Correlation between the Activity of Dopaminergic Neurons of the Ventral Tegmentum and Spectral Power of the EEG Rhythms in Awake Cats

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We studied correlations between the frequency of background impulse activity (BIA) of dopaminergic (DA-ergic) neurons of the ventral tegmentum (VT) and spectral power (SP) of the frequency components of EEG samples recorded in awake cats. The EEG was recorded monopolarly (electrodes were fixed in the cranial bones) from the frontal, occipital, and right and left temporal regions of the cortex. In a great majority of the cases, the BIA frequency of VT DA-ergic neurons demonstrated significant positive correlations with changes in the SPs of the alpha and beta EEG rhythms. The closest correlations of the spiking frequency of DA-ergic cells with the SP of the alpha rhythm was observed in the occipital region, while those with the beta SP were found in the frontal area. Correlations of the activity of DA-ergic neurons with the SPs of the alpha and beta rhythms in the left temporal cortical zone were closer, as compared with those in the symmetrical right zone. Correlations of the SPs of the delta, theta, and gamma EEG components with the discharge frequency of VT DA neurons were of opposite directions, and in most cases such correlations did not reach the level of significance. The results of this study show that, in some cases, specific EEG patterns can be considered indicators of the state of the cerebral VT DA-ergic system.

Keywords: dopaminergic neurons, ventral tegmentum, impulse activity, EEG, spectral composition.

INTRODUCTION

Recording and analysis of EEG are effective means for estimation of the functional state of the CNS; the corresponding techniques are extensively used in research and clinical practice. At the same time, it should be recognized that the mechanisms underlying the formation and modifications of the EEG frequency components (rhythms) remain unclear in many respects [1, 2]. The relations between the activity of neuromodulatory cerebral systems and EEG characteristics attract significant interest from researchers. In 1997, Lubar supposed that the activity of neurons belonging to the aminergic cerebral systems crucially influences the process of formation of EEG rhythms [3]. Our earlier studies showed that rather clear correlations between the parameters of spike activity of brainstem serotonergic neurons of the *nuclei raphe* and noradrenergic cells of the *locus coeruleus*, on the one hand, and changes in the spectral power (SP) of a few main EEG rhythms, on the other hand, do exist [4]. The dopamine (DA)-ergic system is another main aminergic brainstem system. The activity of this system demonstrates clear relations to the organization of motor acts [5, 6], formation of memory engrams [7], changes in the state of systems providing attention [8], and the development of positive emotions [8, 9]. The DA-ergic system is also a component of the mechanisms of motivation [10] and exerts a significant modulatory influence on the psychophysiological state of animals and humans. It can be expected that this system, similarly to other aminergic systems, will definitely contribute to the formation of EEG rhythms and influence their parameters. This is why we examined, in the experiments on unanesthetized cats described below, correlations between the characteristics of impulse activity of DA-ergic neurons (supposedly identified according to their localization and parameters of their spikes) of the ventral tegmentum (VT) of the cat midbrain and changes in the SPs of the main EEG rhythms.

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METHODS

Six awake cats of both sexes weighing 2.5 to 4.0 kg were used in the experiments. Animals were preliminarily operated (Nembutal anesthesia, 40 mg/kg, i.p.); surgery was performed in accordance with the principles of aseptics and antiseptics. A leading cannula (stainless steel) was stereotaxically implanted; its tip was positioned 5 mm above the VT region. To avoid damage to the venous sinus by the cannula, the latter was inserted into the brain tissue at a 10.3 deg angle with respect to the sagittal plane. The region of recording of neuronal activity corresponded to the stereotaxic coordinates A +4…5, L 0…2, and H 4…5. As is known, DA-ergic neurons are most densely localized within this zone. The EEG was recorded monopolarly by four electrodes fixed in the cranial bones above the frontal, right and left temporal, and occipital regions of the cortex (frontal and occipital electrodes were positioned on the sagittal line). A reference electrode was fixed in the frontal sinus. The recording surface of each EEG electrode was positioned in a small nonpenetrating hollow in the cranial bone made using a dental bur; electrodes were rigidly fixed with acryl oxide and connected to the contacts of a miniature switchbox also fixed on the skull. The switchbox could be connected to the contacts of the encephalograph by a thin flexible cable; this arrangement allowed us to record EEG from the awake animal with no intense motor activity.

