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Hippocampal modulation of cardiorespiratory function

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ABSTRACT

Changes in cardiorespiratory control accompany the expression of complex emotions, indicative of limbic brain inputs onto bulbar autonomic pathways. Previous studies have focussed on the role of the prefrontal cortex in autonomic regulation. However, the role of the hippocampus, also important in limbic processing, has not been addressed in detail. Anaesthetised, instrumented rats were used to map the location of hippocampal sites capable of evoking changes in cardiorespiratory control showing that stimulation of discrete regions within the CA1 fields of both the dorsal and ventral hippocampus potently alter breathing and cardiovascular activity. Additionally, tracing of the neuroanatomical tracts and pharmacological inactivation studies were used to demonstrate a role of the basomedial amygdala in hippocampal evoked responses. Collectively, these data support the existence of a hippocampal-amygdala neural circuit capable of modulating bulbar cardiorespiratory control networks and may suggest a role for this circuit in the top-down regulation of breathing and autonomic outflow necessary for the expression of complex emotions.

1. Introduction

Changes in autonomic neural outflow are common during the expression of complex emotions, indicative of descending inputs from higher brain limbic circuits onto bulbar autonomic pathways (Adolphs, 2002; Kreibig, 2010; Quigley and Barrett, 2014). A stereotypical emotion-dependent autonomic response may include changes in the depth or pattern of breathing, heart rate and/or blood pressure (Homma and Masaoka, 2008) secondary to altered respiratory and sympathetic network activity (Guyenet, 2006). Indeed, emotion-dependent autonomic responses are presumably important for meeting metabolic demands during real or perceived threats, and consequently their expression is highly context and experience dependent. In this regard, however, there is limited understanding of neural substrates that might serve to modulate such effects on bulbar autonomic activities.

Previous studies have focussed on the medial prefrontal cortex in autonomic regulation because of its well-established roles in emotional processing (al Maskati and Zbrozyna, 1989; Alexandrov et al., 2007; Bacon et al., 1996; Hassan et al., 2013; Owens and Verberne, 2001; Verberne et al., 1987). However, given the importance of prior experience on emotional expression, it is likely that hippocampal outputs directly or indirectly regulate the bulbar autonomic circuitry (Bannerman et al., 2014; Fanselow and Dong, 2010; Padilla-Coreano et al., 2016; Snyder et al., 2011). Experiments in both rats and humans are consistent with this hypothesis. Thus, in rats, retrograde transynaptic viral tracers injected into the adrenal gland or the stellate ganglion label cells in the ventral hippocampus that are synaptically connected to autonomic networks (Westerhaus and Loewy, 2001), while inactivating the ventral hippocampus, with the nonspecific synaptic blocker cobalt chloride ($CoCl_2$), modulates the cardiorespiratory effects triggered by peripheral chemoreflex activation (Kuntze et al., 2016). The same portion of the hippocampus was shown to modulate the motor expression of augmented breaths (Ajayi and Mills, 2017). Furthermore, using simultaneous measurements of resting state brain activity (via functional magnetic resonance imaging (fMRI)) and heart rate variability in healthy men, Bar et al. (2016) suggested the existence of hippocampal-brainstem connectivity responsible for adjusting vagal autonomic activity.

The studies described above implicate the hippocampus as a source of higher brain input capable of modulating the sympathetic and parasympathetic nervous system. However, little is known about the precise topography of autonomic modulatory sites within the hippocampus or the neuroanatomical circuit connectivity that allows the hippocampus to influences bulbar autonomic processing. It is likely that hippocampal-brainstem connectivity occurs through an intermediary structure such as the amygdala given the dual role of the amygdala in

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mediating emotions and autonomic outflow (Applegate et al., 1983; Kapp et al., 1979). Consistent with this, recent brain imaging studies in humans confirm amygdala and hippocampal connectivity with the brainstem and spinal cord (Arrigo et al., 2016), as well as concurrent activation of the amygdala during autonomic modulation (Napadow et al., 2008; Yoshihara et al., 2016). However, there is no direct evidence of hippocampal neural commands directly regulating the autonomic-related activities of the amygdala.

In the present study, we therefore set out to firstly map hippocampal areas in the rat capable of generating changes to respiratory and cardiovascular outflow using chemical microstimulation techniques and subsequently to provide neurophysiological and neuroanatomical evidence for an essential role of the amygdala in hippocampal-evoked autonomic responses.

2. Materials and methods

Experiments were performed on Sprague-Dawley rats (n = 29; of either sex weighing 350–550 g), in compliance with approvals obtained from accredited institutional Animal Care and Ethics Committees. Prior to surgery, animals were housed in standard plastic cages under 12:12 h light:dark cycle and fed water and food *ad libitum*.

2.1. Physiological experiments

Rats were anaesthetised with urethane (1.5 g/kg i.p), the level of which was determined to be adequate by assessing the palpebral and withdrawal reflexes. Animals were placed in a supine position on a thermostatically controlled heating pad. A midline incision was made along the ventral surface of the neck and the muscles were retracted to expose the trachea and underlying nerves and blood vessels. The left carotid artery was cannulated using a polyethylene tubing (internal diameter = 0.5 mm and outer diameter = 0.9 mm) attached to a pressure transducer filled with heparinised saline (50 U/mL) to measure arterial blood pressure (ABP) and heart rate (HR). In some preparations, the left femoral artery, rather than the carotid artery was cannulated for cardiovascular monitoring. A 14-gauge tracheal cannula was then secured into the lumen of the distal extrathoracic trachea. This cannula was connected via a side-port to a pressure transducer to measure the changes in tracheal pressure (TP) associated with spontaneous respiration. Output from each of the pressure transducers were filtered and amplified (Neurolog Systems, Digitimer, Hertfordshire, UK), digitised (Micro1401 A-D converter, CED, Cambridge, UK) and recorded using Spike II software (CED, Cambridge, UK) for offline analysis. Supplementary fluids (Sterile saline; 2 mL/kg/hr) were administered via vascular lines periodically to maintain intravascular pressure.

