Patterns of Neuronal Functioning in the Hypothalamus and Cerebral Cortex of Rats in Different Motivational/Emotional States

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Firing activity of the same neurons and neuronal pairs in the hypothalamus and cerebral cortex is analyzed in rats during four consecutive motivational/emotional states controlled at the behavioral level (states of motivation, removed motivation, predominantly positive emotions, and predominantly negative emotions). Generalized characteristics of neuronal activity are identified that reflect the four states.

Key Words: *neuronal activity; hypothalamus; cerebral cortex; motivational/emotional states; behavior*

During the elaboration of a conditioned reflex, the cerebral cortex of the animal is believed to receive concordant afferent impulses generated by the stimuli being combined as well as specifically organized reinforcing inputs from motivatiogenic and emotiogenic subcortical structures, including the hypothalamus [3]. It is also thought that one motivational/emotional status of the animal is succeeded by another during the development of a conditioned response, with hypothalamic motivational influences on the cortex predominating at the initial stages of its development and emotional influences at the concluding stage [2]. The specificity of these influences is probably determined by reorganization of the activity of hypothalamic neurons. Dependence of neuronal functioning on a particular motivational/emotional state can be manifested at the level of cortical neurons (specifically, the sensorimotor cortex neurons) experiencing direct hypothalamic influences [5,7]. We are aware of a limited number of studies [4,6,8,9] where firing activities of hypothalamic and cortical neurons are compared in several motivational/emotional states.

This study was designed to detect alterations in the functioning of identified neurons and neuronal pairs in the hypothalamus and cerebral cortex of rats following consecutive changes from one state to another controlled at the behavioral level and characterized, respectively, by motivation, absence of motivation, predominantly positive emotions, and predominantly negative emotions.

MATERIALS AND METHODS

Experiments were performed on 12 unrestrained male white rats. Stimulating electrodes (insulated Nichrome wire 100 μ in diameter) were chronically implanted into the lateral hypothalamus and dorsomedial tegmentum (structures of positive and negative reinforcement, respectively). In preliminary tests carried out 7 days after implantation, the position of each electrode was checked, and the characteristics of stimulating electric current required to elicit the appropriate behavioral responses were determined. Specifically, stimulation of the lateral hypothalamus resulted in approaching the stimulation site and self-

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stimulation, while stimulation of the dorsomedial tegmentum led to avoiding that site. The stimuli were batches of 30 monophasic square 0.1-msec pulses presented at a frequency of 100 Hz. The batches were delivered as 5-sec series at a frequency of 1 Hz, and the series were repeated 10 times over a 5-min period.

Multichannel recording electrodes (insulated Nichrome wire 50 μ in diameter) were chronically implanted into the lateral hypothalamus (9 rats) or into the sensorimotor cortex (3 rats). After experiment, positions of the stimulating and recording electrodes were identified morphologically.

During the experiment, the rats were caused to experience in sequence four different states: *a*) alimentary motivation (hunger), *b*) no alimentary motivation (satiation), *c*) positive emotions, and *d*) negative emotions, the latter two states being induced through stimulation of the emotionally positive and negative structures, respectively. Each state was succeeded by the next after 0.5 to 1.5 h, and the background multineuronal activity was recorded for 5 min in each state. In states (*c*) and (*d*), recording was started after completion of intracerebral stimulation.

During the recording of multineuronal activity, the sequence of spikes pertaining to a single neuron was singled out from this activity by the method of amplitude discrimination. For each recording electrode, up to 3 "windows" were provided so that the pulses entering each window differed by at least 30% from those entering the other two. The separated sequences of times at which neuronal impulses appeared were subjected to statistical analysis. The firing frequency of each neuron was calculated. The stability of impulse traffic from each neuron was controlled on the basis of the mean frequency at a given moment of time. An autocorrelation histogram (ACH) showing the distribution of intervals between all (not only adjacent) neuronal impulses was then constructed for each animal. In addition, a crosscorrelation histogram (CCH) showing the distribution of intervals between all impulses from the neuronal pairs of simultaneously identified neurons was constructed.

