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Modulation of EEG Rhythms and Changes in Spike Activity of Noradrenergic Neurons of the *Locus Coeruleus* Related to Feedback Sessions by EEG Characteristics

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In chronic experiments on awake cats, we studied the dynamics of the spectral power density (SPD) of the α rhythm *vs* SPD of the θ rhythm ratio and also of the characteristics of impulse activity generated by supposedly noradrenergic (NA) neurons of the *locus coeruleus* in the course of feedback (FB) sessions by EEG characteristics (EEG-FB). Trainings were performed using a technique analogous to that in EEG-FB sessions for humans. The level of a sound noise signal presented to the animal decreased with increase in the α/θ SPD ratio in the occipital lead. Changes in the level of the sound signal did not depend on EEG modulation in the control series. The animals were trained to correlate changes in the loudness of the sound signal with the power of EEG rhythms and, in such a way, to control the latter. The α/θ SPD ratio in EEG-FB sessions changed mostly due to a significant increase in the α rhythm power. The frequency of the impulse activity of NA neurons increased in a parallel manner with such EEG modulation. Possible mechanisms of the involvement of the cerebral NA system in the formation of the effects of EEG-FB sessions are discussed.

Keywords: feedback by EEG characteristics, spectral power density of EEG rhythms, noradrenergic neurons, *locus coeruleus*.

INTRODUCTION

The technique of feedback (FB) by characteristics of the electroencephalogram (EEG-FB, neurotherapy) has been recognized as a valid alternative approach with respect to medicament therapy in the treatment of many chronic diseases and for correction of some clinical syndromes. This technique is applied for normalization of the structure of cerebral electrical activity in the case of some pathologies and subclinical disorders of the CNS [1-3]. Effective use of EEG-FB requires more detailed elucidation of the mechanisms of interrelations between the cortex and deep cerebral structures, including those especially significantly involved in the modulation of cortical functions and formation of some behavioral states. Considering this, we carried out some studies in our laboratory that demonstrated the existence of clear correlations of the pattern of EEG oscillations with the functional state of the aminergic brainstem structures. As was found, an increase in the frequency of discharges generated by more than 50% of the supposedly noradrenergic (NA) neurons of the *locus coeruleus* (LC) was accompanied by intensification of the α rhythm in the EEG composition. In experiments on animals using the EEG-FB technique, it was found that the observed modifications of the EEG frequency composition correlated with changes in the frequency of spiking of dopaminergic (DA) brainstem neurons [5]. Analysis of the peculiarities of changes in the EEG rhythms in the course of EEG-FB sessions allowed us to postulate that the DA system is not the only system involved in the formation of the EEG-FB effects; other cerebral mediator systems modulating the activity of cortical cells and that of DA neurons in the ventral tegmentum participate intensely in this process. The brainstem NA system is one of such systems projecting practically to all CNS subdivisions, namely the neocortex, thalamus, hypothalamus, limbic system, and spinal cord [6-10]. The above nuclear structure is a crucially important center of the brainstem where signals of different modalities are integrated, processed, commutated, and exclusively broadly distributed, which allows

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them to influence different regions of the CNS [11, 12]. Axons of NA neurons, together with fibers of the DA midbrain cells, form the medial forebrain bundle, which is a part of the cerebral reward system [13, 14].

Considering this, it is believed that changes in the state of the NA LC system can play a definite role in the formation of the effects of EEG-FB sessions. In these experiments, we studied, in a parallel manner, changes in the EEG frequency composition and the activity of NA neurons of the LC in awake cats; this was made in the course of EEG-FB sessions directed toward an increase in the ratio of the spectral power density (SPD) of the α rhythm *vs* SPD of the θ rhythm.

