J Physiol Sci (2018) 68:567–577
https://doi.org/10.1007/s12576-017-0562-8

ORIGINAL PAPER

Assessment of the septal area neuronal activity during penile erections in rapid eye movement sleep and waking in the rats

Kamalesh K. Gulia[1,2] · Yukihiko Kayama[2] · Yoshimasa Koyama[3]

Received: 22 April 2017 / Accepted: 26 July 2017 / Published online: 2 August 2017

Abstract To understand the central mechanism of penile erections during rapid eye movement (REM) sleep and waking, single units were recorded from the septal area in un-anesthetized head-restrained rats simultaneous with erections. Erectile events were assessed by pressure in the bulb of the corpus spongiosum of the penis and bulbospongiosus-muscle activity. Of 143 recorded neurons, 36% showed increased activity (E-type) and 24% decreased activity (I-type) during different phases of erection in REM sleep, while 10% were E-type and 35% were I-type during erections in waking. Most E-type neurons were recorded from the dorsal and intermediate part of lateral septum, whereas I-type neurons were from the medial septum. The findings illustrate the extensive network of various types of neurons in the septal area that fire in concert in relation to erection during REM sleep and waking. This study provides a unique prospective of the septal area for perpetuation of erectile circuitry during sleep.

✉ Kamalesh K. Gulia
kkguliak@hotmail.com

✉ Yoshimasa Koyama
koyamay@sss.fukushima-u.ac.jp

[1] Present Address: Division of Sleep Research, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala 695012, India

[2] Department of Neurophysiology, Fukushima Medical University School of Medicine, 1-Hikari-ga-oka, Fukushima 960-1295, Japan

[3] Present Address: Department of Science and Technology, Fukushima University, 1-Kanaya-gawa, Fukushima 960-1296, Japan

Keywords Lateral septum · Penile erection · REM sleep · Waking · Neural activity · Medial septum

Introduction

The septal area is a limbic structure in the forebrain that regulates various components of male sexual behavior and sleep [1–6]. In the 1960s, MacLean and Ploog reported that the septum was potentially the brain region for generating penile erections during the waking state [7]. In subsequent years, erections in sleep were documented to occur during the rapid eye movement (REM) phase under normal conditions in humans and other mammalian species [8–11]. For many years, however, no attempts were made to explore whether the septum played any role in erections occurring in sleep. We recently demonstrated that this region controls erections during REM sleep in rats [12, 13]. When various sub-regions were stimulated electrically, erections during REM sleep were evoked from the dorsal and intermediate parts of the lateral septum (LS), whereas the ventral part evoked erections during waking, and the medial septum (MS) did not elicit any erectile response [13].

Within the septal area, the MS neurons have been extensively studied for their role in the hippocampal theta rhythm during waking and REM sleep, but no reports have characterized the neuronal properties of the lateral or medial neurons in the regulation of erections in these two states. In the central nervous system, there is overlap of the circuitries for multiple functions, including sleep and sex, at various levels, which suggests multimodal interaction operating towards homeostasis [14]. It has been observed that REM sleep deprivation in rats produces enhanced male sexual behavior and genital reflexes [15, 16]. Although in

clinical practice, the differential diagnosis between organic and psychogenic impotence in humans is made by monitoring erections during sleep, the neural basis for psychogenic impotence remains unidentified. We previously reported that both excitatory and inhibitory mechanisms for erection are present within the septal area, as different strengths of stimulating current evoked erectile response or inhibition from the same locus [13]. However, information on the putative categories of erection-related neurons operating specifically during different states of vigilance is lacking. To address this question, we proposed to record single neurons in behaving animals under sleep and waking cycles. Therefore, the present study was aimed to: (1) record single units at the time of erection during waking and REM sleep from the septal area in un-anesthetized head-restrained rats; and (2) elucidate the distribution of these neurons in this area. Penile erection was assessed telemetrically by the pressure transducer technique and by electromyography (EMG) of the bulbospongiosus (BS) muscle in order to draw a better temporal correlation between septal neurons and changes in the corpus spongiosum of penis (CSP) pressure.

Materials and methods

Animals

The study was conducted in 14 adult Sprague–Dawley rats (270–375 g) obtained from Japan SLC Inc. (Hamamatsu, Japan), and maintained on a 12-h light/dark cycle at an ambient temperature of 22.0 ± 0.1 °C with food and water given ad libitum.

Surgical procedures

Surgery was performed under sodium pentobarbital anesthesia (50 mg/kg, i.p., supplemented when necessary). Animals were pretreated with atropine (0.4 mg/kg, i.p.) and lidocaine (2%) was applied at the site of incision. Penile erection was assessed by pressure in the bulb of the CSP and the EMG of the BS muscle, as described elsewhere [10, 13]. Briefly, the pressure transducer (TA11PA-C40, Data Sciences International, St. Paul, USA) was placed subcutaneously and sutured to the abdominal muscles. The tip of the transducer catheter was inserted into the bulb of the CSP through an incision made by a needle on the penile shaft, and the incised area on the penile tissue was sealed using one or two drops of veterinary glue (Vetbond). To prevent displacement of the catheter, a small stitch secured it to the fascia overlying the penile shaft. The CSP pressure signal was sent telemetrically to the receiver (RPC-1, Data Sciences International, St. Paul, USA) kept below the animals. To record the BS muscle EMG, a pair of insulated multi-stranded stainless steel wires with bare tips was inserted into the BS muscle, and the free end was passed subcutaneously over the incised skin on the skull to connect them to the socket. After implantation of electrodes for BS EMG and catheter for CSP monitoring, the skin on scrotum was sutured. The heads of the animals were fixed in a stereotaxic instrument and the skin was excised to expose the skull. For electroencephalogram (EEG) recording, stainless steel screws, 1.4 mm in diameter, were screwed into the skull overlying the frontal and parietal cortices. Three or four additional screws were placed into the skull as anchors. Two insulated multi-stranded stainless wires with bare tips inserted into the neck muscle were used as EMG electrodes. Electrodes for EEG and neck EMG activity were implanted for assessment of the vigilant state. The free end of the neck EMG was taken subcutaneously over the skull for connection to the socket. The area where a piece of skull was removed just above the recording area was maintained centrally with a thin layer of dental cement for easy access during experiments. For chronic recording under un-anesthetized head restraining conditions, a U-plate plexiglass plate (width, 20 mm; length, 30 mm; thickness, 5 mm) was placed horizontally and fixed to the skull using dental acrylic cement. During recording, the U-plate was used to immobilize the head painlessly in a stereotaxic position by inserting four holes carved on both sides of the frame to the earbars of the stereotaxic instrument. Gentamycin ointment (0.1%) was applied at the incision sites, and after surgery, penicillin (60 mg) and analgesic voveron (0.5 mg/b wt, i.m.) were given for 4 or 5 days.

