k) and band-pass-filtered (0.3–3 kHz) using a Model-1700 differential amplifier (AM-Systems). In parallel, the electrode signal was integrated ('moving average', τ: 10–50 ms) using a MA-821/RSP unit (CWE) (Fig. 1A). Na^+ spike discharge of LC neuron pairs was recorded with superfusate-filled patch electrodes (dc resistance 4–5 MΩ) via a patch-clamp amplifier (EPC-10, HEKA) and a single-electrode voltage-clamp amplifier (SEC-05L, npi electronic). Signals were sampled at 1–10 kHz into a digital recorder (Powerlab 8/35, ADInstruments).

2.3. Signal analysis

To correlate single LC neuron activity with rFP bursting, spiking in one 'reference' cell was continuously recorded for up to 1 h. A second electrode monitored spiking consecutively in 4–9 further neurons for 2–3 min each while a suction electrode recorded the rFP at the LC boundary (Figs. 2 and 3). Using pClamp10 software (Molecular Devices Corporation), spikes were aligned to the rFP and traces were overlapped and analysed using a numerical matrix technique (Origin 6, Microcal Software). Twenty cycles of rFP bursts plus neuronal spiking