METHODS

Experiments were carried out on two cats. The animals were preliminarily operated under general anesthesia (Nembutal, 40 mg/kg, i.p.) with adherence to aseptic and antiseptic principles; a leading cannula (stainless steel) was implanted in the course of surgery. The cannula tip was positioned at a 5 mm distance above the LC region. The site of recording of neuronal activity corresponded to stereotaxic coordinates A-1, L1-3, and H7-9; as is known, NA neurons are precisely most numerous within this zone. Four active electrodes for monopolar EEG recording were positioned in the cranial bones above the frontal and occipital cortices (along the sagittal axis) and the right and left temporal regions. A reference electrode was fixed in the frontal sinus. The electrodes were fixed using a fast-hardening plastic and connected to the contacts of a miniature contactor also fixed on the skull. The contactor could be coupled with the encephalograph by a thin flexible cable; in such a way, EEG could be recorded from an awake animal. Two to three days after surgery, the state of the animal allowed us to begin EEG-FB sessions. First, we tried to provide a situation where the experimental animal began to correlate the values of the controlled EEG parameter (α/θ SPD ratio) with the noise level; learning was based on the fact that a relatively loud noise was obviously unpleasant for the animal. Fifty to seventy learning sessions without recording of spiking of brainstem neurons were carried out; then, we began sessions where both EEG and impulse activity of LC neurons were recorded in a parallel manner. These two processes were recorded from awake cats in the resting state. A mobile metallic electrode (diameter 12 μm) in glass insulation with the tip sharpened as an injection needle was used. The recorded neurons were qualified as NA cells according to their localization in the brainstem, a relatively low frequency of their background impulse activity (BIA), and a multiphase pattern and rather great duration of action potentials (APs). The BIA frequency did not exceed 8 sec^{-1} in the state of relaxed awakeness of the animal, while the AP duration usually corresponded to 2.5-3.0 msec [15]. The EEG signals, via an interface constructed on the basis of a twin 3-channel 10-order A/D transformer, were entered into computer. The EEG records were subjected to standard spectral analysis with differentiation of the following frequency components: 1-3, 4-7, 8-13, 14-30, and 31-48 Hz (δ, θ, α, β, and γ rhythms, respectively); ongoing values of the SPDs of these rhythms ($\mu V^2/Hz$) were calculated.

The EEG-FB sessions were carried out according to the following scheme: (i) recording of background indices, (ii) presentation of the sound FB signal (white noise, 1st to 5th min of action), and (iii) aftereffects (6th min). The intensity of noise was the controlled parameter; it varied within a 50 to 80 dB range depending on values of the α/θ SPD ratio in the sagittal occipital lead (action sessions, experimental series). After 7 to 10 such sessions, we began to carry out placebo sessions (control series). In these series, the loudness of the sound signal did not correlate with the pattern of EEG; sound signals from the records obtained earlier were used. In the course of recording of the activity of each neuron, we tried to combine one action session and one placebo session. If the action session was carried out during recording of the activity of one neuron and then changed by the placebo session, the consequence was opposite upon recording of the spike activity of the next neuron. We believed that such organization of the experiment will allow us to more clearly identify the differences between reactions of the same neuron to sound signals whose loudness depended on the pattern of the ongoing EEG of the animal (experimental series) or changed chaotically (control series) and also to estimate simultaneously the possible impact of the activity of examined NA neurons on the formation of EEG-FB effects.

Processing and analysis of the experimental data were carried out using STATISTICA-6.0 software. Differences between EEG characteristics and activity of LC NA neurons in action and control sessions were estimated using single-factor dispersion analysis (ANOVA). Values of the studied indices within each 1-min-long recording interval were normalized with respect to the background values taken as 100%.

After termination of the experiments, animals were euthanized by an overdose of Nembutal. Sites of recording of neuronal activity were controlled on brain slices prepared using a routine technique. The experimental technique was described in more detail earlier [5].

RESULTS AND DISCUSSION

In total, 48 EEG-FB sessions and 27 control (placebo) tests were carried out under conditions of parallel recording of impulse activity of LC NA neurons and ongoing EEG. Results obtained on two animals were combined.

