



ISSN: 2456-0057
IJPNPE 2019; 4(1): 301-305
© 2019 IJPNPE
www.journalofsports.com
Received: 28-11-2018
Accepted: 30-12-2018

Goutam Dutta
Department of Physiology,
Prabhat Kumar College, Contai,
West Bengal, India

Tusharkanti Ghosh
Department of Physiology,
UCSTA, University of Calcutta,
West Bengal, India

The EEG power spectrum analysis and sleep-wake behavior after stimulation of acetylcholine receptors in medial septum in rats

Goutam Dutta and Tusharkanti Ghosh

Abstract

Medial septum (MS) in consist of the complex interneuronal circuits of Cholinergic, Glutamatergic and GABAergic receptors containing neurons. In the present study, the muscarinic acetylcholine (m Ach) receptors in MS were stimulated to assess the power spectrum of cortical EEG and sleep-wake behavior. After stimulation of m Ach receptors by putative Ach, the power of Alpha and Beta wave was increased in both wake and slow wave sleep (SWS) condition. But, the power of Delta wave was decreased in both wake and SWS condition. The decreased power of theta wave was found only in SWS condition. No effect was found in power spectrum study after stimulation of the m Ach receptors in REM sleep condition. After micro infusion of Ach into MS, the duration of wake, SWS and REM sleep were not significantly altered compared to that of pre-infusion values. The duration of sleep-wake and the power of EEG remained unaltered after micro infusion of vehicle into MS. Though the duration of sleep-wake cycle is not affected by the stimulation of m Ach receptors, but the power spectrum analysis indicates the there may have some activation / deactivation effects of m Ach receptors on cortical EEG.

Keywords: Medial septum, acetylcholine, receptor, power spectrum analysis, sleep, wake

1. Introduction

As a part of basal forebrain, the regulatory role of the medial septal nucleus on cortical EEG and sleep wake mechanism has been describe (Dutta and Ghosh, 2011, Gerashchenko *et al.*, 2001) [3, 4]. Within Medial Septum (MS), there is a complex interneuronal circuit of cholinergic, GABAergic and glutamatergic neurones (Gritti *et al.*, 1997; Kiss *et al.*, 1997) [5, 7]. The local glutamatergic neurons or the glutamate secreting fibres coming from outside the MS can stimulate the cholinergic, GABAergic and glutamatergic neurones of the MS through AMPA receptors (Manseau *et al.*, 2005) [9]. Besides the presence of glutamate receptors, the cholinergic receptors have also been indentified in MS (Henderson *et al.*, 2001; Milner, 1991) [6, 10]. There are evidences that the acetylcholine (Ach) activates the intrinsic GABAergic, cholinergic and glutamatergic neurons of the MS through muscarinic receptors (Lawson and Bland, 1993; Manseau *et al.*, 2005) [8, 9]. Besides the presence of glutamate receptors, the cholinergic receptors have also been indentified in MS (Henderson *et al.*, 2001; Milner, 1991) [6, 10]. There are evidences that the acetylcholine (Ach) activates the intrinsic GABAergic, cholinergic and glutamatergic neurons of the MS through muscarinic receptors (Lawson and Bland, 1993; Manseau *et al.*, 2005) [8, 9]. The role of Ach receptors of MS on immunomodulation has been described by Dutta *et al.*, 2016 [2]. The behavior controlling role of Ach receptors of MS also have been found in that literature (Dutta *et al.*, 2016) [2], but its effects on sleep-wake duration and power spectrum analysis has not studied significantly. In the present study, the muscarinic acetylcholine (m Ach) receptors in MS were stimulated to assess the role of these receptors on power spectrum analysis of cortical EEG and sleep-wake duration in rats.

2. Methods and Materials

2.1 Animals

Twelve male albino rats weighing 200-220 gm were used in this study. Animals were housed individually in polypropylene animal cage with water and food ad libitum in the animal room

Correspondence
Goutam Dutta
Department of Physiology,
Prabhat Kumar College, Contai,
West Bengal, India

with a 12h light dark cycle (7AM to 7 PM). All adequate measures were taken to minimize the pain and discomfort to the rats.