Electrophysiology

After a post-operative recovery period of at least 7 days, rats were habituated to a recording chamber, a plastic case sized to their body, for 3–5 h a day with their heads fixed to the stereotaxic frame using a U-shaped plate on their head. This was repeated for 2–3 days until the experiment began.

In order to facilitate REM sleep, rats were deprived of sleep on the night before the experiment for 10 h in a slowly rotating wheel (diameter, 37 mm; width, 10 cm; 1.2 rpm) with easy access to food and water. After sleep deprivation, rats were allowed to rest for 2–3 h in their cages. For recording, rats were fixed to the stereotaxic frame with the U-shaped plates on their heads, and their bodies placed in the plastic box. Under these conditions, animals could move their bodies and limbs for postural adjustments.

Throughout the experimental period of 7–8 h, animals generally remained calm and showed no signs of discomfort. A small piece of skull overlying the septum was

trephined. Through a small slit in the dura made under local anaesthesia, single unit discharges were recorded extracellularly with a glass pipette electrode filled with 0.5 M sodium acetate containing 2% Pontamine sky blue (DC resistance, 15–20 MΩ). The electrode was introduced stereotaxically into the septal area either angularly (10–18°) or vertically to target the MS or the LS, respectively, without rupturing the mid-sagittal sinus, using coordinates from Paxinos and Watson [17]. The single units were amplified using conventional methods with a band pass of 50–10,000 Hz. EEG and EMG were amplified and filtered (0.53–120 and 160–1000 Hz, respectively) through conventional amplifiers (AB651J, Nihon Kohden, Japan) and with data on CSP pressure, they were fed to the signal acquisition system (CED1401, Cambridge Electronic Design, Cambridge, UK). Units were recorded simultaneously with EEG and EMG (neck) for assessment of vigilant state, CSP pressure and BS EMG for penile erections.

Histological confirmation of recording sites

Recording sites were verified histologically by iontophoretically ejecting Pontamine sky blue with a cathodal current of 20 μA for 3 min. Four to eight spots were made in each animal and positions of neurons were reconstructed from these spots. At termination of the experiment, the animal was deeply anesthetized with pentobarbital, perfused transcardially with 300 ml of physiological saline followed by same amount of 10% formalin. The brain was removed and post-fixed in the same solution overnight, and was immersed in a 30% sucrose solution. Serial sections (50 μm) in the coronal plane were then cut on a freezing microtome and were stained with neutral red. A photomicrograph showing the tip of the electrode (asterisk), which was marked by ejecting pontamine sky blue, is shown in Fig. 1.

Data analysis

Neurons that could be recorded for at least two states of vigilance and one episode of erection were selected for analysis. Spike-2 software was used for analyzing the neuron firing rate. The mean firing rate (f) of neurons during waking (W), slow wave sleep (SWS) and REM sleep were calculated in order to assess the relationship with the vigilant state. Neurons were classified into R, W, S, W/R and N types based on their most active stage of sleep or waking. R type neurons were those most active during REM sleep f(REM) > f(W) and f(SWS); in these neurons f(W) was lower than or about same as f(SWS) (± 10%) or, in some neurons, was higher than f(SWS), but had a low value (<1.4 Hz). Neurons were classified as W/R type if both f(W) and f(REM) were higher than f(SWS); those whose f(W) and/or f(REM) had low values (<1.4 Hz) were not included in this group, even if they were higher than f(SWS). Neurons most active during W were classified as W type; f(REM) was lowest or lower than 1.4 Hz. S type neurons were those that fired faster during SWS than during W and REM sleep. Neurons showing no clear changes in firing rate based on sleep and waking were grouped as N type.

Erection consisted of a slow rise in CSP pressure from the flaccid baseline (BL), followed by a number of sharp pressure peaks with at least one supra-systolic pressure peak resulting from the BS muscle activity. The start of erection was marked when the CSP pressure reached BL + 30 mmHg. The end of the event was defined as when the CSP pressure fell below BL + 30 mmHg for more than 15 s. The period from the start to the end of erection was erection period (EP). A twenty-second period prior to the EP was defined as pre-EP, and a 20 s period after the EP was taken as post-EP. Non-erection period (non-EP) was defined as the rest of the period of REM sleep or of waking.

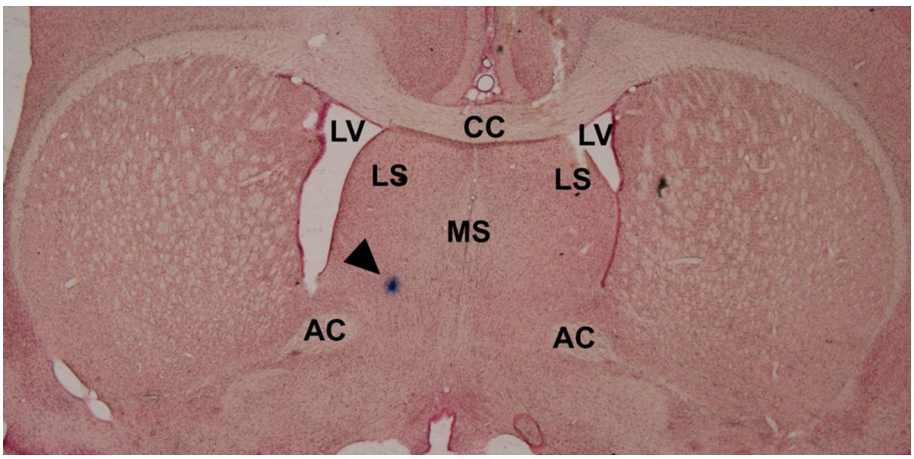

Fig. 1 Photomicrograph of coronal section showing recording site in the septum (*triangle*) marked with Pontamine Sky blue. *AC* anterior commissure, *CC* corpus callosum, *LS* lateral septum, *LV* lateral ventricle, *MS* medial septum

Quantitative criterion to define various neuron types in relation to erection

Erection-related neurons were grouped as excitatory (E) or inhibitory (I) based on their activity in relation to CSP pressure changes during erection. The neuron was defined as E-type if the firing rate during pre-EP or EP became more than 40% higher than that during non-EP. It was defined as I-type if the firing rate during pre-EP or EP decreased to less than 60% of that during non-EP. To identify whether the neuron showed tonic or phasic firing during a particular state, the coefficient of variance (vc) value was calculated. The neuron was defined as tonic if it had a vc value of less than 1.3 and phasic when the vc was more than 1.3. The probability of occurrence of the E or I neurons during waking or REM sleep were compared using Fisher's exact probability test. Differences were considered to be significant at $p < 0.05$.