Analysis of EEG showed that, under the experimental conditions used, statistically significant changes in the SPDs of the EEG rhythms were observed only in the occipital lead. The normalized α/θ SPD ratio in this lead increased in the experimental series as compared with that under control conditions (Fig. 1A). Statistically significant shifts ($P < 0.05$) were observed in this case beginning from the 2nd min. The above-mentioned ratios were 109.8 ± ± 2.8 and 125.3 ± 3.3% in the control realizations and experimental series, respectively. Within the 3rd, 4th, and 5th min, these indices were 104.2 ± 2.9 *vs* 122.4 ± ± 3.0% ($P < 0.01$), 111.5 ± 3.0 *vs* 128.9 ± 3.0%, and 98.6 ± 2.7 *vs* 121.0 ± 3.0% ($P < 0.001$), respectively. Changes in the α/θ SPD ratio were preserved within the aftereffect period (6th min; 102.1 ± 2.9 *vs* 121.0 ± ± 2.9%, $P < 0.001$). Thus, the α/θ SPD ratio increased, as compared with the control, by 17 to 29% within all the above-mentioned time intervals.

To make clearer the nature of the observed changes, we separately analyzed the dynamics of the SPDs of the α and θ rhythms recorded from the occipital lead in the course of EEG-FB sessions. As was found, changes in the intensity of the acoustic signal correlated with modulations of ongoing EEG were accompanied by a rise in the α rhythm SPD (Fig. 1B). In this case, statistically significant ($P < 0.01$) differences between the indices of the control and experimental series began to be manifested from the 3rd min of presentation of the sound signal. Within the limits of this time interval, the normalized α SPDs in control and experimental realizations were, on average, 109.1 ± 3.0 and 128.4 ± ± 3.7% of the background values. Within the 4th, 5th, and 6th min, these indices were 120.7 ± 3.2 *vs* 129.4 ± ± 3.3%, 118.0 ± 3.0 *vs* 147.4 ± 3.5%, and 103.3 ± 3.0 *vs* 121.0 ± 3.3%.

The SPD of the θ rhythm differed from the α SPD in its dynamics. During EEG-FB sessions, this index in the experimental series showed nearly no changes, while in the control series it somewhat increased (Fig. 1C). Differences between the powers of this frequency range of EEG oscillations began to be significant ($P < 0.05$) only from the 5th min. Within the above-mentioned time interval, the normalized SPD of the θ rhythm in the control series was, on average, 119.3 ± ± 3.0, while the respective value was only 105.5 ± 3.5% of the background index in the experimental series.

Thus, the increase of the α/θ SPD ratio in the occipital lead related to EEG-FB sessions was provided mostly by a rather considerable increase in the α SPD.

During the EEG-FB sessions, we recorded the activity of 48 LC neurons classified, according to the above-mentioned electrophysiological criteria, as the NA cells. The respective analysis allowed us to find statistically significant changes in the activity of the studied neurons during EEG-FB sessions directed toward increases in the α/θ SPD ratio (Fig. 1D).

As early as from the 1st min of presentation of the acoustic signal, the discharge frequency of NA neurons in the experimental series increased, while it decreased in the control realizations. Within the 1st min, the normalized frequency of discharges of NA neurons in the control and experimental series was 93.7 ± 3.0 *vs* 105.2 ± 2.7%. Within the 2nd, 3rd, 4th, and 5th min of action of the factor used, these values were 94.7 ± 2.3 *vs* 106.9 ± 2.6%, 90.3 ± 2.5 *vs* 112.9 ± ± 2.5%, 93.3 ± 2.6 *vs* 112.7 ± 2.3%, and 97.7 ±3.1 *vs* 110.9 ± 2.2%, respectively. At the end of application of the sound signal (within the 6th min), we observed some increases in the frequency of spiking of NA neurons in both series (to 103.2 ± 2.3 and 116.4 ± ± 2.0%, respectively).

Therefore, our experiments demonstrated that EEG-FB sessions are accompanied by moderate but rather clearly expressed intensification of the spike activity of LC NA neurons. These modifications differed from transformations of the activity of DA cells observed in the same experimental situation [5]. Statistically significant changes in the frequency of spiking of LC NA neurons was manifested at the 1st min of the action of the feedback signal, i.e., noticeably earlier (by about 1 min). This fact indicates that neurons of the LC are more labile from this aspect.

