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CHARACTERISTICS OF NEURON PROJECTIONS FROM THE ARCUATE NUCLEUS TO THE SUPRACHISMATIC NUCLEUS OF THE HYPOTHALAMUS IN RATS *IN VITRO*

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Abstract. Currently, in the electrophysiology of the brain, there is a point of view about the presence of a physiological connection between the suprachiasmatic and arcuate nuclei. A fully substantiated confirmation of this connection has not been received, however, a number of experiments conducted by scientists from various countries confirm the existence of a certain kind of interaction between the work of the SCN and the ARC of nuclei. The interaction of SCN and ARC regulates the rhythm of metabolic functions. The SCN transmits time-related information to the ARC to perform metabolic functions at the right time. At the same time, SCN affects the sensitivity of ARC to circulating molecules, thus allowing it to respond in a time-of-day manner. As the ARC senses hormones and metabolites from the periphery, it relays this information back to the SCN, allowing the SCN to adapt its output and completing the feedback that the SCN needs to correct the physiology. This interaction between SCN and ARC is necessary to maintain rhythms of food intake, motor activity, temperature, and circulation of corticosterones and glucose. The aim of this work is to characterize the projections of neurons from the arcuate nucleus to the suprachismatic nucleus of the rat hypothalamus in vitro using the electrophysiological technique for constructing a peristimulus temporal histogram (PSTH). Based on the results obtained, make a conclusion about the intensity and nature (excitatory, inhibitory or complex) of axon projections from the arcuate to the suprachiasmatic nucleus, thereby supplementing the theoretical understanding of the functional organization of the SCN of the hypothalamus. Modern methods of electrophysiological studies (extracellular microelectrode recording of neuron activity) were used in the work. All the obtained results indicate that ARC neurons are able to have both excitatory and inhibitory effects on the functional state of the cells of the circadian oscillator of the SCN. These influences may underlie the adjustment of the oscillator in accordance with the level of activity of the center for regulating appetite, metabolism, and diet, located in the arcuate nucleus of the hypothalamus.

Key words: suprachiasmatic nucleus (SCN); arcuate nucleus (ARC); circadian; spike; neuron; peristymal temporal histogram (PSTH); hypothalamus; afferent output.

ХАРАКТЕРИСТИКА ПРОЕКЦИЙ НЕЙРОНОВ ОТ АРКУАТНОГО ЯДРА К СУПРАХИЗМАТИЧЕСКОМУ ЯДРУ ГИПОТАЛАМУСА КРЫС *IN VITRO*

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Резюме. В настоящее время в электрофизиологии мозга существует точка зрения о наличии физиологической связи между супрахиазматическим (СХЯ) и аркуатным (АРК) ядрами. Полностью обоснованного подтверждения

этой связи получено не было, однако ряд опытов, проведенных учеными различных стран, подтверждает существование определенного рода взаимодействия между работой СХЯ и АРК ядер. Взаимодействие СХЯ и АРК регулирует ритмичность метаболических функций. СХЯ передает связанную со временем информацию в АРК для выполнения метаболических функций в нужное время. В то же время СХЯ влияет на чувствительность АРК к циркулирующим молекулам, таким образом позволяя ему реагировать в зависимости от времени суток. Поскольку АРК воспринимает гормоны и метаболиты из периферии, оно передает эту информацию обратно в СХЯ, позволяя СХЯ адаптировать свой вывод и завершая обратную связь, которая необходима СХЯ для корректировки физиологии. Это взаимодействие между СХЯ и АРК необходимо для поддержания ритмов приема пищи, двигательной активности, температуры и циркулирования кортикостеронов и глюкозы. Цель работы — охарактеризовать проекции нейронов от аркуатного ядра к супрахиазматическому ядру гипоталамуса крыс *in vitro* с помощью электрофизиологической техники построения перистимульной временной гистограммы (PSTH). На основании полученных результатов сделать заключение об интенсивности и характере (возбуждающем, тормозном или комплексном) аксонных проекций из аркуатного в супрахиазматическое ядро, тем самым дополнить теоретические представления о функциональной организации СХЯ гипоталамуса. В работе применялись современные методы электрофизиологических исследований (внеклеточная микроэлектродная запись активности нейронов). Все полученные результаты свидетельствуют о том, что нейроны АРК способны оказывать как возбуждающее, так и тормозное влияние на функциональное состояние клеток циркадианного осциллятора СХЯ. Эти влияния могут лежать в основе настройки осциллятора в соответствии с уровнем активности центра регуляции аппетита, метаболизма и режима питания, расположенного в аркуатном ядре гипоталамуса.