Table 1 Number of neurons related to erection during REM sleep and waking

Erections during REM sleep				Erection during waking		
Type	Sub-type	*n*	Total	Sub-type	*n*	Total
E	E1	14	36##	E1	1	6
	E2	17		E2	3	
	E3	5		E3	2	
I	I1	12	24	I1	7	21***
	I2	9		I2	10	
	I3	3		I3	4	
NR		40	40		33	33
All types	100			60		

E excitatory, *I* inhibitory, *NR* not related to erection

Comparison of neuron types between REM sleep and waking state is denoted by#, and comparison of neuron types within one state is shown by*

*** $p < 0.001$

$p < 0.01$

Results

A total of 143 neurons were recorded with penile erection during REM sleep and/or during waking. Of these, 100 were recorded when erection appeared during REM sleep and 60 were recorded with erection during waking. Seventeen neurons were recorded when erection occurred during both states.

Characterization of neurons in relation to erection

Of 100 neurons that were recorded with erection during REM sleep, 36 neurons were E-type, 24 were I-type and 40 were not related to erection (Table 1). Of 60 neurons that were recorded with erection during waking, 6 were E-type, 21 were I-type and the remaining 33 were NR type. Excitatory neurons were grouped into E1, E2 and E3, in which the highest firing rate appeared during pre-EP, EP and post-EP respectively (Fig. 2). A typical example of an E1 neuron that became most active prior to the onset of erection is shown in Fig. 3. This neuron started to increase firing 17 s before erection, remained active at 6.0 Hz during pre-EP, during the first 10 s of erection, and then almost ceased firing during the rest of EP, making the overall mean firing rate during EP 2.8 Hz. The firing of the neuron decreased to 0.79 Hz during post-EP, which was similar to the level during non-EP (0.84 Hz).

Of 15 E1 neurons, firing of 8 (including this neuron) during EP was maintained at more than 40% higher than that during non-EP, while in the remaining seven, firing during EP decreased to non-EP levels (Fig. 2). In E2 neurons, firing started to increase 3.5 s before erection and was maintained at 6.4 Hz during EP (Fig. 4). Of 20 E2 neurons, 10 neurons showed increased in firing during Pre-EP at 40% higher than during non-EP and further peaking during EP, while in remaining half, increases in firing were seen only during EP. In E3 neurons, in addition to increased firing (more than 40% higher than that during non-EP) during EP, firing further increased during post-EP (Figs. 2, 5). It was also observed that a majority of the E neurons (66.6%) displayed tonic firing during erection, while in the remaining neurons, some showed phasic firing during individual supra-systolic peaks of erectile event.

Inhibitory neurons were similarly classified into three types, I1, I2 and I3, in which the lowest firing rate appeared during pre-EP, EP and post-EP, respectively (Fig. 2). I1 neurons firing decreased immediately prior to erection and increased during EP, reaching the level seen during non-EP (Fig. 6). In 10 of 19 I1 neurons, the suppression of firing was restricted to pre-EP, while in others ($n = 9$), firing during EP decreased to less than 60% of that during non-EP (Fig. 2). I2 neurons showed firing that almost completely stopped during EP, and recovered quickly to non-EP levels during post-EP (Fig. 7). In the majority of I2 neurons (17 of 19, 89.5%), the decrease in firing appeared only during EP, while in the remaining neurons ($n = 2$, 10.5%), the decrease in firing started during pre-EP, becoming less than 60% of non-EP values (Fig. 2). I3 neurons showed a decrease in firing during EP, and continued to be suppressed, even during post-EP (Fig. 8).

In 17 neurons, neuronal activity was recorded with erection during two stages (Table 2). Among these, three neurons were specifically related to erection during REM sleep; one was excitatory (E1 type) and two were inhibitory

