Influence of Chemostimulation of Different Nuclei of the Amygdala on the Implementation of the Drinking Conditioned Reflex

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Abstract: In order to study the role of various nuclei of the amygdala in the implementation of the developed drinking habit, chemical stimulation was performed (carbocholine, serotonin, norepinephrine) and temporary shutdown of its basolateral. The results of the studies carried out testify to the modulating role of the basolateral and central nuclei of the amygdala in the formation of complex forms of behavior. Obtained by methods of chemical stimulation and temporary shutdown of AB and AC nuclei of the amygdala, indicate its modulating role in the formation of complex forms of behavior. Since memory and learning are realized through the emotional sphere with the inclusion of serotonergic and noradrenergic fibers, in these cases the involvement of the amygdala in these nervous processes will be determined by the formation of emotionally positive behavior by serotonergic systems, and emotionally negative behavior by noradrenergic ones.

Key words: Carbocholine, serotonin, norepinephrine, novocaine, conditioned drinking reflex.

1. Introduction

The amygdala complex is one of the components of the limbic system and has a modulating effect on the activity of the main brain stem formations, through which the formation of an emotional and motivational state is carried out. The amygdala has numerous connections with various brain structures. So, it receives information from all the senses. This is possible due to its connection with a structure called the thalamus [1]. Direct connection with the amygdala is necessary for a quick response to external stimuli [2, 3]. The amygdala complex has connections with associative zones – areas of the cortex and with the hippocampus. In addition, the amygdala has numerous bilateral connections to subcortical structures such as the basal ganglia and accessory nuclei, as well as to the prefrontal cortex. The amygdala has an effect on the prefrontal cortex in risk/reward assessment in animals [4], and stimulation of projection pathways of certain areas of the prefrontal cortex into the amygdala can enhance or decrease prosocial behavior [5]. Damage to the connections between the amygdala and the prefrontal cortex can lead to post-traumatic stress disorders, depression, etc. [6]. Of particular importance are the connections of the amygdala with the hypothalamus, which plays an important role in the regulation of stress responses, sexual behavior, and aggression [7].

Different nuclei of the amygdala complex are involved in a variety of behavioral responses. Thus, the lateral nucleus plays a role in reactions associated with fear [8]; the basolateral nucleus responds to both negative stimuli (fear, anxiety) and positive ones (reward) [9]; the central nucleus of the amygdala is responsible for reactions to emotional stimuli, the medial nucleus in animals especially important in sexual behavior. In humans, MeA mainly reacts to pungent odors [10] and takes part in their memorization [11].
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Based on the foregoing, the main goal of this study was to study the effect of chemostimulation (carbocholine, serotonin, norepinephrine) and temporary shutdown (novocaine) of the basolateral and central nuclei of the amygdala on the performance of the developed drinking conditioned reflex skill.

2. Material and Methods

The studies were carried out on 25 Chinchilla rabbits weighing 2-3 kg, trained in the drinking conditioned reflex. Animals were subjected to water deprivation for 24 and 48 hours, after which they were taught instrumental drinking habits. In response to a sound signal, the rabbit pressed the pedal, which opened the door, jumped over the barrier from the starting compartment of the chamber to its target compartment to receive water in a strictly defined dose (5-10 ml), and then returned to the cameras of the launch section. The conditioned stimulus was applied at regular intervals (every 45 s) 10 to 15 times during the experiment. During the experimental day, the animals received an average of 100-120 ml of water. The experiments were carried out under conditions of 100% training. In the study of behavioral reactions, the time from the moment the signal was given to the beginning of the jump (latent period), the time of the jump and run, as well as the time spent on returning to the starting box of the camera, were recorded.

The introduction of neurochemical preparations was carried out in a volume of 0.005 ml of saline using a special device consisting of a micromanipulator and a syringe connected to an injection needle with a polyethylene tube, which makes it possible to inject chemical solutions into various brain structures under conditions of free behavior of the animal. The application was made in doses: carbocholine (CH) from 0.5 to 3 mcg; serotonin (5-OT) from 10 to 100 mcg, noradrenalin (NA) from 10 to 50 mcg. Temporary shutdown of the studied areas of the CNS was carried out with a 10% novocaine solution. To control the rabbits, a physiological solution was injected into the study area in a volume equal to the injected solutions.

