Neuroscience 313 (2016) 73–82

URODYNAMIC FUNCTION DURING SLEEP-LIKE BRAIN STATES IN URETHANE ANESTHETIZED RATS

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Abstract—The aim was to investigate urodynamic parameters and functional excitability of the periaqueductal gray matter (PAG) during changes in sleep-like brain states in urethane anesthetized rats. Simultaneous recordings of detrusor pressure, external urethral sphincter (EUS) electromyogram (EMG), cortical electroencephalogram (EEG), and single-unit activity in the PAG were made during repeated voiding induced by continuous infusion of saline into the bladder. The EEG cycled between synchronized, high-amplitude slow wave activity (SWA) and desynchronized low-amplitude fast activity similar to slow wave and 'activated' sleep-like brain states. During (SWA, 0.5–1.5 Hz synchronized oscillation of the EEG waveform) voiding became more irregular than in the 'activated' brain state (2–5 Hz low-amplitude desynchronized EEG waveform) and detrusor void pressure threshold, void volume threshold and the duration of bursting activity in the external urethral sphincter EMG were raised. The spontaneous firing rate of 23/52 neurons recorded within the caudal PAG and adjacent tegmentum was linked to the EEG state, with the majority of responsive cells (92%) firing more slowly during SWA. Almost a quarter of the cells recorded (12/52) showed phasic changes in firing rate that were linked to the occurrence of voids. Inhibition ($n = 6$), excitation ($n = 4$) or excitation/inhibition ($n = 2$) was seen. The spontaneous firing rate of 83% of the micturition-responsive cells was sensitive to changes in EEG state. In nine of the 12 responsive cells (75%) the responses were reduced during SWA. We propose that during different sleep-like brain states changes in urodynamic properties occur which may be linked to changing excitability of the micturition circuitry in the periaqueductal gray.
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Key words: micturition, urethane, sleep-like EEG state, periaqueductal gray, rat, bladder urodynamics.

INTRODUCTION

During the sleeping period urine production normally decreases compared to the waking state. This is due in part to the absence of fluid intake, coupled with a nocturnal increase in secretion of antidiuretic hormone. The storage capacity of the bladder also increases due to functional changes, for example in gap junctions in bladder smooth muscle cells, associated with a diurnal change in expression of connexin43 (Negoro et al., 2012, 2013). During a sleeping period humans typically cycle through a number of sleep stages. These are defined by characteristic changes in the electroencephalogram (EEG) waveform, which cycles between low frequency, synchronized high-amplitude activity (slow wave sleep) and high frequency, desynchronized, low-amplitude activity known as desynchronized or rapid eye movement (REM) sleep. Little is known about the functional activity of the central micturition control circuitry during different sleep states and how this might impact on control of bladder function. Rats, like humans, show a diurnal pattern in sleep and wakefulness (although this is reversed with respect to their light–dark cycle) and different sleep stages can be identified according to characteristic patterns of the EEG waveform (Gottesmann, 1992). Under urethane anesthesia rats show spontaneous changes in the EEG that resemble slow wave and REM brain sleep states seen in unanesthetized animals (Clement et al., 2008). It is thought that urethane promotes unconsciousness by exploiting the brain mechanisms involved in natural sleep, unlike other general anesthetics that induce a pharmacological unitary sleep-like slow wave EEG state (Clement et al., 2008). The urethane-anesthetized preparation has therefore been used as a model to investigate central mechanisms underlying sleep. Another feature of urethane anesthesia is that the micturition reflex is preserved, unlike other commonly used anesthetics (Yoshiyama et al., 2013). Thus this preparation offers the advantage of investigating urodynamics and basic central control mechanisms of micturition during different sleep-like brain states more easily than in chronically instrumented, unanesthetized animals. In the present study we used this preparation as a model system to facilitate mechanistic studies into control of micturition in different sleep-like brain states.

Micturition is dependent on the functional integrity of a spino-midbrain-spinal loop, which relays in the caudal ventrolateral periaqueductal gray matter (vlPAG) (Stone et al., 2011). The vlPAG is thought to act as a neural switch which, in the conscious animal, is under the control

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Abbreviations: EEG, electroencephalogram; EMG, electromyogram; EUS, external urethral sphincter; PAG, periaqueductal gray matter; REM, rapid eye movement; SEM, standard error of the mean; STFT, short-term Fourier transform; SWA, slow wave activity; vlPAG, ventrolateral periaqueductal gray matter.

http://dx.doi.org/10.1016/j.neuroscience.2015.11.027
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of higher centers that can switch the circuitry between storage and voiding modes (see de Groat et al., 2015 for a recent comprehensive review). We carried out continuous cystometry while simultaneously making urodynamic measurements and monitoring activity in the micturition control circuitry in the PAG.