2.2 Experimental design

The power spectrum analysis and duration of sleep-wake were measured in 3 groups of rats (control, vehicle control, Ach micro infused groups). Different groups are described as follows: vehicle (PBS, pH 7.4) infused control (CV), 0.06 μ M Ach infused (ACH). The optimum dose of Ach was determined on the basis of locomotor activities and immunological parameters of different dose of Ach (Dutta *et al.*, 2016)^[2].

2.3 Implantation of cannula and EEG electrode

The cannula was implanted into MS stereotaxically on AP + 1.00 mm from bregma and L \pm 0.00 mm, with bregma and lamda in the horizontal plane in anesthetized rats (Nathiopentone, 50 mg/kg body wt, i.p). The guide cannula (C 313-G, Roancke, Virginia, USA) was inserted 3.00 mm below the skull surface (AP + 1.00 mm from bregma and L \pm 0.00 mm) with the help of stereotaxic apparatus and was fixed on the skull with dental acrylic (Dutta and Ghosh., 2011)^[3]. For EEG recording, two stainless steel screw electrodes (0.8 X 1/8 inch) were implanted over the visual cortex (6.0 mm posterior to bregma and 3.5 mm lateral to the midline on both side) and one stainless steel screw electrode was placed 11.0 mm anterior to bregma on the midline served as ground following the method of Ghosh *et al.* (1989). For EMG recording, pairs of insulated wire electrodes were inserted into dorsal neck muscles. The electrodes were soldered to a miniature socket and the whole assembly was fixed to the skull with dental acrylic.

2.4 Microinfusion of acetylcholine or vehicle into medial septum

Acetylcholine (Sigma, USA) was dissolved in sterile phosphate buffer saline (PBS) (pH 7.4) for the micro infusion into MS. The sterile PBS (pH 7.4) was used as vehicle in this experiment. The Ach or vehicle was micro infused into MS on 21st, 23rd and 25th day of cannula implantation for this experiment.

2.5 Sleep-wake durations and power spectrum analysis of cortical EEG

The sleep-wake durations were determined from the EEG and

EMG recording in both the pre-infused and post-infused (after microinfusion of Ach or vehicle on 25th day of cannula implantation) conditions. On 25th day of cannula implantation (last day of microinfusion) the pre-infusion sleep-wake durations (from 12.30 PM to 1.30 PM) were expressed in four 15 min episodes (pre 1, pre 2, pre 3 and pre 4) and the post-infusion sleep-wake durations (1.30 PM to 2.30 PM) were expressed in another four 15 min episodes (post 1, post 2, post 3 and post 4). The sleep-wake durations were expressed as min/15 min (in wake and SWS) and in sec/15 min (in REM sleep). Eight episodes of 15 min duration were termed as pre 1 (0 to 15 min), pre 2 (15 to 30 min), pre 3 (30 to 45 min), pre 4 (45 to 60 min), post 1 (60 to 75 min), post 2 (75 to 90 min), post 3 (90 to 105 min) and post 4 (105 to 120 min).

The power spectrum of EEG was analysed from the four 15 min episodes in pre-infused condition (pre 1, pre 2, pre 3 and pre 4) and from another four 15 min episodes in post-infused condition (post 1, post 2, post 3 and post 4). Nine 10 s EEG samples during wake or SWS in each of 15 min episode (pre 1, pre 2, pre 3, pre 4 and post 1, post 2, post 3, post 4) were used to determine the power of different EEG waves in decibel Volt (dbV). In case of REM sleep, nine 10 sec EEG samples from 1 hr EEG recording (as the durations of REM sleep was not sufficient to take nine 10 sec EEG samples during REM sleep in each 15 min episode) in pre-infused condition and another nine 10 sec EEG samples from 1 hr EEG recording in post-infused condition were used to determine the power of different waves of EEG in dbV. The powers of the different waves of EEG after microinfusion of Ach or vehicle into MS were expressed as the percentage of the power of that wave (100%) in pre-infused condition.