3. Results and Discussion

The results of the conducted studies showed that the introduction of carbocholine into the basolateral nucleus of the amygdala at a dose of 0.5 μg did not lead to a violation of the skill. The animals responded in a timely manner to the sanctioned signal. The introduction of carbocholine into the basolateral nucleus of the amygdala at a dose of 2–3 μg led to an aggressive reaction: the animal became shy, shuddered at the sound signal, and could hardly be picked up. At the same time, the performance of the skill was not violated. An intense emotional reaction develops: constant hissing, grumbling, continuous walking around the room, and piloerection. There was an increase in the latent period of the reaction to the conditioned stimulus (from 1.23 ± 0.07 to 3.57 ± 0.08 sec) and return (from 4.2 ± 0.07 to 8.5 ± 0.07 sec) (Fig. 1). The remaining components remained at the level of background indicators. Registration of the amount of water consumed showed that it did not differ from those in intact animals. Similar changes in the behavior of animals were also noted after the introduction of the indicated doses of 5-OT to the amygdala core AB.

The application of 5-OT to the amygdala core AB in doses of 10-20 μg did not lead to a violation of the conditioned reflex drinking habit. The rabbits placed in the starter compartment of the experimental chamber showed signs of emotional disturbance in anticipation of a sanctioned signal. When a conditioned stimulus was given, the rabbit reacted to it in a timely manner. The time spent on the jump-run, lapping led to the appearance of an alert reaction, pronounced salivation, and return did not change either. Increasing the dose to 30-40 μg, the
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Animals did not show signs of emotional anxiety, which was reflected in an increase in the time of the latent period of reaction to the conditioned stimulus (from $1.27 \pm 0.07$ to $3.37 \pm 0.09$ sec). The introduction of serotonin did not affect the overall motor activity. The animal made a run-jump, lapping and return. The time parameters of these components of the conditioned reflex remained at the level of the background indicators and were respectively: $3.37 \pm 0.1$ sec; $20.23 \pm 0.15$ sec and $4.23 \pm 0.07$ sec.

Increasing the dose to 50-100 µg led to complete inhibition of the conditioned reflex. The amount of water consumed did not change either.

Similar changes in the behavior of animals were noted after the introduction of 5-OT into AC (as shown in Figure 1).

The introduction of NA (20 µg) into the amygdala nucleus AB led to the appearance of a state of lethargy, stupor in animals, which resulted in an increase in the time of the latent period of the reaction to the conditioned stimulus. The amount of water consumed did not change either.

![Figure 1](image-url)

**Fig. 1** Influence of electrical and chemical stimulation of the AB of the amygdala nucleus on the time parameters of the execution of the conditioned reflex drinking habit. 1: background; 2, 3: low- and high-frequency stimulation; 4: CH application; 5: introduction of 5-OT; 6: NA application; 7: the introduction of novocaine.
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Fig. 2  Influence of electrical and chemical stimulation of the AB of the amygdala nucleus on the time parameters of the execution of the conditioned reflex drinking habit. 1: background; 2, 3: low- and high-frequency stimulation; 4: CH application; 5: introduction of 5-OT; 6: NA application; 7: the introduction of novocaine.

conditioned stimulus (from 1.23 ± 0.07 to 3.57 ± 0.08 sec). There was also an increase in the time spent on a jogging jump (from 3.27 ± 0.07 to 6.2 ± 0.07 sec), lapping (from 20.27 ± 0.12 to 34.93 ± 0.15 sec) and vice versa return (from 4.17 ± 0.09 to 7.6 ± 0.09 sec) (Fig 1). The application of NA did not affect the amount of water consumed, which remained at the level of background indicators. Similar changes in the behavior of animals were also noted after the introduction of NA (20 μg) into the AC nucleus of the amygdala. There was an increase in the time of the latent period of the reaction - from 1.2 ± 0.07 to 3.6 ± 0.08 sec; jogging jump - from 3.3 ± 0.07 to 6.2 ± 0.07 sec; lapping - from 20.3 ± 0.12 to 34.93 ± 0.15 sec and return return - from 4.2 ± 0.09 to 7.6 ± 0.09 sec.