Two to three days after surgery, the state of the animal allowed us to begin the main experiments with parallel recording of impulse activity of VT neurons and of EEG in awake animals under conditions close to unrestricted behavior (under conditions of motor resting) (Fig. 1).

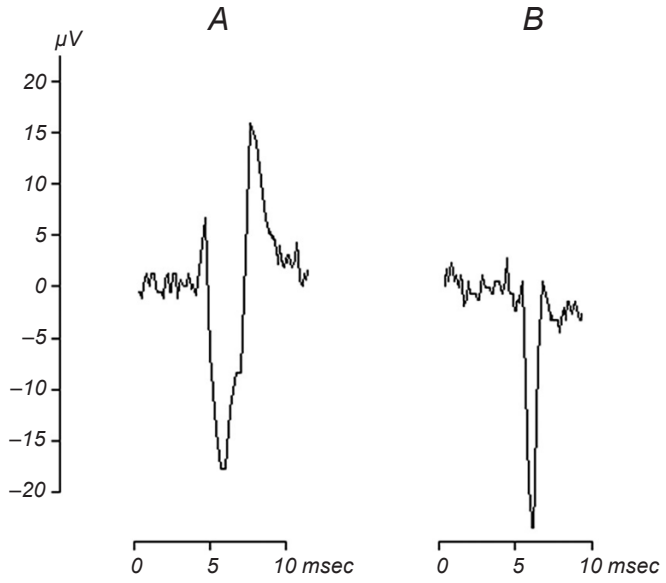

Fig. 2. Action potentials of a supposedly dopaminergic units (A) and a non-dopaminergic (B) neuron of the ventral tegmentum of the cat under conditions of extracellular recording.

To record the activity of single VT neurons, we used a movable electrode made from a silver microwire (diameter 12 μm) in glass insulation, with the end obliquely sharpened similarly to an injection needle. The bandpass of the recording/amplification tract of neuronal spike activity was 10 Hz to 10 kHz. The signal came to the soundmap of a computer (digitization frequency up to $4 \cdot 10^4$ Hz) and, in a parallel manner, to a monitor providing visual control. The neurons under study were qualified as supposedly DA-ergic cells according to their localization in the brainstem, a relatively low frequency of their background impulse activity (BIA) that did not exceed 8 sec^{-1} under

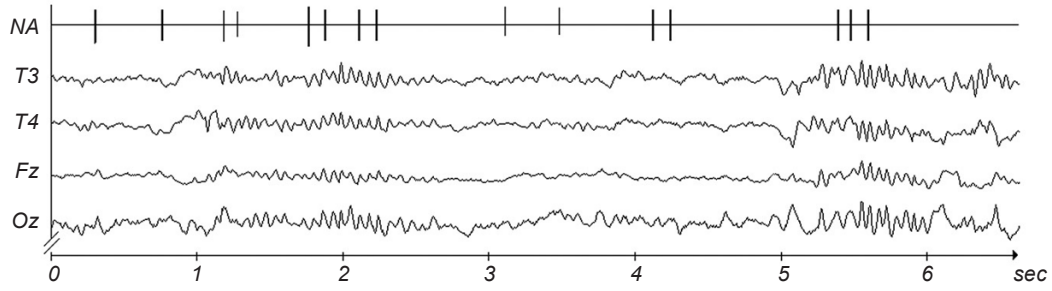

Fig. 1. Example of parallel recording of background impulse activity of a dopaminergic neuron of the ventral tegmentum and field neocortical activity in real time using the EEG Mapping 3 software. Horizontal scale) Time of recording, sec; from above to below) NA is neuronal activity; T3, T4, Fz, and Oz are EEG recordings from the left temporal (T3), right temporal (T4), frontal (Fz), and occipital (Oz) leads.

conditions of the resting awake state of the animal, a polyphasic pattern of action potentials (APs), and their long duration (2.5-5.0 msec) under conditions of extracellular recording (Fig. 2) [11].