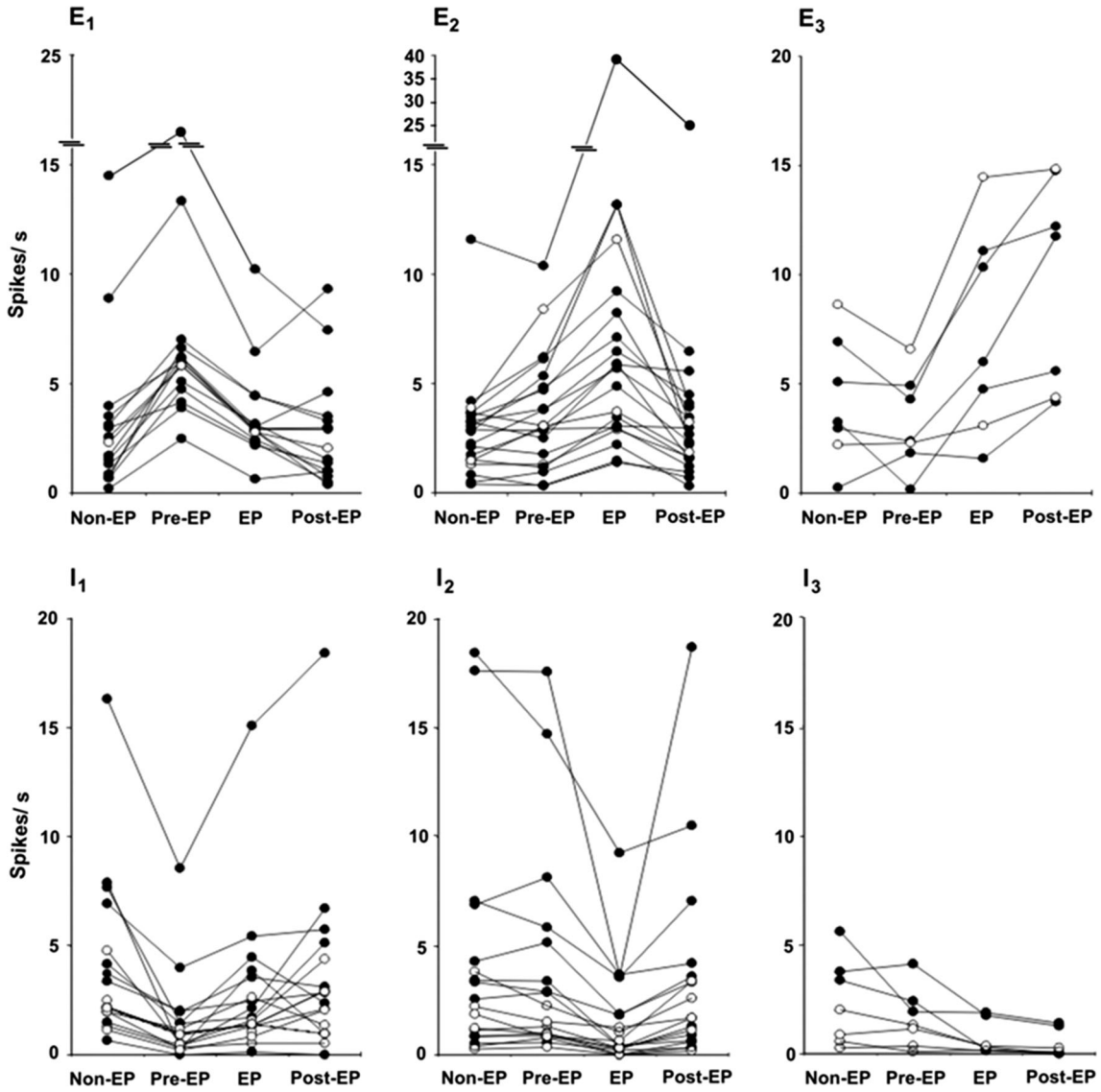

Fig. 2 Changes in mean firing rate of erection-related neurons at different phases of erection. *E1*, *E2* and *E3* subclasses of E-type neurons, *I1*, *I2* and *I3* subclasses of I-type neurons, *closed circles* REM sleep, *open circles* Waking, *EP* erection period

(I1 type). Six neurons showed the same activity pattern in erection during both stages (one was E type, 5 were I type). Five neurons showed different activity patterns in erection during REM sleep and during waking (four were E-type during REM sleep and I-type during waking, and one was I-type during REM sleep and E-type during waking). The neuron in which firing increased before erection during REM sleep (E1 type) and decreased during and after erection in waking (I2 type) is shown in Fig. 9.

Table 1 summarizes the number of neuron types in relation to erection during waking and REM sleep, indicating that the ratio of E type neurons was significantly larger during REM sleep (36/100) than during waking (6/60) (Fisher's Exact test, $p = 0.003$), and that during waking, the ratio of I type neurons (21/60) was significantly larger than that of E type neurons (6/60) ($p = 0.001$).

Classification of erection-related neurons according to sleep–wake states

Of 36 E-type neurons recorded with erection during REM sleep, the majority (33 neurons, 91.6%) showed higher activity during REM sleep (REM active); 28 (77.7%) were R type and 5 (13.8%) were W/R type (Table 3). Similarly, of 24 I type neurons, 22 (16 R type and 6 W/R type, 91.6%) were also REM active. With regard to neurons recorded with erection during waking, all E-type neurons

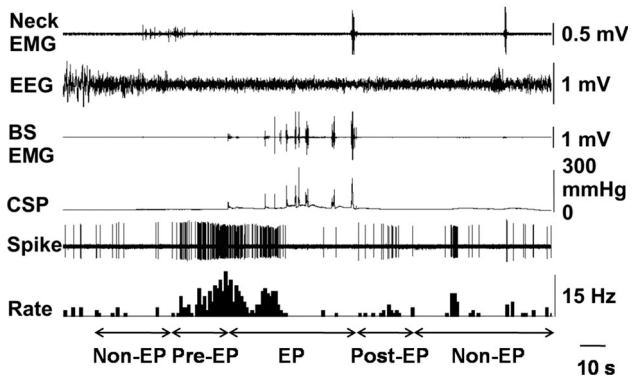

Fig. 3 Illustration of E1 neuron firing in relation to erection during REM sleep. The neuron showed an increase in firing prior to the onset of erection, and this tonic firing continued to the early-phase erection period. *BS* bulbospogiosus, *CSP* corpus spongiosum of penis, *EEG* electroencephalogram; *EMG* electromyogram, *Rate* mean firing rate, *Spike* neuronal firing

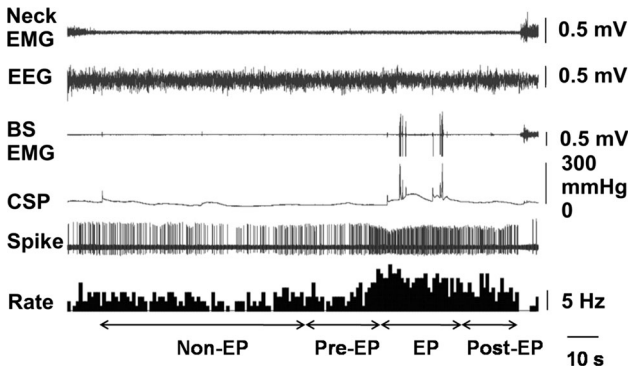

Fig. 4 Illustration of E2 neuron firing in relation to erection during REM sleep. The neuron showed a tonic increase in firing prior to the onset of erection, and this was maintained throughout the erection period. *BS* bulbospogiosus, *CSP* corpus spongiosum of penis, *EEG* electroencephalogram, *EMG* electromyogram, *Rate* mean firing rate, *Spike* neuronal firing

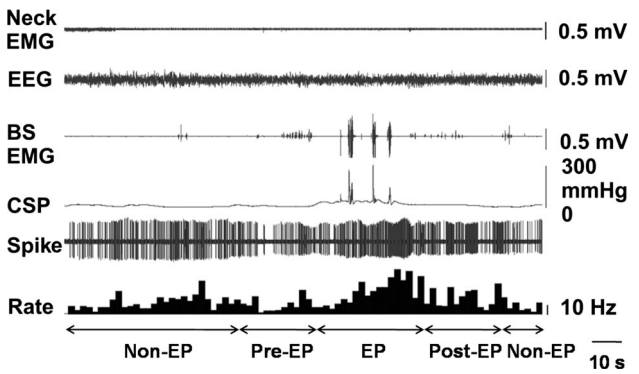

Fig. 5 Illustration of E3 neuron firing in relation to erection during REM sleep. The neuron showed maximum firing in the post-erection period. *BS* bulbospogiosus, *CSP* corpus spongiosum of penis, *EEG* electroencephalogram, *EMG* electromyogram, *Rate* mean firing rate, *Spike* neuronal firing