2.6 Statistical analysis

Data are expressed as mean \pm SEM. The duration of wake or SWS or REM sleep in each rat of a group (i.e. Ach or vehicle microinfused rats) were analysed by one way ANOVA followed by post Dunnett test to determine the significant difference between pre-infusion episode and post-infusion episodes. Absolute power of each wave band in wake or SWS or REM sleep were analysed by one way ANOVA followed by Dunnett test to determine the significant difference between each of the pre-infusion episode and post-infusion episode.

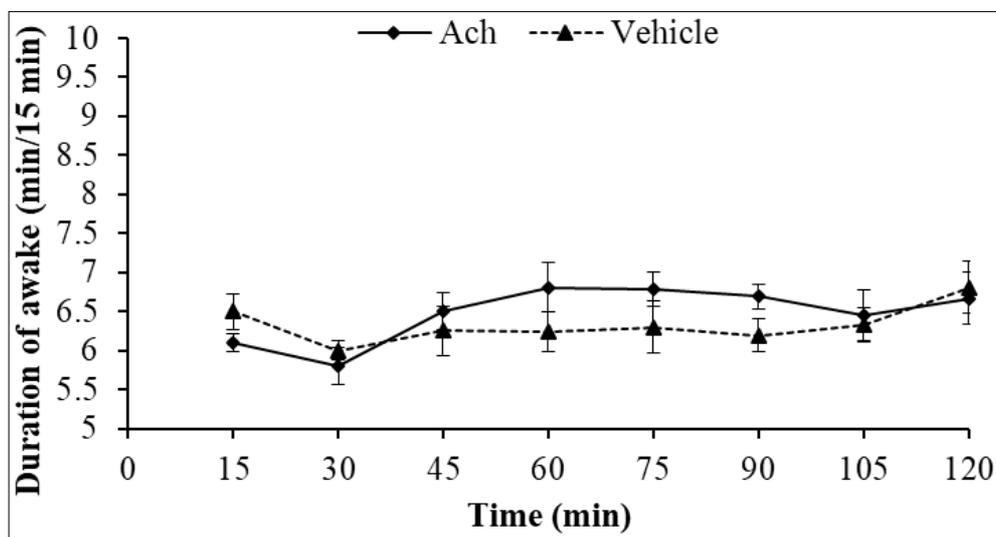


Fig 1: The duration of wake, before and after microinfusion of Ach or vehicle into MS in rats. 0-60 min represent pre-infused durations and 60-120 min represent post-infusion durations of wake. Mean of each 15 min episode is presented at the end point of that episode. Values are expressed as mean \pm SEM. n = 6 in each group of rats.

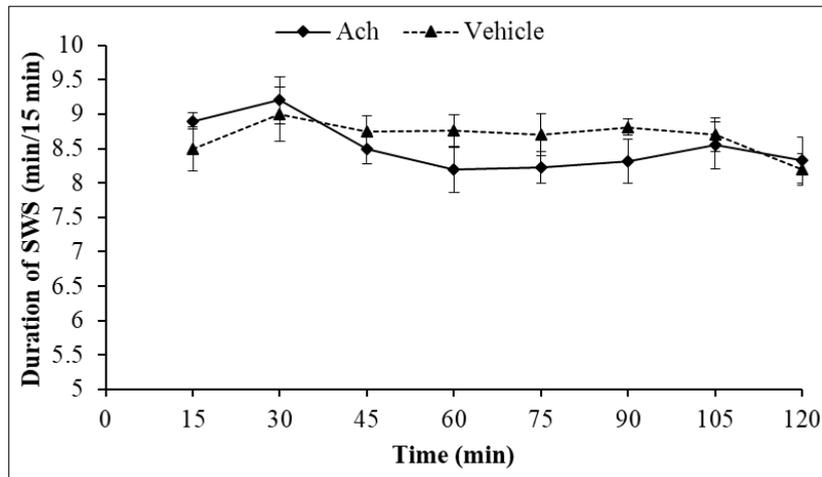


Fig 2: The duration of slow wave sleep, before and after microinfusion of Ach or vehicle into MS in rats. 0-60 min represent pre-infused durations and 60-120 min represent post-infusion durations of wake. Mean of each 15 min episode is presented at the end point of that episode. Values are expressed as mean ± SEM. n = 6 in each group of rats.