The EEG signals were fed into the computer via an interface constructed on the basis of a doubled three-channel 10-order A-D converter. The frequency of digitization of EEG signals was 200 sec^{-1}. Segments of constant recording of the analyzed EEG and BIA of VT neurons were 60 sec long. The data were processed off-line using the EEG Mapping 3 software specially prepared for realization of the above-described techniques (programmer E. Zinchenko). The EEG samples were subjected to standard spectral analysis; the frequency components 1-3, 4-7, 8-13, 14-30, and 31-48 Hz (delta-, theta-, alpha-, beta-, and gamma rhythms, respectively) were classified, and current values of their SPs were calculated.

After termination of the experiment, the animals were sacrificed by injection of Nembutal in a lethal dose, and localization of the region of recording was verified. A nichrome needle electrode was inserted into the corresponding depth instead of the leading cannula, and a 2 mA current of positive polarity was passed through this electrode for 1 min. Then the brain was fixed in formalin solution, and frontal slices were prepared using a freezing microtome. The position of the coagulated zone was verified on these transverse slices (Fig. 3) according to the stereotaxic coordinates (atlas of Reinoso-Suarez [12]).

Further processing and analysis of the experimental data were performed using STATISTICA-6.0 software. Spearman's coefficients of rank correlation (r) were calculated for characterization of correlations between the BIA frequency of VT DA-ergic neurons and values of the SPs of separate EEG rhythms.

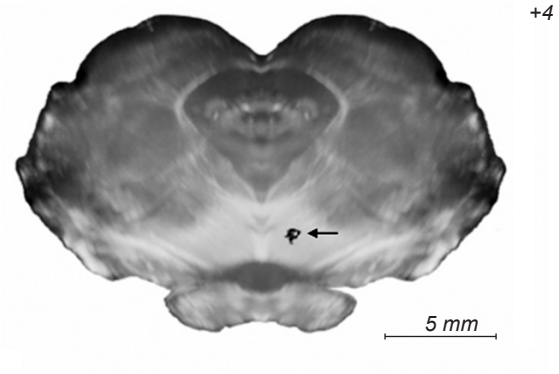

Fig. 3. Frontal slice of the brain transecting the zone of localization of dopaminergic neurons of the ventral tegmentum. An electrolytic label (shown by arrow) corresponds to the region where the spike activity of supposedly dopaminergic neurons was recorded.

RESULTS AND DISCUSSION

In the course of experiments, we recorded the activity of 62 units, which, according to the above-mentioned criteria, were supposedly classified as DA-ergic neurons. In a parallel manner with recording from such neurons, whose APs were, as a rule, three-phase and long-lasting (longer than 2.5 msec), units with short (about 1 msec) two-phase or practically monophase APs were also recorded (Fig. 1). The latter cells were not included in the studied group of supposedly DA-ergic neurons. When analyzing correlations of the frequency of impulsation of VT DA-ergic neurons with the SPs of different EEG rhythms, we estimated the normalized numbers of the cases where the BIA frequency of the above neurons significantly correlated with the values of the SPs of the EEG rhythms in each of the four leads and also the level of such correlations. The correlations were classified as moderate, considerable, close, and extraclose in the cases where r values were within 0.31 to 0.50, 0.51 to 0.70, 0.71 to 0.90, and ≥0.91 ranges, respectively [13].