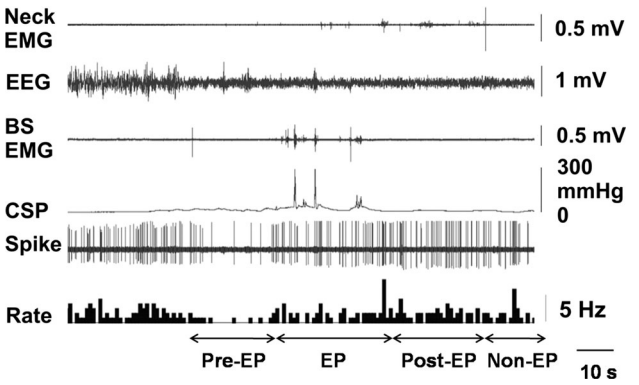

Fig. 6 Illustration of I1 neuron firing in relation to penile erection during REM sleep. The neuron showed a decrease in firing prior to erection

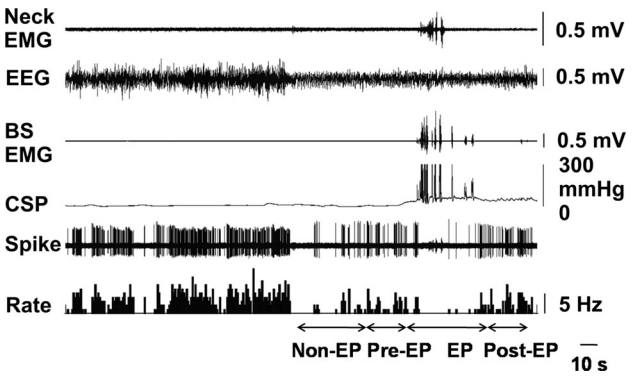

Fig. 7 Illustration of I2 neuron firing in relation to erection during waking. The neuron showed a decrease in firing during the erection period

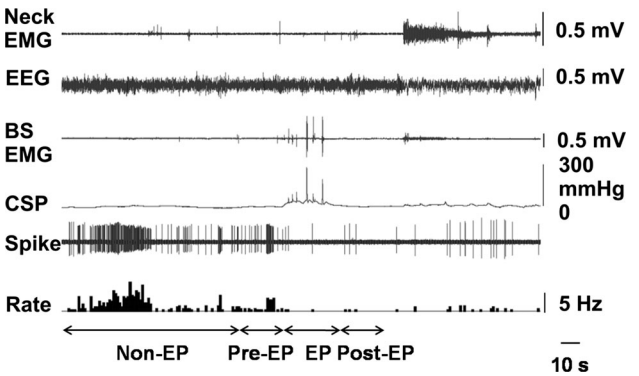

Fig. 8 Illustration of I3 neuron firing in relation to erection during REM sleep. The neuron showed a decrease in firing during the erection period and continued to be suppressed during the post-erection period

were waking active (2 were W type and 4 were W/R type). The majority of I-type neurons (18/21; 85.7%) were REM active (15 R type and 3 W/R type).

Table 2 Number of erection-related neurons recorded with erection during both REM sleep and waking

Neuron types in relation to erection during		Numbers of neurons (*n*)
REM sleep	Waking	
E	NR	1
I	NR	2
E	E	1
I	I	5
E	I	4
I	E	1
NR	NR	3

E excitatory, *I* inhibitory, *NR* no relation, *n* number of neurons

Distribution of erection related neurons in the septal area

The locations of the recorded neurons are shown in the coronal sections of the forebrain at four levels in the anterior–posterior region (Fig. 10). During both REM sleep and waking, E type neurons were largely recorded from the LS (46.4%) during REM sleep and 13.6% during waking), but not at all from the MS (Table 4). On the other hand, higher percentages of I-type neurons were recorded from the MS (60%) during REM sleep, and 62.5% during waking than from the LS (21.7%) during REM sleep and 34.1% during waking.

Within the LS, the majority of E-type neurons were recorded from LSd and LSi, (11/16, 69 and 19/37, 51%, respectively) during REM sleep, but not during waking (0/9, 0 and 4/26, 15%, respectively), as shown in Table 4. Higher percentages of I type neurons were recorded in the LSv both during REM sleep (6/16, 38%) and waking (5/9, 56%). Few erection-related neurons were recorded from the diagonal band (DB) and adjoining areas (Table 4).

Discussion

The present study documents, for the first time, the firing properties of erection-related neurons in the septal area recorded during different states of vigilance, and highlight

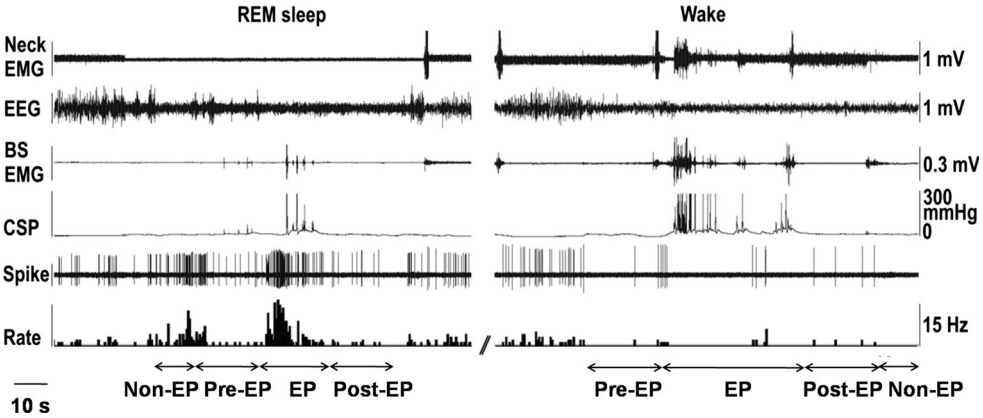

Fig. 9 Firing of a neuron recorded during both states, REM sleep and waking. The neuron is specifically active in relation to erection during REM sleep, showing tonic firing prior to erection during REM sleep, but did not show such firing in relation to erection during waking

Table 3 Number of erection-related neurons classified according to relationship with sleep/wakefulness

Neuron type in relation to PE during REM sleep	Neuron type in relation to sleep–wake stages					
	W	S	R	W/R	N	Total
E	1	0	28	5	2	36
I	1	1	16	6	0	24
NR	3	3	20	10	4	40
Total	5	4	64	21	6	100
Neuron type in relation to PE during waking	Neuron type in relation to sleep-wake stages					
	W	S	R	W/R	N	Total
E	2	0	0	4	0	6
I	0	3	15	3	0	21
NR	3	3	16	7	4	33
Total	5	6	31	14	4	60

E excitatory, *I* inhibitory, *N* not related to sleep/wakefulness, *NR* not related to erection, *R* REM sleep related, *S* slow wave sleep related, *W* wake related, *W/R* wake and REM sleep related