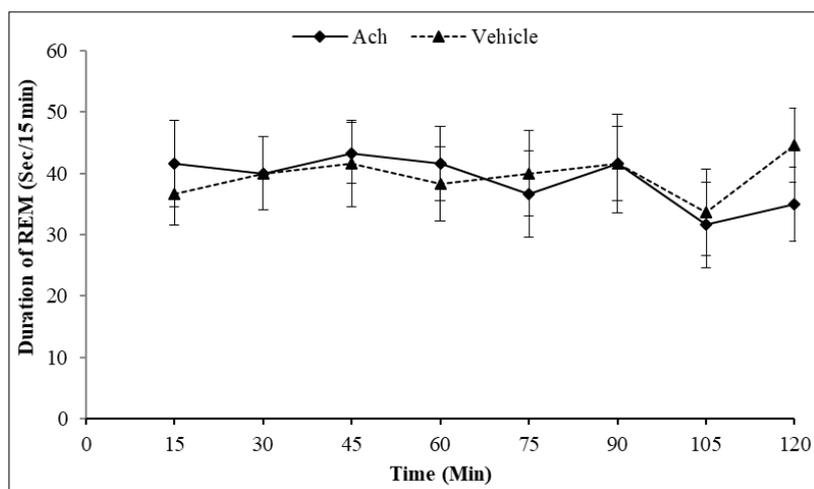


Fig 3: The duration of REM sleep, before and after microinfusion of Ach or vehicle into MS in rats. 0-60 min represent pre-infused durations and 60-120 min represent post-infusion durations of wake. Mean of each 15 min episode is presented at the end point of that episode. Values are expressed as mean ± SEM. n = 6 in each group of rats.

Table 1: Mean power of different waves of EEG expressed as percentage of pre-infusion values in wake and SWS after microinfusion of Ach into MS in rats.

Condition	Time (min)	Beta (13-35 Hz)	Alpha (8-13 Hz)	Theta (4-8 Hz)	Delta (0.05-4 Hz)
Wake	0-15	116.10±0.868***	114.00±0.706*	101.69±1.020	73.89±1.502***
	15-30	114.67±0.505***	112.12±0.286***	99.81±0.662	75.78±0.615***
	30-45	109.93±0.615***	104.79±0.190	101.87±0.185	83.94±0.589***
	45-60	102.44±0.267	100.47±0.350	101.55±0.370	82.94±0.526***
SWS	0-15	117.69±0.500***	77.70±1.148***	74.32±0.684***	95.38±1.278
	15-30	114.17±0.422***	73.80±0.687***	74.92±0.684***	80.32±1.521*
	30-45	105.32±0.627*	85.53±0.616**	76.28±1.044**	84.49±1.285*
	45-60	99.51±0.591	91.06±0.365	88.48±2.272	95.48±1.150

Values are expressed as mean ± SEM. SWS: slow wave sleep. *** p < 0.001, ** p < 0.01 and *p < 0.05 denotes the significantly different from pre infusion mean. The values have been taken from 6 animals (the value of one animal has been calculated from nine 10 sec EEG sample in each 15 min episode).

Table 2: Mean power of different waves of EEG expressed as percentage of pre-infusion values in wake and SWS after microinfusion of vehicle into MS in rats.