Analysis of correlations between the BIA frequency of DA-ergic neurons and SPs of the EEG rhythms performed for the entire number of recording sites (Fig. 4) allowed us to conclude that the intensity of discharges of VT DA-ergic neurons correlates most closely with the alpha SP. In general, the proportion of DA-ergic neurons with the BIA frequency manifesting significant positive correlations with the SP of the alpha EEG rhythm was 40.7% (101 cases of 248 possible for 62 neurons and 4 EEG leads). Among these cases, the number of situations where the discharge frequency of DA-ergic neurons was in moderate correlations with the alpha-rhythm SP was 49 (19.8%). In 14.1% of the cases (35 of 248), correlation was considerable, in 4.4% (11 cases) it was close, and in 2.4% (6 cases) it should be classified as extraclose. The level of statistically significant positive correlations between the BIA frequency of VT DA-ergic neurons and SP of the beta rhythm was also rather high. The proportion of such cases in the entire population of recordings was 37.1% (92 significant correlations among 248 possible; see above). The intensities of correlations between the frequency of spiking of DA neurons and beta SPs were distributed within the entire population of recordings in such a way. Correlations were

moderate in 38 cases (15.3%), they were considerable in 48 cases (19.4%), and they were close in 6 cases (2.4%). It should be noted that negative correlations between the studied parameters were rarely found. In contrast to correlations with the SPs of the alpha and beta rhythms, situations with a positive sign of correlations of spiking of DA-ergic neurons with the powers of the delta, theta, and gamma rhythms were not dominant. In most cases, the latter correlations did not reach the significance level.

As is supposed [14], the existence of sufficiently well expressed alpha and beta rhythms in the pattern of current EEG is related to a definite level of activation of thalamo-cortical neuronal networks. It was shown [15] that the DA-ergic cerebral system possesses rather highly developed projections to the thalamic, limbic, and cortical brain regions. Using a positron tomography technique, a positive correlation between an increase in the activity of the VT in humans and transition from the prevalence of the low-frequency EEG rhythms to that of the alpha rhythm was demonstrated [16]. Considering these data and results of our study, we can suppose that the DA-ergic system of the VT plays an important role in modulation of the activity of intracortical and thalamo-cortical pathways involved in generation of the alpha and beta rhythms.

The numbers of VT DA-ergic neurons of the examined group, whose activity positively and significantly correlated with the SPs of the alpha and beta rhythms of EEG samples recorded from the frontal sites, were 22 (35.5%) and 24 (38.7%), respectively. At the same time, the respective figures for the "occipital" EEG activity were 29 (46.8%) and 23 (37.1%). As could be noticed, a high level of correlations of the frequency of BIA of VT DAergic neurons with the SP of the beta rhythm recorded from the frontal leads was observed more frequently than that for occipital leads. For "frontal" EEG, considerably more intense positive correlations were found for the activity of 12 DA-ergic neurons (19.4%) (Fig. 5A). Some authors believe that a high level of the beta rhythm is related to intensification of the motor cortex activity richly innervated by the DA-ergic system [11, 17]. The studies of Storozhouk [18] demonstrated that DA-ergic influences play a significant role in the organization of the motor function at the cortical level. In addition, as was found in a few studies on rats, activation of the DA-ergic system (e.g., upon application of an agonist of dopamine, midantan) was accompanied by a rise in the SP of EEG within a 19-30 Hz range, while the power of these oscillations within this frequency range decreased under the influence of an antagonist of the above amine, haloperidol [19].

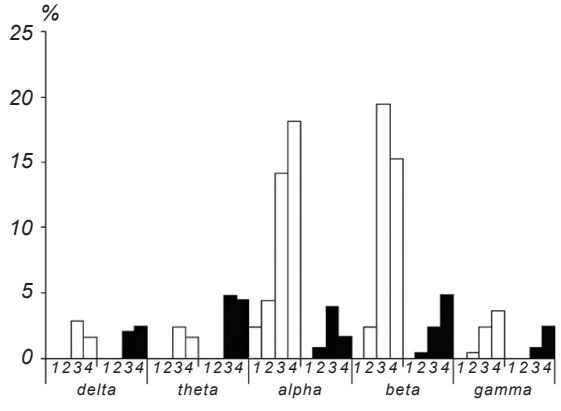

Fig. 4. Normalized numbers of the examined dopaminergic neurons (%) whose activity manifested significant positive and negative (open and filled columns, respectively) correlations of different intensities with the spectral power of the EEG rhythms within the entire totality of recordings. 1-4) Extraclose ($r \geq 0.91$), close ($r = 0.71$ to 0.90), considerable ($r = 0.51$ to 0.70), and moderate ($r = 0.31$ to 0.50) correlations, respectively.