If we take into account that the intensity of NA release in the target structures depends approximately linearly on the frequency of discharges of LC neurons [16] and that axons of these NA neurons are characterized by exclusively rich branching [11], we can suppose that the release of NA in cerebral regions

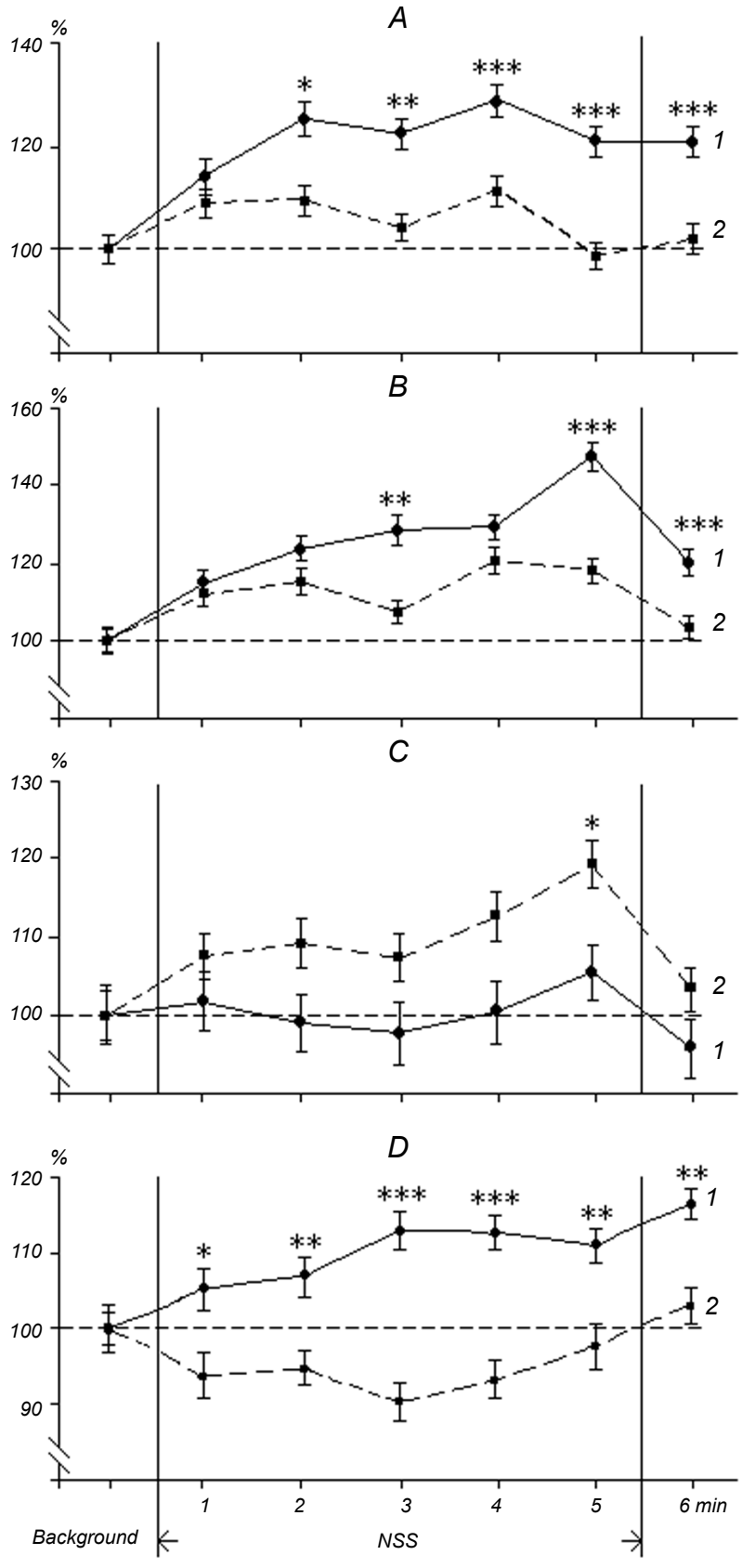

Fig. 1. Dynamics of the mean intragroup values of the examined indices of EEG and spike activity of noradrenergic (NA) neurons during feedback sessions (EEG-FB, 1) or their imitation (2). A) Ratio of the spectral power densities (SPDs) of the α *vs* θ rhythm in the occipital lead; B and C) SPDs of the α and θ rhythms, respectively; D) frequency of impulsation of NA neurons localized in the *locus coeruleus*. Data are averaged for 48 EEG-FB sessions and 27 placebo sessions. Abscissa) Time of recording, min; vertical lines show the interval within which a noise sound signal (NSS) was applied; ordinate) normalized values of the indices, % (initial levels of the latter are taken as 100%). One, two, and three asterisks show cases of significant differences from the control with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

involved in realization of the respective conditioned reflex noticeably increases with rise in the frequency of discharges generated by the above LC neurons. It seems probable that the observed increase in the frequency of NA activity can induce a rise in the efficacy of perception of internal and external stimuli [17] and provide more effective synthesis and processing of information [18, 19].

The above-described changes in the neuronal activity were accompanied by some substitution of relatively low-frequency waves by a high-amplitude α rhythm in the pattern of ongoing EEG. These modifications agree with the results of our preliminary study on cats where we noticed significant positive correlations of the level of activity of LC NA neurons with the SPD of α EEG rhythm [4]. Intensification of the α rhythm observed in this our study can be explained as follows. The task resolved by the animal needed sufficiently stable, selective, and focused attention. Taking into account the role of NA produced by LC neurons in these processes, we can suppose that transformation of the activity of these cells