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Functional topography of respiratory, cardiovascular and pontine-wave responses to glutamate microstimulation of the pedunculopontine tegmentum of the rat

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ABSTRACT

Functionally distinct areas were mapped within the pedunculopontine tegmentum (PPT) of 42 ketamine/xylazine anesthetized rats using local stimulation by glutamate microinjection (10 mM, 5–12 nl). Functional responses were classified as: (1) apnea; (2) tachypnea; (3) hypertension (HTN); (4) sinus tachycardia; (5) genioglossus electromyogram activation or (6) pontine-waves (p-waves) activation.We found that short latency apneas were predominantly elicited by stimulation in the lateral portion of the PPT, in close proximity to cholinergic neurons. Tachypneic responses were elicited from ventral regions of the PPT and HTN predominated in the ventral portion of the antero-medial PPT. We observed sinus tachycardia after stimulation of the most ventral part of the medial PPT at the boundary with nucleus reticularis pontis oralis, whereas p-waves were registered predominantly following stimulation in the dorso-caudal portion of the PPT. Genioglossus EMG activation was evoked from the medial PPT.Our results support the existence of the functionally distinct areas within the PPT affecting respiration, cardiovascular function, EEG and genioglossus EMG.

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1. Introduction

The pedunculopontine tegmentum (PPT) is known to participate in a wide range of state-regulating and behavioral functions. PPT plays an important role in rapid eye movement (REM) sleep and REM sleep-related phenomena, including EEG activation, hippocampal theta rhythm and pontine-waves (p-waves) (Datta and Hobson, 1995; Garcia-Rill, 1991; Rye, 1997; Shouse and Siegel, 1992; Steriade and McCarley, 1990; Vertes et al., 1993). Injection of glutamate into the PPT increases REM sleep and wakefulness for hours in unanesthetized rats (Datta et al., 2001a,b) and injection of carbachol increases REM sleep for days in cats (Calvo et al., 1992; Datta et al., 1991) and rats (Carley and Radulovacki, 1999). Lesions or pharmacological blockade of the PPT diminishes wakefulness and eliminates or alters expression of the tonic and phasic components REM sleep, including p-waves and rapid eye movements (Shouse and Siegel, 1992). The PPT also participates in regulation of motor control (Garcia-Rill, 1991; Winn, 2006), modulation of sensation (Reese et al., 1995), orientation and attention (Rostron et al., 2008), reaction time, and learning and memory (Garcia-Rill, 1991; Datta, 1997; Datta and Hobson, 1995).

The PPT also may play an important role in autonomic regulation. Continuous electrical stimulation within the PPT evoked respiratory depression in anesthetized cats (Lydic and Baghdoyan, 1993), whereas we demonstrated that carbachol injection into the PPT increased respiratory dysrhythmia during sleep in conscious rats (Carley and Radulovacki, 1999; Radulovacki et al., 2004). We further showed that microinjection of glutamate into the PPT of anesthetized rats initiated respiratory disturbances characterized by irregular alternations between tachypnea and bradypnea/apnea (Saponjic et al., 2003, 2005, 2006). Stimulation of the PPT can also evoke cardiovascular reactions, characterized by increased blood pressure (BP) (Padley et al., 2007).

A variety of evidence shows that PPT neurons are, to some extent, anatomically organized into subregions according to their function (Rye, 1997). For example, anatomically a specific antero-

Abbreviations: PPT, pedunculopontine tegmentum; EEG, electroencephalogram; ECG, electrocardiogram; EMG, electromyogram; BP, blood pressure; HTN, hypertension; p-wave, spontine waves; REM, rapid eye movement; AP, antero-posterior; ML, medio-lateral; DV, dorso-ventral; NTS, nucleus of the solitary tract; VLM, ventro-lateral medulla; RVLM, rostro-ventro-lateral medulla; PB, parabrachial complex.

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ventro-medial region of the PPT reciprocally innervates the basal ganglia (Rye et al., 1987), whereas a dorso-caudal portion of the PPT has specific connectivity to the limbic ventral tegmental area (Oakman et al., 1995; Rye, 1997; Rye et al., 1987). Lesions of the anterior and posterior PPT produced opposite effects on nicotine-induced locomotion and self-stimulation behavior in rats (Alderson et al., 2008), and electrical stimulation specific to the anterior PPT prior to training improved subsequent learning (Andero et al., 2007).

Our laboratory has demonstrated that local injection of glutamate in anesthetized rats can evoke respiratory dysrhythmia, electroencephalogram (EEG) activation, hippocampal theta-rhythm, or increased phasic events such as p-waves (Saponjic et al., 2003, 2005, 2006). Further, these studies suggested that such phenomena are at least partially differentiable, according to stimulation site, suggesting a functional topography. However, a detailed functional mapping of these activities within the pedunculopontine tegmentum has not been performed. The aim of the present study was to use glutamate microinjections to determine the functional map of respiratory, cardiovascular and electroencephalographic phenomena within the PPT.

2. Methods

Experiments were performed on 42 spontaneously breathing adult male Sprague–Dawley rats, weighing 200–300 g, maintained on a 12-h light–dark cycle, and housed at 25 °C with free access to food and water. Principles for the care and use of laboratory animals in research were strictly followed, as outlined by the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences Press, Washington, DC, 1996).