The intensity of correlations between the frequency of background discharges of VT DA-ergic neurons and the SP of the alpha EEG rhythm in the occipital lead was higher than that of the BIA frequency of these neurons with the SP of the beta EEG components in the same lead. Correlations between the unit discharge frequency and the alpha SP in this lead were moderate in 15 neurons (24.2%) and considerable in 11 DA-ergic cells (17.7%); impulsation of three DA-ergic neurons (4.8%) demonstrated extraclose positive correlation with the SP of the above rhythm recorded from the mentioned region (Fig. 5B). These data appear somewhat unexpected because it was shown that the density of DA innervation of the occipital neocortical zones is the lowest [11]. At the same time, oscillations of the alpha range, as is known, demonstrate the greatest amplitude and stability precisely in the occipital and parietal cortical regions [17]. It seems logical to suppose that dopamine released by cells of the DA-ergic system exerts modulatory effects not only in a direct mode (on the neocortical cells) but also on the thalamic neurons playing an important role in the formation of the alpha rhythm [14].

Analysis of correlations between the frequency of BIA generated by VT DA-ergic neurons and SP of the rhythms of EEG recorded from the contralateral (in our case, right) and ipsilateral (left) temporal loci also allowed us to demonstrate the predominance of positive statistically significant correlations with the SPs of the alpha and beta EEG rhythms. The BIA frequency of 28 examined DA-ergic neurons (45.2%)