Ключевые слова: супрахиазматическое ядро (СХЯ); аркуатное ядро (АРК); циркадианность; спайк; нейрон; перистимульная временная гистограмма (PSTH); гипоталамус; афферентный выход.

INTRODUCTION

There is a lot of evidence that the suprachiasmatic nucleus (SCN) of the hypothalamus is a master circadian clock in mammals, responsible for controlling most, if not all, biochemical, physiological, and behavioural processes. Individual cells of the SCN constitute a self-sustaining molecular clock, but their synchronisation, precision, and stability of the circadian rhythm are derived from intercellular interactions mediated by network mechanisms [8]. The period of the endogenous free SCN rhythm must be synchronised with the period of the environment (exactly 24 h), matching the phase of the internal clock with the corresponding solar time. In addition to the most physiologically important photic engagement of the SCN clock in the light-dark cycle, which is mediated by direct input to the retina via the retinohypothalamic tract, relevant non-photic cues may also contribute [11].

The SCN has a wide range of reciprocal neural connections (e.g., with the arcuate nucleus (ARC), dorsomedial hypothalamus, intergeniculate leaflet, dorsal suture) that provide non-convex feedback from these nuclei to the SCN to entrain the underlying circadian clock.

Among the neural connections of the SCN, reciprocal connections with the ARC are of particular interest. The arcuate nucleus is critical for maintaining energy homeostasis and metabolic balance, being an integrator of hunger and satiety signals that may be involved in the mechanisms of non-photic setting of the SCN clock [14]. Projections from the SCN to the ARC have been demonstrated in autoradiographic and electrophysiological studies, but little is known about the transmitters involved.

Circadian rhythms of functional activity in mammals, manifested at the level of both individual cells and the whole organism, are formed under the influence of pacemaker neurons localised in the hypothalamic SCN [10, 13]. Pacemakers act as biological clocks, with the help of which the body is able to control various rhythms of such processes as metabolism, cell proliferation, behavioural reactions (sleep, wakefulness, food intake, drinking, locomotor activity, etc.). In the nervous system, at the level of individual neurons, this is manifested in rhythmic fluctuations in the production and release of physiologically active substances, as well as the level of expression of receptors to these substances, for example, to serotonin, norepinephrine, acetylcholine, melatonin [10, 16, 17].

Previous studies have shown that neural connections between the SCN and the ARC are essential for circadian function. These connections integrate metabolic information into the circadian system and thereby adapt circadian rhythms of behaviour, food intake, body temperature, and circulating corticosterone and glucose levels. It has been demonstrated that the ventromedial ARC can modulate SCN activity. The suprachiasmatic nucleus, in turn, can induce rhythms in the ARC [7]. Lesion of a specific (leptin-sensitive) population of neurons in the ARC leads to disruption of rhythms of sleep, body temperature, and food intake. Isolation of the ARC from the SCN in

stab-injured rats resulted in complete arrhythmia of locomotor activity, body temperature, and corticosterone secretion under conditions of constant darkness, despite the rhythmicity of the SCN and persistent rhythmic secretion of melatonin. It was concluded that the interaction between the SCN and the ARC is critical for the expression of circadian rhythms [5, 6, 12].

AIM

To characterise the projections of neurons of the ARC to SCN in rat's hypothalamus *in vitro* using the electrophysiological technique of peristimulus time histogram (PSTH).

MATERIALS AND METHODS

48 male Wistar rats weighing 75–170 g were used in the experiments. At the beginning of the experiment, the animals were anesthetised with urethane (1.2 g/kg intraperitoneally) and decapitated. The brain was excised and a vibratome (Vibroslice NVSL, WPI, USA) was used to make 500 μm sagittal sections through the suprachiasmatic and arcuate nuclei. The sections were pre-incubated in artificial cerebrospinal fluid (aCSF) oxygenated with 95 % oxygen and 5% carbon dioxide for at least 1 hour at physiological temperature (37 °C). The composition of aCSF consisted of 124 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 0.5 mM NaH_2PO_4, 30 mM glucose. After that, the sections were transferred to the recording chamber and perfused with aCSF. The recording chamber was maintained at room temperature of 24–26 °C. The flow of aCSF through the recording chamber was maintained using a peristaltic pump (Minipuls3, Gilson, France) at a constant rate of 1.5–2.0 ml/min.

Extracellular recordings were made from SCN neurons using glass electrodes with a tip diameter of approximately 0.5 μm, filled with aCSF to give a tip resistance of approximately 10 MΩ.