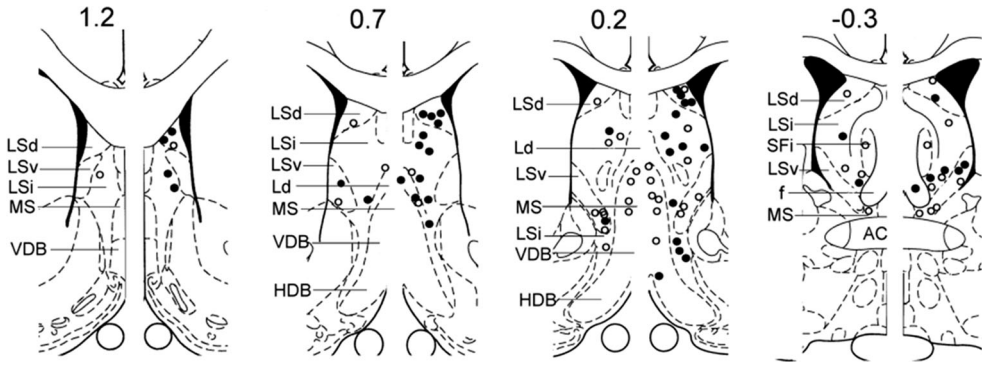

Fig. 10 Histological representation of erection-related neurons plotted on diagrams of serial coronal sections around the septal area. Left hemisection shows neurons recorded during waking; Right hemisection shows neurons recorded during REM sleep; • Neurons excitatory to erection; o Neurons with reduced firing during erection. Numbers above each section indicate distance from the bregma. *AC* anterior commissure, *f* fornix, *HDB* horizontal limb of diagonal band of Broca, *Ld* lambdoid septal zone, *LSd*, *LSi* and *LSv* indicate dorsal, intermediate and ventral parts of the lateral septum, respectively, *MS* medial septum, *SFi* septofimbrial nucleus, *VDB* vertical limb of diagonal band of Broca

Table 4 Number and ratio of erection-related neurons in the septal area

Area	REM sleep				Walking			
	E	I	N	Total	E	I	N	Total
LSd	11 (69.8)	3 (19.7)	2 (12.5)	16	0 (0.0)	3 (33.3)	6 (66.7)	9
LSi	19 (51.4)	6 (16.2)	12 (32.4)	37	4 (15.4)	7 (26.9)	15 (57.7)	26
LSv	2 (12.5)	6 (37.5)	8 (50.0)	16	2 (22.2)	5 (55.6)	2 (22.2)	9
LS	32 (46.4)	15 (21.7)	22 (31.9)	69	6 (13.6)	15 (34.1)	23 (52.3)	44
MS	0 (0.0)	6 (60.0)	4 (40.0)	10	0 (0.0)	5 (62.5)	3 (37.5)	8
SFi/f/Ld	3 (42.8)	2 (28.6)	2 (28.6)	7	0 (0.0)	1 (33.3)	2 (66.7)	3
DB	1 (7.1)	1 (7.1)	12 (85.8)	14	0 (0.0)	0 (0.0)	5 (100.0)	5
								60
				100				

The values represent number of neurons in each area and the ratio of each type of neuron to recorded neurons in the corresponding area (in bracket)

E excitatory, *I* inhibitory, *N* no relation, *LSd LSi* LSv dorsal, intermediate and ventral part of the lateral septum respectively, *MS* medial septum *DB* diagonal band of Broca, *SFi/f/Ld* septofimbrial nucleus/fornix/lambdoid septal zone

the probable neural mechanisms for penile erections operating in this area. In addition, the functional significance of such neurons in erectile responses is discussed.

Presence of both E- and I-type neurons in relation to erection during waking and REM sleep suggests dynamic erectile machinery that yields activation or suppression of erections. A considerable number of E-neurons during REM sleep in comparison to erections during waking indicate an erectile mechanism predominantly active during REM sleep in the absence of external stimuli. Majority of the E-type neurons (27/35, 77%) were REM sleep-specific (R type), and 65% (15/23) of I-type neurons were also R type, suggesting that one group of R type neurons has an excitatory role, whereas activation of another pool results in inhibition of erections during REM sleep. In erections during waking, a relatively large number of I-type neurons were R type, indicating that these neurons possibly had inhibitory inputs.

Successful recording of activity in 17 neurons in two stages during erection indicated that a population of septal neurons is specific to erections during REM sleep (one was E-type, and two were I-type). It is also true that another

population is involved in erections in general; both during REM sleep and waking (one was E type, and 5 were I type). E1 neurons that increased their firing during pre-EP appear to play an important role in initiating erections. The tonic increase in their firing may be important in building the slow rise in CSP pressure before erection. E2 neurons that increased firing during EP would be involved in subsequent full achievement of the erectile event. The initial increase in firing rate in some E2 neurons during pre-EP would be significant for initiating the slow CSP rise similarly to E1 neurons. These neurons would thus be participating in both initiating and establishing a full erection. E3 neurons showing increased peak firing after EP would be involved in processing the sensory information generated during erectile events. Association of the septum with pleasure and sexual excitement is evident from the intracranial self-stimulation studies in rats [18] and humans [19]. Recent functional magnetic resonance imaging studies have also depicted activation in the septum after ejaculation in healthy human subjects [20]. The septum is connected with other pleasure/reward areas, including the medial forebrain bundle and ventral tegmental area [21, 22]. Thus, activation of the E3 type neurons may also contribute to such pleasure circuits.

Some of the phasic firing neurons in which firing was closely related to sharp CSP peaks may be responsible for driving the CSP pressure peaks during erection. However, phasic firing was not related to erection in the majority of phasic firing neurons. These neurons would primarily receive sensory feedback rather than driving individual suprasystolic peaks.

Decreased firing in I1, I2 and I3 type neurons at different phases of erection suggests the presence of an inhibitory network in vicinity. Release from inhibition (disinhibition) of these neurons in appropriate order would allow the smooth occurrence of erection. It is also probable that I2 and I3 type neurons receive sensory inhibition from erection.