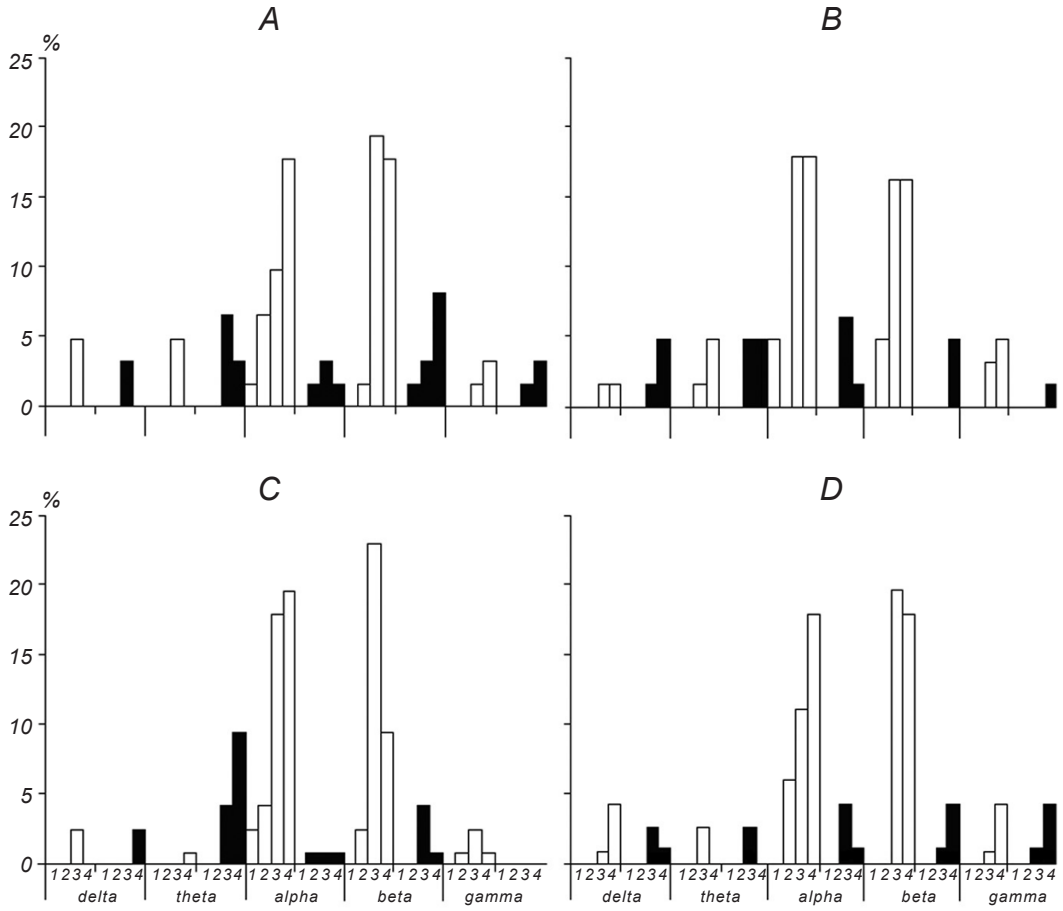

Fig. 5. Normalized numbers of the examined dopaminergic neurons (%) whose activity manifested significant positive and negative (open and filled columns, respectively) correlations of different intensities with the spectral power of the EEG rhythms recoded from the frontal (A), occipital (B), left temporal (C), and right temporal (D) leads.

most closely correlated with the alpha SP in the left temporal locus (this index was higher than the indices in all other leads). In most cases, these correlations were characterized by moderate intensity (Fig. 5C). In particular, the BIA frequency was in moderate positive correlation with the alpha SP in the left temporal lead in 21.0% of the cases (13 cells). Correlation of considerable strength was noticed in 17.7 (11 neurons), while close and extraclose correlations were found in 3.2% (2 and 2 neurons with such levels) (C). The number of cases of significant correlations between the BIA frequency of DA-ergic neurons and SPs of the beta rhythm observed in different temporal leads was approximately the same, 35.5% (22 neurons) and 37.1% (23 neurons) for the ipsi- and contralateral leads, respectively. At the same time, recording was performed from the left temporal lead, high r values for correlations of the discharge rate and SP of the beta EEG component were found more frequently. The distribution of the intensities of the respective correlations was the following. Moderate, considerable, and close correlations were typical of 6 (9.7%), 14 (22.6%), and 2 (3.2%) neurons. At the same time, correlations for the right temporal lead were moderate in 11 cases (17.7%) and considerable in 12 cases (19.4% of DA-ergic neurons) (C, D). The closer correlations between the BIA frequency of DA-ergic neurons and the SPs of the alpha and beta rhythms in the left temporal lead can be related to the design of our experiments; the unit neuronal activity was recorded from the left VT, and this structure, as is known, sends ipsilateral (uncrossed) projections to the neocortex. There are also indications that the left hemisphere is to a greater extent subjected to modulatory influences of the catecholaminergic systems, while the right hemisphere is more influenced by the serotonergic system [20]. The content of DA was found to be higher in the left hemisphere [21].

Thus, the correlations between the BIA frequency of VT DA-ergic neurons and the SPs of the alpha and beta

EEG rhythms observed in our study demonstrate that the DA-ergic cerebral system significantly contributes to the formation of a specific EEG pattern. The findings in our work show that such specific patterns can be used as certain indicators of the state of the above-mentioned system (brainstem VT). It should be taken into account that a great part of the effects of catecholaminergic brainstem systems in general and of the DA-ergic system in particular are not realized in a "classic" mode of discrete synaptic transmission from a given neuron to another neuron (other neurons); these effects are, to a considerable extent, provide by the so-called volume transmission, e.g., by neurosecretion of the corresponding transmitters from non-synaptic structures (varicosities and others). Therefore, creation of an increased level of the transmitter (in our case of dopamine) in rather extensive brain regions is a crucial factor determining the effects of such systems; this results in simultaneous modifications of the state of large neuronal populations in the target cerebral structure. An understanding of the significant role of such neurochemical phenomena has led a number of researchers working in neuroscience to recognize the necessity of a closer integration of "purely electrophysiological" and biochemical approaches in interpretations of the mechanisms of formation and modulation of the EEG rhythms. We believe that the "bridging" of "dry electrophysiology" and "liquid" (i.e., neurochemical) methods of analysis of cerebral functions has become obviously more urgent [17].

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