EXPERIMENTAL PROCEDURES

The study conforms to the national guidelines for the care and use of animals and was carried out under the authority of UK Home Office Project Licence PPL30/3200.

29 Male Wistar rats (270–450 g) were used for the study. They were anesthetized with urethane (1.4 g kg^{-1} i.p.) and instrumented to record cortical EEG, femoral arterial blood pressure, heart rate and respiratory airflow. The trachea was cannulated to maintain a patent airway and a femoral venous catheter was inserted for infusion of fluids; rectal temperature was maintained at 37 °C by a homeothermic blanket system (Harvard Apparatus, Holliston, Massachusetts, USA). Following the prepar atory surgery, animals were positioned in a stereotaxic frame and a craniotomy was performed to give access to the left periaqueductal gray matter (PAG). The lower body was rotated to allow access to the abdomen. Following a midline laparotomy to expose the bladder, a 25-G needle tip attached to a length of saline-filled polythene tubing was inserted through the bladder dome. A T-piece in the line enabled simultaneous recording of intravesical pressure while infusing saline into the bladder. Two insulated needle electrodes were inserted into the external urethral sphincter (EUS) muscle to record electromyogram (EMG) activity. The time and volume of voids evoked by infusing saline into the bladder was measured either by a drop counter positioned to register the passage of drops of urine falling from the penis or by collecting urine in a beaker placed on the platform of an electronic balance with output to the data acquisition system. All data were captured and displayed using a PowerLab 8SP running Chart v5 software, and exported to Matlab R1013a for further analysis.

Neuronal recording

Single units. In six rats activity of single neurons within the vlPAG and surrounding tegmentum during voiding was recorded with a single-channel electrode. The dura mater was removed from the cortical surface and a parylene coated tungsten microelectrode (A-M Systems Inc, Carlsborg, Washington, USA) was lowered into the PAG. Single-unit activity was amplified (5000×) and bandpass filtered (300–5000 Hz) using a Neurolog system (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). If necessary, residual electrical interference was eliminated using a Hum Bug device (Quest Scientific, North Vancouver, British Columbia, Canada). Activity was digitally sampled at 20 kHz and sorted offline by a computer running Spike2 (v7) software.

Multichannel recording. In 14 animals, neuronal activity in the PAG was sampled using silicon probes (10-mm shank length, 50-μm probe thickness, width 1.25–1.48 mm with a bevelled end, Poly3 and Edge designs) with 10–32 active channels (NeuroNexus, Ann Arbor, Michigan, USA). Activity was amplified and digitized with a PZ2 Preamplifier (Tucker Davis Technologies, Alachua, Florida, USA) and bandpass filtered (300–5000 Hz) using a RZ5D processor (Tucker Davis Technologies, USA). Spike sorting was carried out offline using KlustKwik (Ken Harris, Rutgers University, Newark, NJ) with evaluation and correction as required using MClust (A. David Redish, University of Minnesota, Minneapolis MN). In all experiments microelectrodes and silicon probes were coated with DiI (50 mg ml^{-1} in ethanol, Biotium, Hayward, California, USA) and allowed to dry prior to insertion into the brain. Fluorescent electrode tracks were later located in histological material.

Experimental protocol

Once the surgical preparation was completed, a period of 30 min was allowed before starting continuous infusion of saline into the bladder (6 ml h^{-1}). Once repeated cycles of filling and voiding had established, the recording electrode was inserted into the brain at approximately 7.3–7.8 mm caudal and 0.8–1.2 mm lateral to the bregma. To target the PAG the electrode was angled 0–10° caudally and 0–5° medially and advanced so the tip was 5 mm below the cortical surface. The electrode was then moved slowly within the target area until single unit spiking activity was clearly distinguishable on the single-channel electrode or on at least one site on the multichannel probes. Continuous recordings of unit activity were carried out over periods of up to 3 h.

Analysis of EEG waveform

For each animal, analysis of urodynamic and EEG state was conducted over a 2-h period after the micturition reflex had been reliably established. The EEG state was quantified by measuring the power in the 0.5–1.5-Hz bandwidth of the EEG signal using a short term Fourier transform (STFT), with a window length of 2.025 s, and a hop size of 0.5 s. The 'slow wave power' was obtained by calculating the mean power in the 0.5–1.5-Hz band and smoothing using a moving average over 25 s (Fig 1). Slow wave power values at the time of voiding were divided into upper and lower quartiles and used to assign voids to 'slow wave activity (SWA)' and 'Cortical Activation' brain states, respectively.

Analysis of urodynamic data

For each void, the following measurements were obtained:

Void pressure threshold. The pressure within the bladder at the end of the filling phase, prior to the sharp pressure rise at the onset of a void.

Void volume and voiding volume threshold. The volume of fluid expelled per void was assessed from the weight of the fluid expelled, assuming a specific gravity

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