Once instrumented, animals were placed in a stereotaxic frame, the skull exposed by a midline incision in the scalp and the level of Lambda and Bregma aligned in the dorsoventral axis (i.e. skull flat position). Burr holes were then drilled over regions spanning 2.16–4.80 mm caudal to Bregma for the dorsal hippocampus and 4.56–5.20 mm caudal to Bregma for the ventral hippocampus (Paxinos and Watson, 2007) in both cerebral hemispheres and the brain was covered with paraffin oil to prevent drying. Cardiorespiratory responses evoked by focal excitation of different hippocampal regions were assessed using microinjections of the excitatory amino acid, D, L–Homocysteic acid (DLH; Sigma-Aldrich; 50 mM, 200–300 nL) via calibrated glass micropipettes (~20 µm tip diameter) connected to a pneumatic pressure injector (Pneumatic PicoPump; WPI, Sarasota FL) (Fig. 1). Rhodamine-B fluorescent microspheres (Sigma, Australia) were added to the DLH solution to label injection sites and the glass micropipette was left in place for 5 min after each injection to minimise leakage of the injectate along the needle tract. Each animal (n = 15) received up to 12 individual injections (no more than 6 injections in each hemisphere). A minimum of 20 min was allowed between each injection for effects to return to baseline. Isotonic saline injections were used as control and produced no cardiorespiratory effects in all instances.

In a separate series of experiments, we assessed the role of the amygdala in the cardiorespiratory effects evoked by hippocampal activation. Animals received bilateral microinjections of the $GABA_A$ agonist, muscimol (Sigma-Aldrich; 20 ng in 150 nL, n = 6) or vehicle (saline, n = 3) into the basomedial amygdala (Bregma −2.76 mm, ML 4.00 mm, DV 9.20 mm). Ten minutes after injecting muscimol, the animals then received a single injection of DLH into the ventral hippocampal responsive area (CA1 pyramidal cell layer at Bregma −4.56 to −5.20 mm, ML 5.20–5.80 mm, DV 7.00–8.20 mm), as described above (Fig. 1).

At the end of all physiological experiments, animals were transcardially perfused with phosphate buffered saline followed by 4% paraformaldehyde (PFA) and brains removed for histological examination of injection sites (see below).

2.2. Retrograde neuronal tracing

Cholera toxin-b conjugated with Alexa Fluor 488 (CTb-488) injections were made bilaterally into the basomedial amygdala (n = 5) to map the anatomical organisation of hippocampal inputs to this region. In brief, rats were anaesthetised with isoflurane (2.5% in medical oxygen) via a nose cone and their heads were placed into a stereotaxic frame (as described above). A single microinjection of CTb-488 (0.2%, 100–150 nL; Molecular Probes, Thermo Fisher Scientific) was performed into the amygdala at 2.76–3.96 mm caudal to Bregma (Paxinos and Watson, 2007) and the glass micropipette was left in place for 20 min to minimise non-specific spread of the tracer. Wounds were sutured and animals were left to recover for 10 days.

2.3. Tissue processing and imaging

Brains were collected from all animals in order to map injection sites and localise traced neurons. Animals were first deeply anaesthetised with sodium pentobarbitone (150 mg/kg i.p) and then transcardially perfused with 300 mL of 0.1 M PBS, pH 7.4, followed by 300 mL of 4% PFA in phosphate buffered saline (PBS). Brains were removed and post-fixed in 4% PFA for up to 16 h at 4 °C. Coronal sections were cut at either 100 µm (physiological studies) or 50 µm (tracing studies) thickness using a vibratome (Leica VT1000S) and collected serially in 0.1 M PBS. Every second section was mounted on gelatin-coated slides and visualised for fluorescence (Olympus BX51 microscope). When fluorescently labelled neurons and injection sites were identified, the corresponding adjacent section was immunohistochemically stained for the calcium binding protein calretinin to delineate tissue architecture. Immunostaining was performed on free-floating sections that were first blocked with 10% goat serum in 0.1 M PBS for 1 h, following which they were incubated overnight at room temperature in rabbit anti-calretinin (diluted 1:1000 in 2% goat serum and 0.3% Triton-X 100 in 0.1 M PBS; Swant Inc., Switzerland). Sections were then washed and incubated for 1 h at room temperature with goat anti-rabbit Alexa Fluor 594 (1:500; Thermo Fisher Scientific, Australia), mounted on gelatin-coated slides and cover-slipped with Fluroshield mounting media (Sigma-Aldrich, Australia). Sections were viewed and photographed using a fluorescent microscope (Olympus BX51 equipped with an Olympus DP72 camera). Digital images were optimised by minimally adjusting contrast and brightness using Adobe Photoshop CC (version 2017.1.1).

2.4. Data analysis

DLH evoked physiological responses were calculated per minute over 10 min from chart recordings and compared to pre-injection baseline data (defined as the 60 s period immediately prior to the microinjection). Measurements collected included the inspiratory (Ti) and expiratory (Te) duration, respiratory rate (RR), tracheal pressure (TP),