observed in the course of EEG-FB sessions are related to "search" for the state corresponding to the possibility of effective performance of the behavioral task by the animal. The observed effects can be compared to an earlier observed reverse U-like relation between the intensities of tonic and phasic activity of LC neurons, on the one hand, and level of performance of the operant task dependent on the attention level provided by the animal, on the other hand [20, 21]. This dependence looks like a classic Yerkes–Dodson curve. Most tasks are realized more adequately when some intermediate level of behavioral excitation (arousal) is maintained. The result is less successful at excessively low or excessively high levels of excitation. In our experiments, the animal was in a relatively comfortable state during recording of initial EEG samples; this corresponds, in general, to a relatively low level of tonic and phasic activity of NA neurons. In cats, this state is accompanied by domination of relatively low-frequency waves in the EEG composition. When the animal resolves the task providing decrease in the noise intensity and searches for an optimum comfort state, some intermediate level of the tonic activity of the above-mentioned neurons related to the maintenance of focused attention is probably reached. Such a level of excitation is manifested in the α rhythm domination, as compared with the θ activity. When an excessively high level of tonic activity of NA cells is attained (this is the phenomenon underlying the increased lability of attention; it can lead to disorders in the conditioned reflex activity), the animal, based on the feedback principle, decreases the above activity, thus providing more comfortable conditions (decrease in the noise intensity).

Thus, not only activation of DA neurons in the ventral tegmentum [5], but also creation of a relatively increased NA level in sufficiently extensive cerebral regions, is one of the crucial factors determining the efficacy of EEG-FB sessions. These events lead to simultaneous modification of the state of great neuronal populations related to the formation of the EEG α rhythm. Such shifts promote the formation of the conditioned reflex and facilitate the animal's learning to control the rhythms of its own EEG.