Condition	Time (min)	Beta (13-35 Hz)	Alpha (8-13 Hz)	Theta (4-8 Hz)	Delta (0.05-4 Hz)
Wake	0-15	100.23±2.12	101.22±1.34	102.67±2.87	98.67±3.76
	15-30	100.87±2.43	102.33±2.87	100.23±2.60	99.45±2.87
	30-45	101.77±1.98	99.98±2.12	101.65±2.99	100.55±2.09
	45-60	101.11±2.22	100.89±2.87	100.87±1.97	101.44±2.09
SWS	0-15	100.34±2.34	100.32±2.68	102.30±3.33	100.34±2.33
	15-30	99.67±2.89	101.23±3.54	102.45±2.77	100.12±2.98
	30-45	101.22±2.67	100.10±2.98	100.23±2.22	102.32±2.89
	45-60	100.98±2.67	99.78±2.12	101.98±2.19	102.77±2.01

Values are expressed as mean ± SEM. SWS: slow wave sleep. The values have been taken from 6 animals (the value of one animal has been calculated from nine 10 sec EEG sample in each 15 min episode).

3. Results and Discussion

3.1 Power spectrum analysis of cortical EEG

The power of beta wave was increased [$F(4, 40) = 9.89, p < 0.001$] compared to that of pre-infusion values after microinfusion of Ach into MS. The increased value of beta wave persisted for 45 min in wake condition. In SWS condition, the power of beta wave was also increased [$F(4, 40) = 9.89, p < 0.001$] and persisted for 45 minutes. The power of alpha wave was increased [$F(4, 40) = 9.89, p < 0.001$] after microinfusion of Ach into MS and persisted for 30 min in wake. In SWS, the power of alpha wave was also increased [$F(4, 40) = 9.89, p < 0.001$] and persisted for 45 min of microinfusion. The power of theta wave remained unaltered in wake condition after microinfusion of Ach but it was significantly decreased [$F(4, 40) = 9.89, p < 0.001$] in SWS condition and persisted for 45 min. The power of delta wave was decreased [$F(4, 40) = 9.89, p < 0.05$] in SWS (15 min after the microinfusion was started) condition after microinfusion of Ach into MS and persisted for 30 min. In wake condition, the power of delta wave was decreased [$F(4, 40) = 9.89, p < 0.001$] and the decreased value of delta wave was persisted for 60 min (Table 1). The power of all the four measured waves of EEG was not altered in REM sleep after microinfusion of Ach into MS in rats. After microinfusion of vehicle into MS, the power of all the waves in wake, SWS and REM sleep were not altered in rats compared to that of the pre-infused values (Table 2).

3.2 Durations of sleep-wake

After microinfusion of Ach into MS, the duration of wake, SWS and REM sleep were not significantly altered compared to that of pre-infusion values. The duration of wake, SWS and REM sleep remained unaltered after microinfusion of vehicle into MS compared to that of pre-infusion values of vehicle (Fig 1, 2 and 3).

4. Discussion

In the present experiment, the durations of awake, SWS and REM was not altered after microinfusion of Ach into MS in rats. But the power of the different waves of EEG was altered in those rats. The power of beta and alpha wave were increased in both wake and SWS condition after stimulation of Ach receptors of MS in rats. The maximum persistent time for these two waves is 45 min after the infusion. The change of the power of delta wave was found in both wake and SWS condition. The power of delta wave was decreased after infusion. But the power of theta wave was only decreased in SWS condition after stimulation. The present observed pattern of the power change indicate a desynchronization of EEG after stimulation of Ach receptors of MS by putative Ach.

Within MS, there is a complex interneuronal circuit of cholinergic, GABAergic and glutamatergic neurones (Gritti *et al.*, 1997; Kiss *et al.*, 1997) [5, 7]. The local glutamatergic neurons or the glutamate secreting fibres coming from outside the MS can stimulate the cholinergic, GABAergic and glutamatergic neurones of the MS through AMPA receptors (Manseau *et al.*, 2005) [9]. The role of the glutamate receptors of MS on sleep-wake behavior and EEG power spectrum has been investigated in rats after microinfusion of putative glutamate into MS. The duration of wake was increased and the duration of SWS was decreased in those rats (Dutta and Ghosh, 2011) [3]. In the same study they observed an activation of EEG after stimulation of glutamate receptors of MS by power spectrum analysis. The observations on power spectrum by Dutta and Ghosh, (2011) [3] has some similarities

