PHYSIOLOGY

Involvement of Cingulate Cortex in the Formation of Defensive Behavior in Rats

A. F. Meshcheryakov and K. V. Sudakov

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Changes in behavior patterns in rats during electric stimulation of the ventromedial hypothalamus (the center of defensive behavior) and cingulate cortex were studied. Electric stimulation of the frontomedial cingulate cortex reduced the latency for anxiety and escape reactions during electric stimulation of the ventromedial hypothalamus. Electrolytic destruction of the frontomedial cingulate cortex substantially increased the threshold of escape behavior during stimulation of the ventromedial hypothalamus. These findings demonstrate the involvement of the frontomedial cingulate cortex into the development of defensive (anxiety and escape) behavior in rats.

Key Words: *behavior; ventromedial hypothalamus; cingulate cortex; electric stimulation; coagulation*

In recent years, the cingulate gyrus attracts close attention of investigators [1,3,4,6,8,9]. Cingulate gyrus was shown to be the key structure in the formation of emotions [1,2,7,10].

Destruction of the medial cingulate gyrus in humans impairs emotional behavior [6] and leads to the absence of mimic and vocal emotional reactions [9].

In rats, cingulate gyrus consists of cincture and cingulate cortex [1]. Destruction of the cingulate cortex in rats disturbs sexual behavior [5] and reduces anxiety [11].

The most pronounced defensive behavior (runaway) in animals appears after stimulation of the hypothalamic ventromedial nuclei. In light of this, the objective of this study was to discover the role of the medial cingulate cortex in the formation of defensive behavior in rats during electric stimulation of "fear centers" in the ventromedial hypothalamus.

P. K. Anokhin Institute of Normal Physiology Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** alex-maf@mail.ru. A. F. Meshcheryakov

MATERIALS AND METHODS

The study was performed on 36 male Wistar rats weighing 180-210 g. The animals were kept 6 rats per cage under conditions of artificial illumination (12 h light period) with free access to water and standard combined feed.

The experiments were performed in accordance with "Rules for the Use of Experimental Animals"approved in P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, and conforming to regulations of World Society for the Protection of Animals (WSPA) and European Convention for Protection of Experimental Animals.

Anesthetized (chloral hydrate, 400 mg/kg body weight, intraperitoneally in 2 ml physiological solution) rats were scalped and calvarial bones were trephined. Monopolar Ni-Cr electrodes (50 μ in diameter) were implanted unilaterally into the ventromedial hypothalamic nuclei and bilaterally in the frontomedial cingulate cortex according to stereotaxic coordinates

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of rat brain (ventromedial hypothalamus: AP=2.2-2.4 mm, DS=0.5 mm, h=9.2-9.6 mm; frontomedial cingulate cortex: AP=1.6-1.8 mm, DS=1.4-1.6 mm, h=2.5-2.6 mm). Indifferent electrode was implanted into the nasal bone. The electrodes were fixed to the bones using a Super Moment glue.

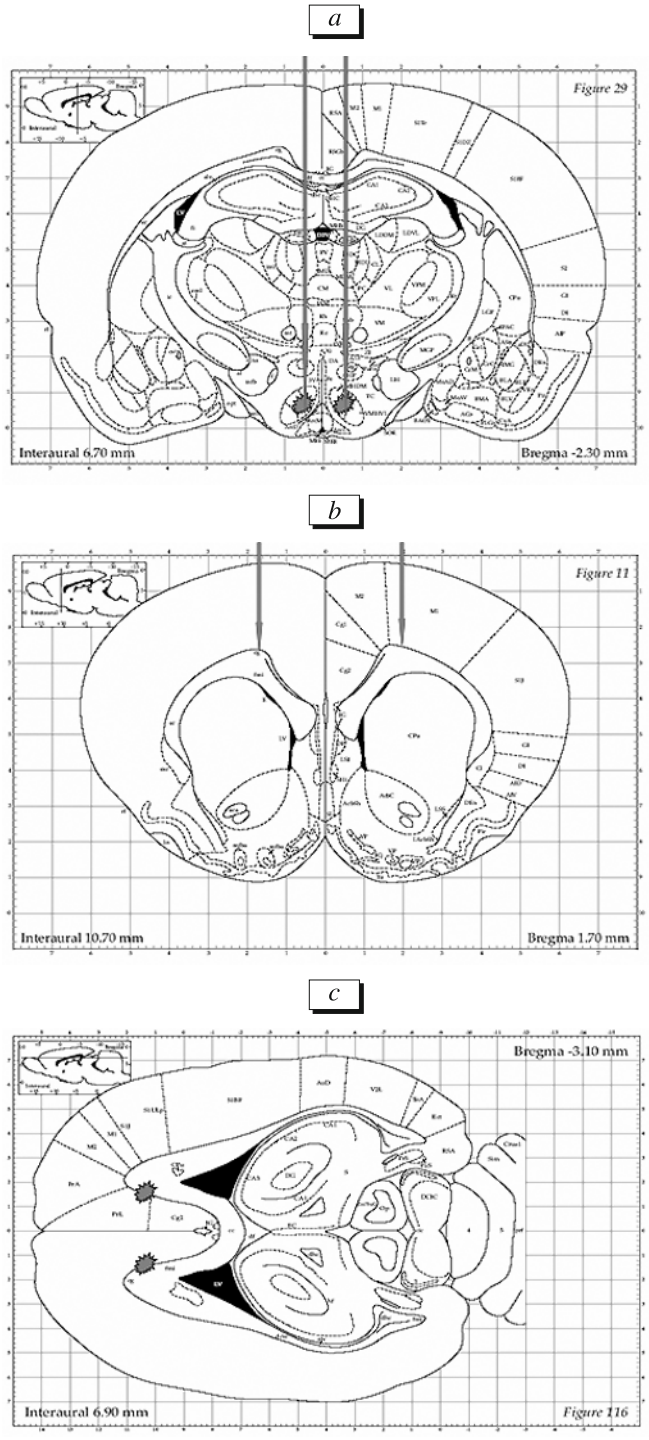

Fig. 1. Areas of electric stimulation in the ventromedial hypothalamus (*a*; dark dots) and in the frontomedial cingulate cortex (*b*) and destruction areas in the frontomedial cingulate cortex (*c*, dark dots).