The electrostimulation, recording of neuronal activity, amplitude discrimination of neuronal impulses, and subsequent statistical analysis of impulse sequences were all performed using an IBM PC/AT computer linked to the stimulatory and amplifying units of the experimental setup through an interface containing analog-digital and digital-analog converters.

RESULTS

A total of 41 hypothalamic and 12 cortical neurons were identified; of these, 23 and 4, respectively, were recorded in all four motivational/emotional states. The mean firing frequencies of hypothalamic and cortical neurons ranged 0.5-35 Hz and 0.4-17 Hz, respectively. Disordered generation of solitary impulses, when interimpulse intervals of any duration occurred with equal probability, was observed for 70% of the hypothalamic neurons and 75% of the cortical neurons at least in one of the four states; the corresponding ACH then had a statistically "flat" form, i.e., did not contain significant deviations from the mean level (with the exception of a trough in the environs of the zero interimpulse interval, reflecting a refractory period in neuronal firing). Approximately the same proportions of hypothalamic and cortical neurons were observed to show ordered firing at least in one state, and the corresponding ACHs then had more or less marked peaks — an indication that the probability of impulse generation by the neuron at a given interval was increased. In particular, neuronal impulse trains contained indistinct groups of impulses (manifested on the ACH as a broad peak located in the region of short interimpulse intervals and gently sloping in the direction of longer intervals) as well as short impulse volleys (a narrow peak with steep fronts in the region of short intervals on the ACH). Where impulse volleys followed each other in an orderly manner, a second,

TABLE 1. Statistical Characteristics of Firing Activity Recorded for Individual Hypothalamic Neurons and Neuronal Pairs in Rats with Different Motivational/Emotional States

Neuronal activity	Total	States				
		All four	*a*	*b*	*c*	*d*
Number of identified neurons	41	23	36	40	32	34
Neurons generating groups/volleys of impulses, %	68.2	26.1	41.6	45	53.2	53.0
Number of identified neuronal pairs	37	21	31	33	30	31
Neurons showing dependent functioning, %	40.6	9.5	32.3	27.3	33.3	25.9

Note. Here and in Table 2: *a* = motivational state; *b* = "neutral" state; *c* = positive emotional state; *d* = negative emotional state.

wider and lower peak appeared on the ACH. If the volleys were generated rhythmically, then the ACH had a periodic form.

Joint firing activity was recorded for 37 pairs of hypothalamic and 9 pairs of cortical neurons, 23 and 3 of which, respectively, were identified in all four states. Pulse trains of one neuron in a pair depended on those of the other neuron at least in one state in 40% of the hypothalamic neuronal pairs and 33% of the cortical neuronal pairs. Increased probabilities of synchronous generation by neurons of solitary impulses (a narrow peak on the CCH in the environs of the zero interimpulse interval), impulse volleys (a wider central peak on the CCH), and groups of impulses (a broad and low central peak on the CCH) were noted. As a rule, the central peaks covered the zero interimpulse interval by their base. In a few cases, a narrow peak appeared against the background of a broader peak on the CCH. Other peaks and troughs reproducing characteristics of shape seen on the respective ACH could be present on both sides of the narrow central peak on the CCH — the main sign that the impulse traffics of the two neurons were interdependent.

The characteristics of firing by hypothalamic neurons and neuronal pairs could undergo substantial changes as the animal passed from one state to another. For instance, during sequential transition from states *a* to *d*, the disordered firing by a hypothalamic neuron transformed into the firing first of pulse volleys and then of rhythmic volleys followed by complete disappearance of volleys in the final (*d*) state. The initial firing of groups of impulses by the adjacent neurons was succeeded by its firing first of volleys and then again of groups of impulses, and finally by the firing of disordered impulses. The mean firing frequency in the different states differed by 20%. The initial synchronization of impulses generated by hypothalamic neurons was superseded by synchronization of volleys, followed by the reappearance of synchronized impulses, after which the neurons began discharging impulses independently of each other. Sometimes the narrow peak present on the CCH against the background of a wide peak disappeared and then reappeared, while the wide peak was still present or, on the contrary, the wide peak disappeared and the narrow peak was retained. These patterns indicate the existence of different and independent sources of two types of synchronization of impulse trains from hypothalamic neurons.