REFERENCES

1. R. Kall, J. Kamiya, and G. E. Schwartz, *Applied Neurophysiology and Brain Biofeedback*, Future Health, Bensalem (2000).
2. J. F. Lubar, "Neocortical dynamics: implication for understanding the role of neurofeedback and related techniques for the enhancement of attention," *Appl. Psychophysiol. Biofeedback*, **22**, No. 2, 111-126 (1997).
3. M. Linden, T. Habib, and V. Radojevic, "A controlled study of the effects of EEG biofeedback on cognition and behavior of children with attention deficit disorder and learning disabilities," *Biofeedback Self-Regul.*, **21**, No. 1, 35-49 (1996).
4. O. I. Kolotilova, A. M. Kulichenko, Yu. O. Fokina, et al., "Effect of the brainstem structures on the pattern of mass electrical activity in awake cats," *Uch. Zap. Tavricheskii Nats. Univ.*, **18**, No. 2, 34-42 (2005).
5. A. M. Kulichenko, Yu. O. Fokina, and V. B. Pavlenko, "Changes in EEG rhythms and spike impulse activity of brainstem dopaminergic neurons induced by neurofeedback sessions in cats," *Neurophysiology*, **41**. No. 3, 196-200 (2009).
6. A. Yu. Budantsev, *Monoaminergic Brain Systems* [in Russian], Nauka, Moscow (1976).
7. T. I. Belova, E. L. Golubeva, and K. V. Sudakov, *Homeostatic Functions of the Locus Coeruleus* (in Russian), Nauka, Moscow (1980).
8. A. I. Semenyutin, "On the topography of catecholamine-containing neurons of the *locus coeruleus* in the cat brain," *Fiziol. Zh.*, **35**, No. 2, 7-12 (1989).
9. A. I. Semenyutin and V. A. Maiskii, "Distribution of noradrenaline-containing neurons projecting in the parietal associative cortex and spinal cord, in the *locus coeruleus* of the cat," *Neirofiziologiya*, **21**, No. 1, 112-121 (1989).
10. Yu. P. Limanskii, "Morphofunctional organization of the aminergic systems and their role in the motor activity controlled by the brain," *Usp. Fiziol. Nauk*, **21**. No. 2, 3-17 (1990).
11. G. Nistico and G. Nappy, "*Locus coeruleus*, an integrative station involved in the control of several vital functions," *Funct. Neurol.*, **8**, No. 1, 5-25 (1994).

12. V. B. Pavlenko and A. B. Kulichenko "Self-initiated motor behavioral act-related neuronal activity in the cat *locus coeruleus*," *Neurophysiology*, **35**, No. 1, 29-37 (2003).
13. M. E. Olds, "Effect of intraventricular norepinephrine on neuron activity in the medial forebrain bundle during self-stimulation behavior," *Brain Res.*, **3**, No. 3, 461-477 (1974).
14. L. Stain, J. D. Belluzzi, S. Ritter, and C. D. Wise, "Self-stimulation reward pathways: norepinephrine vs. dopamine," *J. Psychiat. Res.*, **11**, 115-124 (1974).
15. K. Rasmussen and J. L. Barry, "Single units activity of *locus coeruleus* neurons in the freely moving cat. 1. During naturalistic behaviors and in response to simple and complex stimuli," *Brain Res.*, **371**, No. 2, 324-334 (1986).
16. C. W. Berridge and E. D. Abercrombie, "Relationship between *locus coeruleus* discharge rates and rates of norepinephrine release within neocortex as assessed by *in vivo* microdialysis," *Neuroscience*, **93**, No. 4, 1263-1270 (1999).
17. C. W. Berridge and B. D. Waterhouse, "The *locus coeruleus*–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes," *Brain Res. Rev.*, **42**, No. 1, 33-84 (2003).
18. S. Sara, "The *locus coeruleus* and cognitive function: attempts to relate noradrenergic enhancement of signal," *Physiol. Psychol.*, **13**, No. 3, 151-162 (1985).
19. Yu. P. Limanskii, *Reflexes of the Brainstem* [in Russian], Naukova Dumka, Kyiv (1987).
20. J. Aston-Jones, J. Rajkowski, and J. Cohen, "*Locus coeruleus* and regulation of behavioral flexibility and attention," *Prog. Brain Res.*, **126**, No. 11, 155-182 (2000).
21. J. Aston-Jones and J. Cohen, "An integrative theory of *locus coeruleus*-norepinephrine function: adaptive gain and optimal performance," *Annu. Rev. Neurosci.*, **28**, No. 1, 403-450 (2005).