After surgery and electrode implantation, the rats were kept under standard vivarium conditions for 72 h and then electrical stimulation of the ventromedial hypothalamus and frontomedial cingulate cortex was performed for 3 days with current intensity 50 and 150 μA (3-5 V), stimulation frequency 100 Hz, and duration of stimulation 1 sec. For electrolytic destruction of the frontomedial cingulate cortex, 30 μA current (voltage 3.5-4.5 V) was applied for 15-30 sec.

During electric stimulation of the ventromedial hypothalamus and frontomedial cingulate cortex, the spectra of behavioral reactions were analyzed and the thresholds and latencies of defensive behavior, escape, grooming, orientation and exploratory behavior, freezing, piloerection, and vocalization were determined; fecal boluses and urinations were also counted.

On day 4 after electrode implantation, the bilateral electrolytic coagulation of the frontomedial cingulate cortex was performed and during the subsequent 3 days, electric stimulation of ventromedial hypothalamus was performed again and the studied types animal behavior were recorded.

The position of electrode tips in structures of the ventromedial hypothalamus was verified and morphological control of the destruction area in medial cingulate cortex was performed on serial 100-μ brain sections (Fig. 1). Analysis of experimental data was performed using ANOVA software.

RESULTS

Subthreshold (50 μA) electric stimulation of the ventromedial hypothalamus induced a complex of reactions: orientation and exploratory behavior, active avoidance, piloerection, freezing, vocalization, defecation and urination, and grooming, orientation and exploratory behavior, freezing, and grooming being the most pronounced forms (Table 1).

Electric stimulation of the ventromedial hypothalamus with 150-μA current induced similar reactions, but grooming, orientation and exploratory behavior and freezing became less pronounced, while piloerection, vocalization, and active avoidance increased (Table 1).

During electric stimulation (50 and 150 μA) of the frontomedial cingulate cortex, freezing, orientation and exploratory behavior, and grooming predominate (Table 1) against the background of the above listed reactions. Stimulation of the cingulate cortex with 150-μA current was associated with decreased levels of orientation and exploratory behavior, urination, and grooming and increased levels of active avoidance, piloerection, and vocalization in comparison to stimulation with 50 μA current. The reactions virtually corresponded to stimulation of the ventromedial hypothalamus. However, the latencies of these behavioral

TABLE 1. Parameters of Rat Behavior during Electric Stimulation of Ventromedial Hypothalamus (VMH) and Frontomedial Cingulate Cortex (VMCC) with Current 50 and 150 μA and after Destruction of Frontomedial Cingulate Cortex

Type of reaction, constancy	Electric stimulation with current 50 μA			Electric stimulation with current 150 μA		
	VMH, %	VMCC, %	VMH after VMCC destruction, %	VMH, %	VMCC, %	VMH after VMCC destruction, %
Orientation and exploratory behavior	30	31.67	30	15*	15*	15*
Active avoidance	0	0	0	8.33*	3.33*	5*
Piloerection	13.33	13.33	5+	25*	25*	23.33*
Freezing	26.67	18.33	25	11.67*	18.33	21.67
Vocalization	1.67	0	0+	21.67*	20*	11.67*+
Boluses	5	6.67	3.33	6.67	5	6.67*
Urinations	8.33	10	5	8.33	5*	8.33
Grooming	15	20	31.67+	3.33	8.34*	8.33*+

Note. $p<0.05$ *compared to stimulation of VMH and VMCC with current 50 and 150 μA, +during VMH stimulation after VMCC destruction.

TABLE 2. Latency (sec) of Anxiety and Avoidance Reactions during VMH Stimulation before and after VMCC Destruction

Stimulation current intensity, μA	Stimulation side	VMH stimulation	VMH stimulation after VMCC destruction
50	D	9.8±0.9	>60**
	S	9.6±0.7	>60**
150	D	4.3±1.1	16.7±1.5**
	S	4.1±0.9	16.4±1.3**

Note. Right-side (D) and left-side (S) stimulation. **$p<0.001$ compared to latency of anxiety and avoidance reactions during VMH stimulation after electrolytic destruction of the medial cingulate cortex.

forms after stimulation of the frontomedial cingulate cortex were significantly ($p<0.05$) longer, than after right- and left-side stimulation of the ventromedial hypothalamus (Table 2).

After electrolytic destruction of the frontomedial cingulate cortex, the latencies of active avoidance and anxiety reactions during stimulation of ventromedial hypothalamus with current 50 and 150 μA were significantly ($p<0.001$) longer than during stimulation of the ventromedial hypothalamus before electrolytic destruction of the medial cingulate cortex (Table 2).

Thus, bilateral electrolytic destruction of the frontomedial cingulate cortex led to reorganization of animal behavior induced by electric stimulation of the ventromedial hypothalamus. Electrolytic destruction of the frontomedial cingulate cortex was associated with a substantial increase in the threshold of anxiety and avoidance behavior after stimulation of the ventromedial hypothalamus.

Our findings suggest that the frontomedial cingulate cortex is involved into the mechanisms responsible for the formation of defensive behavior of anxiety and avoidance.

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