Recorded signals were amplified (2400 A, Dagan, USA), passed through a 50 Hz noise suppressor (Hum Bug; Quest Scientific, Canada) and interface device (Micro 1401; CED, Cambridge, UK) to a PC. Spike 2 software (CED, Cambridge, UK) was used for data acquisition. The raw waveform was sampled at 20 kHz and autonomous discrimination between peak events and stimulus artefacts was performed using dedicated data analysis software [1, 2]. ARCs were stimulated with biphasic rectangular stimulation pulses of 8 × 8 V (corresponding to 0.2 × 0.2 mA). The stimulation protocol consisted of single biphasic pulses of 1 × 1 ms duration delivered at a frequency of 1 Hz using a model 2100 stimulator (AM Systems, USA). The distance between the stimulating electrode and recording zone in the SCN was 2.5–3 mm.

The experiments were conducted to study the spike activity of SCN neurons *in vitro* and its modulation arising during electrical stimulation of the ARC and to characterise electrophysiologically the state of axon projections to suprachiasmatic neurons. In this case, methods for constructing and analysing a peristimulus time histogram (PSTH) were used.

RESULTS

A total of 65 stable single recordings of neuronal responses were made from the SCN. After spontaneous activity of each cell had been recorded for at least 5 min in normal aCSF, the cells were tested for the effects of ARC stimulation. The 42 of 65 cells (64.6 %) responded and showed simple excitatory, simple inhibitory, or complex responses to PSTH. Fig. 1 illustrates the procedure for generating PSTH of two SCN cells showing simple short-latency excitation (Fig. 1a) and simple short-latency inhibition (Fig. 1b) after ARC stimulation. 11 cells (16.9%) showed simple short-latency (< 14 ms) excitation, 13 cells (20.0 %) showed simple short-latency (< 14 ms) inhibition, 2 cells (3.1 %) showed simple long-latency (> 40 ms) excitation, 2 cells (3.1 %) had simple long-latency inhibition (> 25 ms), and 23 cells (35.4 %) did not respond. The remaining 14 cells (21.5 %) showed complex responses.

In the research, 9 cells (13.8 %) showed a complex response consisting of short-latency inhibition followed by excitation, 2 cells (3.1%) showed a complex response consisting of antidromic excitation followed by inhibition, 1 cell (1.5 %) showed a complex response consisting of short-latency excitation followed by inhibition, and 2 cells (3.1 %) showed a complex response consisting of three components: short-latency excitation followed by inhibition and delayed excitation.

Most cells (38 of 42; 90.5 %) responding to stimulation showed a short-latency response (including an early component of the complex response) with the onset of 5.8 ± 0.9 ms of the stimulus pulse. The remaining 4 cells (9.5 %) showed a delayed response with an onset of the stimulus pulse > 25 ms.

DISCUSSION

In the present *in vitro* electrophysiological study on 500 μm sagittal slices, peristimulus time histograms (PSTH) were used to assess the responses of SCN neurons to ARC stimulation. 65 % of orthodromically identified SCN neurons responded to ARC stimulation with simple monophasic or complex responses, indicating the existence of rich neuronal projections from the ARC to the SCN. In an earlier in vivo electrophysiological study, simple or complex responses to ARC stimulation were detected in 86% of SCN neurons [15]. The lower proportion of neurons responding to stimulation