We also found that 88% (51/58) of the NR neurons showing no relationship to erection were sleep related (R, WR, S, or W type) during REM sleep or waking, thus indicating that one group of NR neurons regulates only sleep–wakefulness. Localization of E-type neurons, particularly in the LSd and LSi, clearly demonstrated functional segregation within the LS for erection during REM sleep. This notion supports our previous findings in which erections were electrically evoked only from the LSd and LSi but not from the LSv [13]. Neuroanatomical studies have revealed the ascending projections from the mesopontine tegmental REM sleep generating areas; the pedunculopontine and laterodorsal tegmentum nuclei to the LSd and the LSi sparing the LSv [23–25]. The LSi sends projections to the lateral proptic area [26], the area known to regulate erections during REM sleep [27]. The LSD/LSi would thus be a relay center mediating excitatory drive for erection during REM sleep from the mesopontine tegmental area to the lateral preoptic area. An abundance of I-type neurons in the MS during both states suggests the presence of inhibitory networks in this area for erectile response. This finding supports earlier reports, in which lesions involving MS neurons produced activation in copulatory behavior and erection [1, 5, 12]. The septum is richly innervated by noradrenergic fibers arising from the locus coeruleus and caudal brainstem [28, 29]. The noradrenergic system in the LS has a stimulatory role in male sexual behavior in rats [2]; it is possible that a group of E-type neurons during waking are activated by NE. The role of glutamate cannot be ruled out, as the LS receives massive glutamergic fiber inputs from the hippocampus via the fornix [30], an area that participates in erection [31]. The dorsal and intermediate parts of the LS receive cholinergic afferents from the LDT [32], indicating a plausible role of acetylcholine in erection during REM sleep. The MS is rich in GABA neurons, and sends GABAergic fibers to the hippocampus [33]. In light of these findings, it is possible that in the MS, GABA may be a key neurotransmitter in the I neurons.

Erection during waking is regulated by a number of brain areas including the amygdala, bed nucleus of the stria terminalis, hippocampus, medial preoptic area, olfactory bulbs, paraventricular nucleus and septum [13, 31, 34–38]. The lateral preoptic area regulates the erections appearing only during REM sleep and not during waking [27]. We reported in a single neuronal recording study that the mesopontine tegmental area has a role in erection during REM sleep [39], and this was further confirmed by an electrical stimulation study [40]. The present study supports the notion that the septum is involved in erection during REM sleep. The potential connections between these areas (septo-preoptic-mesopontine system) for erectile function need to be explored further.

The function of erection during REM sleep remains a matter of speculation, but it may be helpful in securing a successful erection during waking. In humans, erection patterns during REM sleep are so regular that they are clinically used to differentiate organic and psychogenic impotency. Erections have also been reported during the REM sleep in human fetus and infants [41, 42]. REM sleep deprivation in the neonatal period produces deficits in sexual behavior in later life stages in mammalian species [43, 44], while REM sleep deprivation in adult rats activates male sexual behaviour [15]. Erections during REM sleep may have a role in the development of a normal erectile circuit. It is also possible that hyper-activity of I-type neurons or hypo-activity of E-type neurons results in ineffective occurrence or suppression of erection. As the septum is also involved in the control of emotion and

motivation [26, 45], it may be a potential target for erectile dysfunction of psychogenic origin.

This is a unique study providing insight into the functional prospective of the septal neurons in relation to both sleep and erection. The findings illustrate the extensive network of various types of neurons in the septal area that fire in a concerted manner in correlation to complex physiological phenomena erection during different vigilant states of sleep and waking. The present knowledge will contribute to understanding the interrelationship between sleep and erectile function under conditions such as stress, anxiety, depression and insomnia, which are on the increase in modern society. Crucial information on the activity of I or E-type neurons may also facilitate our understanding of the neural basis of psychogenic impotency.

A future study could be targeted to identify the neural phenotype in the septal area using optogenetic techniques to selectively activate and deactivate the neurons in free moving rats that are involved in erectile processes [46–48]. Even though, there have been attempts to elicit erections by peripheral stimulation of using optogenetic tools, the central mechanism needs to be studied in detail [46]. Thus, retrograde tracing techniques along with cell-lineage genetic tracing approaches would be useful in identifying the neuronal pathways and functions of the septal area in relation to sleep and erectile functions [49, 50].

Acknowledgements The authors greatly thank Dr. Markus Schmidt for his critical comments and helpful suggestions to our study. This study was supported by International Brain Research Organization Research Fellowship to KK Gulia and from Japan Society for the Promotion of Science (No. 15590211) to Y Koyama. The authors thank Ms Nobuko Anzai for her technical assistance.

Author contribution Research Conception: KKGulia, Y Koyama. Experimentation and Analysis: KKGulia, Y Koyama. Manuscript Draft: KKGulia, Y Koyama, Y Kayama. Critical Review: KKGulia, Y Koyama, Y Kayama.

Compliance with ethical standards

Ethical approval All procedures were carried out under the control of the Animal Research Committee in accordance with the Guidelines for Animal Experiments of Fukushima Medical University and the Animal Protection and Management Law of the Japanese Government (No. 105), and in accordance with NIH guidelines.

Conflict of interest The authors declare no competing financial interests.

References

1. Gogate MG, Brid SV, Wingkar KC, Kantak NM (1995) Septal regulation of male sexual behavior in rats. Physiol Behav 57:1205–1207
2. Gulia KK, Kumar VM, Mallick HN (2002) Role of lateral septal noradrenergic system in the elaboration of male sexual behavior in rats. Pharmacol Biochem Behav 72:817–823
3. Gulia KK, Mallick HN, Kumar VM (2005) Atenolol or butoxamine injection at the lateral septum doesn't inhibit male sexual behavior in rats. Indian J Physiol Pharmacol 49:103–107
4. Kondo Y, Shinoda A, Yamanouchi K, Arai Y (1990) Role of septum and preoptic area in regulating masculine and feminine sexual behavior in male rats. Physiol Behav 24:421–434
5. Kumar VM, Khan NA, John J (1996) Male sexual behaviour not abolished after medial preoptic lesion in adult rats. NeuroReport 7:1481–1484
6. Srividya R, Mallick HN, Kumar VM (2004) Sleep changes produced by destruction of medial septal neurons in rats. NeuroReport 15:1831–1835
7. MacLean PD, Ploog DW (1962) Cerebral representation of penile erection. J Neurophysiol 25:29–55
8. Fisher C, Gorss J, Zuch J (1965) Cycle of penile erection synchronous with dreaming (REM) sleep, Preliminary Report. Arch Gen Psychiatry 12:29–45
9. Karacan I, Williams RL, Salis PJ (1970) The effects of sexual intercourse on sleep patterns and nocturnal penile erections. Psychophysiology 7:338
10. Schmidt MH, Valatx JL, Schmidt HS, Wauquier A, Jouvet M (1994) Experimental evidence of penile erections during paradoxical sleep in the rat. NeuroReport 5:561–564
11. Morlet A et al (1990) Effects of acute alcohol on penile tumescence in normal young men and dogs. Urology 35:399–404
12. Gulia KK, Mallick HN, Kumar VM (2004) The septum modulates REM sleep related penile erections in rats. Iran J Pharmaceutical Res 3(supple 1):21
13. Gulia KK, Jodo E, Kawauchi A, Miki T, Kayama Y, Mallick HN, Koyama Y (2008) The septal area, site for the central regulation of penile erection during waking and REM sleep in rats: a stimulation study. Neuroscience 156:1064–1073
14. Gulia KK, Mallick HN, Kumar VM (2005). Sleep and sexual behavior: a crosstalk. In: International Proceeding Division, 2nd Interim Congress of the World Federation of Sleep Research and Sleep Medicine Societies—WFSRSMS. (ISBN: 88-7587-210-4) pp 7–13
15. Verma S, Chhina GS, Kumar VM, Singhm B (1989) Effect of rapid eye movement sleep deprivation on sexual behaviour of male rats. Indian J Exp Biol 27:892–894
16. Andersen ML, Bignotto M, Tufik S (2003) Cocaine-induced genital reflexes during paradoxical sleep deprivation and recovery. Physiol Behav 78:255–259
17. Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates. Academic Press, San Diego
18. Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419–428
19. Heath RG (1972) Pleasure and brain activity in man. J Nerv Ment Dis 154:3–17
20. Mallick HN, Tandon S, Jagannathan NR, Gulia KK, Kumar VM (2007) Brain areas activated after ejaculation in healthy young human subjects. Indian J Physiol Pharmacol 51:81–85
21. Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluoescence study in the rat. Brain Res Bull 9:321–353
22. Kaufling J, Yeinante P, Pawlowski SA, Freund-Mercier MJ, Barrot M (2009) Afferents to the GABAergic tail of the ventral tegmental in the rat. J Comp Neurol 513:559–565
23. Satoh K, Fibiger HC (1986) Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J Comp Neurol 253:277–302