with the present observation. Therefore, the glutamate or Ach receptors of MS may have some effect on the cortical EEG power. The cortical arousal was also found after microinfusion of GABA_A agonist muscimol (Osborne 1994) [11] or β -adrenergic agonist ISO (Berridge 1996) [1] into MS indicates the regulatory role of MS on cortical EEG. Moreover, there are reports that the stimulation of Ach projecting neurons from PPT causes desynchronization of cortical EEG (Steriade *et al.*, 1993) [12]. If we summarized the stimulatory effects of different receptors (glutamate, Ach, adrenergic and GABAergic) of MS on cortical EEG activities, we can find the generalized cortical desynchronization and an interesting point is that, one of the potential neural pathways that transmit these effects to cortex is Ach. There are reports about the septocortical projections to the cingulate cortex. Other than septo-cortical fiber, there are septo-hippocampal pathways which are the regulator of the cortical theta wave (Gerashchenko *et al.*, 2001) [4]. In the present observation, the duration of REM sleep remain unaltered after stimulation of Ach receptors. Moreover, the power of theta wave in wake condition was also remained unchanged after stimulation. It appears that the cortical activation after stimulation of Ach receptors of MS may be through the cholinergic septocortical fibers or through the complex interneuronal circuits of MS.

5. Conclusions

In the present observation, the duration of slow wave sleep, REM sleep and wake are not altered after stimulation of the cholinergic receptors of medial septum. The power spectrum analysis of cortical EEG indicates a desynchronization of EEG after stimulation of Ach receptors of MS. This cortical activation may be through the cholinergic septo-cortical fibers or through the complex interneuronal circuits of medial septum.

6. References

- Berridge CW, Bolen SJ, Manley MS, Foote SL. Modulation of forebrain electroencephalographic activity in halothane-anesthetized rat via actions of noradrenergic β -receptors within the medial septal region. *Journal of Neuroscience*. 1996; 16:7010-7020.
- Dutta G, Ghosh T. Effects of stimulation of muscarinic acetylcholine receptors in medial septum on some immune responses in rats. *Neurosci Lett*. 2016; 619:155-161.
- Dutta Gand Ghosh T. Effects of stimulation of glutamatergic receptors in medial septum on power spectrum analysis of EEG in rats. *Proc Zool Soc*. 2011; 64:109-116.
- Gerashchenko D, Salin-Pascual R, Shiromani PJ. Effects of hypocretin-saporin injections into medial septum on sleep and hippocampal theta. *Brain Research*. 2001; 913:106-115.
- Gritti I, Mainville L, Mancia M, Jones BE. GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. *J Comp Neurol*. 1997; 383:163-177.
- Henderson Z, Morris NP, Grimwood P, Fiddler G, Yong HW, Appenteng K. Morphology of local axon collaterals of electro physiologically characterised neurons in the rat Medial septal/ diagonal band complex. *J Comp neurol*. 2001; 430:410-432.
- Kiss J, Magloczky Z, Somogyi J, Freund TF. Distribution

- of calretinin-containing neurons relative to other neurochemically identified cell types in the medial septum of the rat. *Neuroscience*. 1997; 78:399-410.
8. Lawson VH, Bland BH. The role of the septohippocampal pathway in the regulation of hippocampal field activity and behavior: analysis by the intraseptal microinfusion of carbachol, atropine, and procaine. *Exp Neurol*. 1993; 120:132-144.
 9. Manseau F, Danik M, Williams S. A functional glutamatergic neurone network in the medialisepum and diagonal band area. *J Physiol*. 2005; 566:865-884.
 10. Milner TA. Cholinergic neurons in the rat septal complex: ultrastructural characterisation and synaptic relations with catecholaminergic terminals. *J Comp Neurol*. 1991; 314:37-54.
 11. Osborne PG. A GABAergic mechanism in the medial septum influences cortical arousal and locomotor activity but not a previously learned discrimination task. *Neuroscience Lett*. 1994; 173:63-66.
 12. Steriade M, McCormick DA, Sejnowski TJ. Thalamocortical oscillation in the sleeping and aroused brain. *Science*. 1993; 262:679-685.