2.1. Surgical preparation

The rats were anesthetized with a combination of 80 mg/kg ketamine (Abbott Laboratories, North Chicago, IL) and 5 mg/kg xylazine (Phoenix Scientific Inc., St. Joseph, MO) by intraperitoneal injection. After a surgical plane of anesthesia was achieved, rats were placed in the stereotaxic apparatus (David Kopf Inst., model 962 A Tujunga, CA). The level of anesthesia was monitored by BP, heart rate and reflexes (corneal and toe-pinch). Supplemental dose of 40 mg/kg ketamine/2.5 mg/kg xylazine were used as necessary to maintain a stable plane of anesthesia. No injections were made for at least 15 min after any supplemental dose of anesthesia.

Animals were instrumented to record bilateral electroencephalograms (EEGs) using stainless steel screw electrodes located in the frontal (AP, +2.5 mm to bregma, ML, 2 mm to midline; DV, 1 mm ventral to the surface of the brain) and parietal cortex (AP, −2.5 mm to bregma; ML, 2 mm to midline; DV, 1 mm ventral to the surface of the brain), referenced to a screw electrode in the nasion. A bipolar twisted electrode made of teflon-coated stainless steel wires with uninsulated tips of 1 mm and with a 1 mm separation between the uninsulated tips was stereotaxically targeted into the contralateral PPT to record the pontine EEG (AP, −7.8 mm to bregma; ML 1.8 mm to midline; DV, 7 mm ventral to the surface of the brain) (Paxinos and Watson, 2004). The genioglossus electromyogram (EMG) was obtained using teflon-coated wire electrodes with uninsulated tips of 2 mm inserted bilaterally into the base of the tongue 1–2 mm lateral to the frenulum. The electrocardiogram (ECG) was registered by needle electrodes placed in the left axilla and the right flank. The femoral artery was catheterized to monitor blood pressure using Transpac IV transducer (Hospira, Lake Forest, IL).

A unilateral burr-hole osteotomy provided access to the rostral lateral pons, contralaterally to the twisted electrode and the dura was carefully removed. Three-barrel micropipettes were built using standard glass with filament (1 mm × 0.25 mm, A-M Systems, Carlsborg, WA) and a vertical puller (Model No 50-239, Harvard Apparatus Ltd., Kent, England) to achieve an overall tip diameter of 10–20 μm. A milli-pulse pressure injector (MPPI-2, ASI, Eugene, OR) was used to inject glutamate (10 mM L-glutamic acid monosodium salt in 0.2 M PBS; ICN Biomedicals, Aurora, OH), 0.2 M PBS, or oil red-O dye (Sigma, St. Louis, MO; solution of 7 mg in 1 ml ethanol) to aid histological verification of injection sites.

The MPPI-2 was outfitted with a backpressure unit to prevent injected fluids or extracellular fluid from entering the multibarrel assembly between injections. Typical injection pressure was ∼70 psi, while back pressure was ∼0.1 psi.

2.2. Recording procedure

Throughout each experimental protocol, we performed a 10-channel recording comprising: right and left monopolar frontal cortical EEG; right and left monopolar parietal cortical EEG; pontine bipolar EEG; genioglossus EMG; respiration recorded by a thoracic piezoelectric sensor (Velcro® Tab-Infant-Ped; Sleepmate® Technologies); ECG; arterial BP and an injection marker (logic level pulse) provided by the pressure injector.

After conventional amplification and filtering (1–20 Hz bandpass for EEG and BP, 1–100 Hz for EMG and ECG, and 1–10 Hz for respiration; CyberAmp 380, Axon instruments/Molecular Devices, Sunnyvale, CA) the analog data were digitized (sampling frequency 200/s) and recorded using SciWorks for Windows software (Datawave Systems, Longmont, CO). Each recording began with a 10 min registration of the baseline activity prior to any injections, which were separated by at least 5 min.

2.3. Experimental protocol

The pipette was stereotaxically positioned at the expected dorsal margin of the PPT region (Paxinos and Watson, 2004) and was advanced ventrally in 100 μm increments. At each site 5–12 nl of L-glutamate was injected. Injection volumes were directly measured using a dissecting microscope (Wild Heerbrugg, model M5) with a calibrated reticule to observe the movement of the fluid meniscus within the pipette barrel. The dose of the glutamate was chosen according to our previous work (Saponjic et al., 2003, 2005, 2006), which showed the effectiveness of this volume and concentration to evoke a prominent respiratory reaction from the PPT. Further, work by Nicholson (1985) suggests that within the first 35 s the effective diffusion radius is limited to approximately 150 μm.

EEG (cortical and pontine), EMG, BP and respiration were registered prior to and after each glutamate injection. An “effective site” was determined by visually evident perturbations in any of these parameters after the glutamate injection. In this case a minimum 5-min interval was provided prior to advancing the pipette. In all animals at least two repeated glutamate injections were made at any effective site to better document the duration and reproducibility of the response. A sham control also was obtained at each effective site by injection 5–12 nl of PBS. The PBS injection was made either prior to or after the second glutamate injection at the effective site. In every experiment, subsequent glutamate injections reproduced the initial response, whereas all PBS injections failed to do so. Although application of the back pressure to the barrel containing O-red dye raises potential concern of alcohol-leakage to the injection site between injections, the reproducibility of the responses after the repeated glutamate injections argues that this effect was not of significant concern to the interpretation of the present observations.

The effective sites were injected with oil O-red dye for histological verification. In some cases the point of the reaction was marked