24. Hallanger AE, Wainer BH (1988) Ascending projections from the pedunculopontine tegmental nucleus and the adjacent mesopontine tegmentum in the rat. J Comp Neurol 274:483–515
25. Staiger JF, Nurnberger F (1991) The efferent connections of the lateral septal nucleus in the guinea pig: projections to the diencephalon and brainstem. Cell Tissue Res 264:391–413
26. Risold PY, Swanson LW (1997) Connections of the lateral septal complex. Brain Res Brain Res Rev 24:115–195
27. Schmidt MH, Valatx JL, Sakai K, Fort P, Jouvet M (2000) Role of the lateral preoptic area in sleep-related erectile mechanisms and sleep generation in the rat. J Neurosci 20:6640–6647
28. Moore RY (1978) Catecholamine innervation of the basal forebrain. 1 The septal area. J Comp Neurol 177:665–684
29. Swanson LW, Hartman BK (1975) The central adrenergic system, An immunofluorescence study of the location of cell bodies and their efferent connecctions in the rat utilizing dopamine-beta-hydroxylase as a marker. J Comp Neurol 163:467–505
30. Jakab RL, Leranth C (1995) Septum. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic Press, San Diego, pp 405–442
31. Chen KK, Chen JY, Chang LS, Chen MT, Chan SH (1992) Elicitation of penile erection following activation of the hippocampal formation in rat. Neurosci Lett 141:218–222
32. Srebro B, Mellgren SI, Hardmark SW (1976) Acetycholinesterase histochemistry of the septal region in the rat. In: DeFrance JF (ed) The septal nuclei. Plenum Press, New York, pp 65–76
33. Gritti I, Mariotti M, Mancia M (1998) GABAergic and cholinergic basal forebrain and preoptic-anterior hypothalamic projections to the mediodorsal nucleus of the thalamus in the cat. Neuroscience 85:149–178
34. Fernandez-Fewell GD, Meredith M (1995) Facilitation of mating behavior in male hamsters by LHRH and AcLHRH5-10: interaction with the vomeronasal system. Physiol Behav 57:213–221
35. Giuliano F, Rampin O, Brown K, Courtois F, Benoit G, Jardin A (1996) Stimulation of the medial preoptic area of the hypothalamus in the rat elicits increases in intracavernous pressure. Neurosci Lett 209:1–4
36. Kondo Y (1992) Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. Physiol Behav 51:939–943
37. Melis MR, Stancampiano R, Argiolas A (1994) Prevention by NG-nitro-L-arginine methyl ester of apomorphine- and oxytocin-induced penile erection and yawning: site of action in the brain. Pharmacol Biochem Behav 48:799–804
38. Valcourt RJ, Sachs BD (1979) Penile reflexes and copulatory behavior in male rats following lesions in the bed nucleus of the stria terminalis. Brain Res Bull 4:131–133
39. Koyama Y, Schmidt M, Takahashi K, Kayama Y (2001) Cholinergic neurons in the laterodorsal tegmental nucleus regulate penile erections during paradoxical sleep. Proc Soc Neurosci 522:268
40. Salas JC et al (2008) Penile erection and micturition triggered by electrical stimulation of the mesopontine tegmental area. Am J Physiol Regul Integr Comp 294:R102–R111
41. Koyanagi T, Horimoto N, Nakano H (1991) REM sleep determined using in utero penile tumescence in the human fetus at term. Biol Neonatol 60(suppl 1):30–35
42. Shirozu H, Koyanagi T, Takashima T, Horimoto N, Akazawa K, Nakano H (1995) Penile tumescence in human fetus at term- A preliminary report. Early Hum Devl 41:59–66
43. Mirmiran M, Scholtens J, van de Poll NE, Uylings HB, van der Gugten J, Boer GJ (1983) Effects of experimental suppression of active (REM) sleep during early development upon brain and behavior in the rat. Brain Res 283:277–286
44. Feng P, Ma Y, Vogel GW (2001) The critical window of brain development from susceptive to insuspective. Effects of clomipramine neonatal treatment on sexual behavior. Brain Res Dev Brain Res 129:107–110
45. Heimer L, Van Hoesen GW (2006) The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. Neurosci Biobehav Rev 30:126–147
46. Kim T, Folcher M, Doaud-El Baba M, Fussenegger M (2015) A synthetic erectile optogenetic stimulator enabling blue-light-inducible penile erection. Angew Chem Int Ed Engl 54:5933–5938
47. Yamada K, Takahashi S, Karube F, Fujiyama F, Kobayashi K, Nishi A, Momiyama T (2016) Neuronal circuits and physiological roles of the basal ganglia in terms of transmitters, receptors and related disorders. J Physiol Sci. 66:435–446
48. Vandecasteelea M, Vargaa V, Berényia A, Pappc E, Barthóc P, Venanceb L, Freundc TF, Buzsákia G (2014) Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. PNAS 111:13535–13540
49. Hahn JD, Swanson LW (2015) Connections of the juxtaventromedial region of the lateral hypothalamic area in the male rat. Front Syst Neurosci. 9:66
50. Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. Science 350:957–961