Changes of states also involved substantial alterations in the characteristics of firing by cortical neurons and neuronal pairs. For instance, one cortical neuron discharged pulse volleys in states *a* and *b* but not in state *c*, when its firing frequency was two times lower than in the two other states; in state *d*, the neuron restored the former firing frequency and began discharging impulses in groups. The adjacent neuron showed approximately equal mean firing frequencies and showed weak group activity in all four states. The synchronization of pulse volleys by one neuron and of pulse groups by the adjacent neuron observed in states *a* and *b* practically disappeared in state *c*, being partially restored in state *d*.

Of the hypothalamic neurons recorded in all four states, 31% demonstrated only disordered firing, 26% only ordered, and 43% disordered or ordered firing depending on the animal's state. Upon transition to a new state, the number of neurons whose disordered firing was succeeded by ordered firing was considerable, but comparable to that whose ordered firing was replaced by disordered activity, so that the different states differed only little with regard to the proportion of neurons firing in an orderly fashion, although the proportion showing orderly activity in the emotionally colored stated (*c* and *d*) was somewhat greater than in the two other states (Table 1).

Of the hypothalamic neuronal pairs recorded in all states, the neurons in 66% of the pairs were discharging only independently, 10% only dependently, and 24% independently or dependently in different states. The proportion of dependently functioning neurons in the total number of recorded pairs also differed only slightly in the different states, but was somewhat higher in the presence of motivation (state *a*) and positive emotions (state *c*) (Table 1).

Taking into account the largest differences between the values presented in Table 1, each of the four states of the animal can be identified from two variables — the proportion of hypothalamic neurons with ordered activity and the proportion of neuronal pairs with dependent pulse trains. The first variable divides all states into two classes — *a* and *b* with 41-45% of neurons and *c* and *d* with 53% of neurons. The second variable identifies particular states in each class — *a* and *c* with 32-33% of neuronal pairs and *b* and *d* with 26-27% of neuronal pairs.

Individual states of the animal were recognized more reliably with the use of generalized indicators of changes in functional characteristics of hypothalamic neurons occurring upon transition from one state to another, because the relevant numerical values were more scattered, while showing a stronger tendency towards grouping. Thus, an informative indicator proved to be the proportion of neurons that increased or decreased the mean firing frequency when the animals passed to a new state (Table 2). This factor immediately divides all possible transitions into three classes; thus, the proportion of neurons that increased their firing frequency was about 40%

TABLE 2. Effects of Transition from One Motivational/Emotional State to Another on Statistical Characteristics of Firing Activity by Hypothalamic Neurons and Neuronal Pairs in Rats

Neuronal activity	Transition from state to state					
	$a\to b$	$a\to c$	$a\to d$	$b\to c$	$b\to d$	$c\to d$
Number of identified neurons	35	27	29	31	33	29
Neurons that increased mean firing frequency, %	42.8	40.7	41.4	64.5	60.6	27.6
Neurons whose mean firing frequency changed ≥2 times, %	17.2	29.7	34.5	25.9	21.3	0.0
Groups/volleys of impulses, %:						
appeared	11.4	18.5	24.1	16.1	18.2	17.2
disappeared	11.4	7.4	17.2	9.6	12.1	17.2
Number of identified neuronal pairs	28	24	25	22	29	28
Dependence of impulse trains, %:						
appeared	0.0	4.2	4.0	9.1	3.4	3.6
disappeared	10.7	4.2	12.0	0.0	0.0	7.1

after the transitions from state a ($a\to b$, $a\to c$, $a\to d$), about 60% after the transitions from state b ($b\to c$, $b\to d$), and about 28% after the transition from state c ($c\to d$).

A second factor is the proportion of neurons that altered the mean firing frequency by 2 times or more (Table 2). This factor sharply divides the transition from state c to state d (0%), and it also differentiates the transition $a\to b$ (17%) and the transitions $a\to c$ and $a\to d$ (30-34%). Although the transition $a\to b$ is poorly differentiated from $b\to c$ and $b\to d$ with this factor, they are well recognizable with the use of the first factor. The second factor also distinguishes, though less well that the first, $a\to c$ and $a\to d$ from $b\to c$ and $b\to d$. Using only these two factors, it is possible to identify the transitions $a\to b$ and $c\to d$ and to divide the other transitions into two classes — $a\to c$ & $a\to d$ and $b\to c$ & $b\to d$.