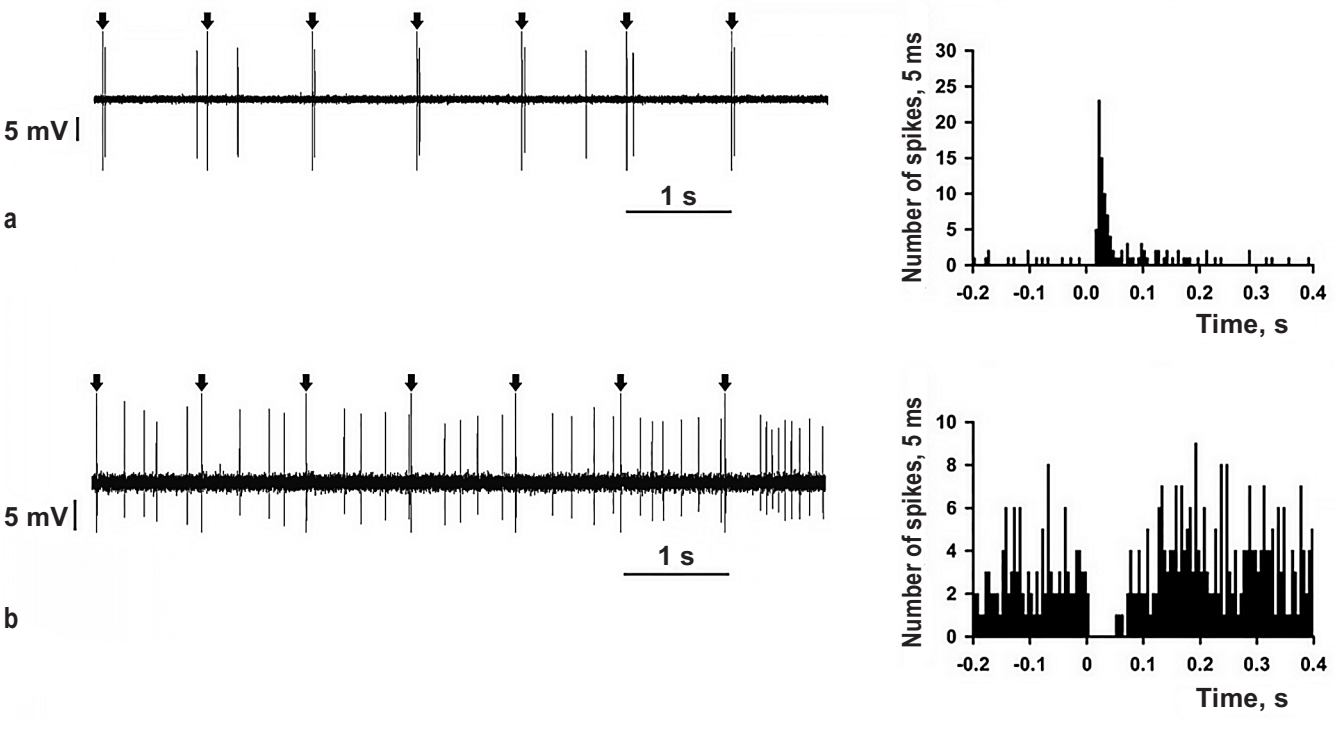

Fig. 1. Examples of response of neurons in the suprachiasmatic nucleus to stimulation of the arcuate nucleus in the form of short-latency excitation (*a*) and inhibition (*b*). The upper part of the figure shows fragments of neuronograms, with arrows marking stimulation artefacts. The lower part shows three peristimulus time histograms of the activity of these neurons, constructed based on data on the distribution of spike generation moments during the action of 325 and 150 consecutive stimuli, respectively. The abscissa axis shows time (ms) (the "0" mark corresponds to the stimulus moment); the ordinate axis shows the total number of spikes for each 5 ms time interval

in vitro may be explained by a decrease in the number of intact axonal projections from the stimulation zone to the SCN due to projection damage during slice preparation. In our earlier in vitro study performed on 300 μm sagittal sections, only 48% of the tested SCN neurons responded to ARC stimulation [9]. The reduced number of responding cells in the thinner section compared to the present study could be explained by the possible trajectory of some fibres connecting the ARC and SCN, which may have initially passed laterally and exited the section.

Stimulation of the ARC caused both simple (monophasic) excitatory, simple inhibitory, and complex responses in the SCN. Simple excitatory and inhibitory responses were observed in 20 and 23.1% of the suprachiasmatic neurons tested, respectively. Complex responses consisting of multiple components (excitatory, inhibitory, or both) were observed in 21.5% of neurons. This significant number of complex responses may indicate that significant proportion of SCN cells receive 2 or more distinct inputs from cells in the ARC or pathways passing through the ARC.

In conclusion, the present study demonstrated reciprocal connections between the ARC and SCN using electrophysiological methods. The data confirm previous electrophysiological and anatomical studies demonstrating reciprocal neural connections between the ARC and the SCN [3, 4]. Projections from the ARC are thought to convey peripheral metabolic information to the SCN. It has been suggested that the SCN is important component in a larger network that controls homeostasis by changing physiological parameter sets daily.

CONCLUSION

In the study, reciprocal connections between the ARC and SCN were demonstrated using electrophysiological methods. The data confirm previous electrophysiological and anatomical studies demonstrating reciprocal neural connections between the ARC and SCN. Projections from the ARC presumably transmit peripheral metabolic information to the SCN.

The results indicate that the arcuate nucleus contains a population of cells that are the source of excitatory and inhibitory projections to neurons of the suprachiasmatic nucleus. Due to the presence of these projections, synchronisation of the circadian oscillator may be achieved, in particular, in accordance with the level of metabolism and severity of food motivation.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisi-tion, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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All procedures complied with ethical standards approved by legal acts of the Russian Federation, the principles of the Basel Declaration, and the recommendations of the bioethics committee of the biological faculty of Samara National Research University (protocol no. 11 from 3.10.2013).

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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