A third factor is the proportion of neurons changing their firing from orderly to disorderly relative to those changing it vice versa following transition from one state to another. The total number of such neurons could reach 40% upon the transition $a\to d$, although the total number of neurons with a particular type of firing activity differed by only several percent between the states. This factor identifies additional differences from the other transitions for $a\to b$ and $c\to d$ in which the number of neurons that changed their activity from orderly to disorderly proved to be the same as the number of neurons whose activity changed in the opposite direction. Accordingly, the total number of neurons with ordered firing activity remained unchanged. In all other cases the number of neurons whose firing activity became ordered was larger, so that the total number of such neurons in the new states increased. According to the total percentage of neurons with altered firing activity, the transition $a\to c$ (25.9%) can be differentiated from the transition $a\to d$ (41.3%).

A fourth factor is the proportion of neuronal pairs in which a dependence of pulse trains from one neuron on those of the other appeared or disappeared upon transition from one state to another (Table 2). This factor affords an additional opportunity for distinguishing the transition $a\to c$, after which the dependence of impulse trains appeared and disappeared in the same number of neuronal pairs, from the transition $a\to d$, after which the number of independent neuronal pairs became greater. The total proportion of dependent and independent neuronal pairs significantly differed between the transitions $a\to c$ and $a\to d$ (8.4% *vs.* 16%). Finally, this factor also permits separation of $b\to c$ (9.1%) from $b\to d$ (3.4%). The fourth factor, however, is less reliable than the other three because the number of neuronal pairs that reversed the pattern of their joint functioning was small.

Thus, the four factors, which reflect the functioning of hypothalamic neurons, enabled us to identify transitions from the motivational state to the "neutral" ($a\to b$), emotionally positive ($a\to c$), and emotionally negative ($a\to d$) states, from the "neutral" state to the emotional states ($b\to c$ and $b\to d$), and from the emotionally positive to emotionally negative state ($c\to d$). These factors less well differentiated transitions from the "neutral" state to the emotionally positive ($b\to c$) and emotionally negative ($b\to d$) states. Nevertheless, since the percentage of neurons in which particular functional characteristics changed following the transition from one state to another was high, and since all transitions between the states under study were specific, it can be assumed that the mosaic of active neurons and of interneuronal connections is specific for each particular motivational/emotional state of the animal.

It was impossible to carry out a detailed analysis for cortical neurons and neuronal pairs because of their small number. However, the state of food motivation can be considered specific, since 7 out of 8 identified neurons generated pulses in groups or volleys; after this motivation was removed, only 2 out of 7 neurons generating such impulses remained. The state of alimentary motivation was also the one in which the largest number of dependent neuronal pairs was identified (3/7).

Our findings indicate that different motivational/emotional states may differ in the proportion of neurons showing ordered firing (e.g., generating impulses in groups, volleys or rhythmically) or in the proportion of neuronal pairs with dependent impulse traffics relative to the total number of recorded neurons or neuronal pairs. Thus, a state characterized by alimentary motivation is clearly defined by these variables of cortical neurons. These results agree with those of others [4,6,8,9]. In addition, the orderliness (dependence) of impulse traffics appeared in some neurons (neuronal pairs) and disappeared in others as the animal passed to a new state. Such opposite changes in functional characteristics could be exhibited by a considerable proportion of neurons (neuronal pairs), although the proportion of neurons with ordered firing activity or that of neuronal pairs with dependent impulse traffics in the total number of neurons and neuronal pairs was slightly changed. Therefore, multivariational analysis of functional characteristics of the same neurons recorded in different states of an animal is required for a more complete description of how a particular state is specifically reflected at the neurophysiological level so that it can be identified. Useful information was provided by generalized data on the proportion of neurons for which the mean firing frequency was found to change (increase or decrease) following the transition from one state to another; also useful were data on the proportion of neurons which started or ceased to generate ordered impulses and on the proportion of neuronal pairs whose impulse traffics changed from dependent to independent or vice versa.

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