The introduction of a 10% solution of novocaine in AB led to some changes in the behavior of the animals. Animals placed in the experimental chamber did not show, as usual, signs of emotional arousal. The animal did not show any aggressive or defensive reactions and became tame. On the whole, such an inhibited state did not disturb the performance of the developed
drinking habit. The animal reacted to the applied sound signal with a long latent period of 3.7 ± 0.07 instead of 1.27 ± 0.07 sec. The time spent on a jogging jump also increased - from 3.23 ± 0.07 to 6.5 ± 0.11 seconds, lapping - from 20.07 ± 0.1 to 39.07 ± 0.21 seconds, reverse return – from 4.27 ± 0.07 to 8.8 ± 0.13 sec (Figure 2). Registration of the amount of water consumed under conditions of novocaine blockade of AB in the amygdala nucleus did not reveal its change compared to the background indicator. Similar changes in the behavior of animals were also noted after the introduction of novocaine into the central nucleus of the amygdala.

Our studies have shown that with the introduction of 2 μg of CH in both AB and AC, the amygdala nucleus at animals appeared aggressive reactions: the animal became shy, shuddered at the sound signal; it was difficult to pick it up. At the same time, the performance of the skill as a whole did not change. There was an increase in the time of the latent period of the reaction to the conditioned stimulus and the reverse return (2 times). The appearance of a search motor reaction, a reaction of alertness, a pronounced salivation, accompanied by an increase in the time of the latent period of the reaction to the conditioned stimulus, was also noted by us when 30 μg of serotonin was applied to the AB and AC of the amygdala nucleus. A similar behavioral response was observed by some scientists when 5-OT was administered to the AB nucleus of the amygdala complex in cats. An increase in the time of the latent period of the alimentary conditioned reflex was also noted in experiments on rabbits when the precursor of serotonin, 5-hydroxytryptophan, was administered intravenously [12]. At the same time, other scientists, when serotonin was administered, noted in animals a violation of the coordination of movements, the development of a stupor state, and the phenomena of catalepsy [13]. The depth of inhibition and the duration of the effect depended on the dose of the substance. Increasing the dose to 50-100 mcg in our studies led to complete inhibition of the conditioned reflex. The emergence of a state of inhibition was noted by us with the introduction of NA (20 μg), which was accompanied by an increase in all temporal parameters of the conditioned reflex habit. A similar behavioral response was obtained by some scientists when NA was introduced into the amygdala [14] - in rabbits, inhibition of food conditioned reflexes is observed, in dogs it partially inhibits motor food reflexes.

We noted a more pronounced inhibition of the conditioned drinking reflex during novocaine blockade of the studied nuclei of the amygdala, while the animals became tame. In this case, there was an increase in all time parameters of the execution of the conditioned reflex drinking reaction, but more pronounced than with the application of NA. The disappearance of the fear reaction without any special disturbances, motor reactions was obtained with damage to the amygdala. The disappearance of aggressive reactions after the destruction of the amygdala and the fact that the animal becomes tame after such an operation suggests that the amygdala has a facilitated effect on the low mechanisms of aggressive behavior. The most pronounced aggressive reaction was observed when the tonsils were irritated. Moreover, the lowest threshold for the alertness reaction and the orienting-exploratory reaction was evoked in our studies with nuclear AB, while the aggressive-defensive reaction was observed only with AC stimulation [15]. Thus, our data on the participation of the amygdala in conditioned reflex drinking behavior, obtained by the methods of electrical, chemical stimulation and temporary switching off of the AB and AC of its nuclei, indicate its modulating role in the formation of complex forms of behavior. Since memory and learning are realized through the emotional sphere with the inclusion of serotonergic and noradrenergic fibers, in these cases, the involvement of the amygdala in these nervous processes will be determined during the formation of
emotionally positive behavior by serotonergic systems, and emotional-negative-noradrenergic.

The effects of chemostimulation of the AB and AC nuclei of the amygdala showed that its different nuclei are associated with opposite manifestations of emotional states, which can be divided into three groups: search behavior of an emotionally positive type (licking, sniffing, and salivation), reaction of alertness and aggression. The data obtained indicate the modulating role of the amygdale in the formation of complex forms of behavior. Since memory and learning are realized through the emotional sphere with the inclusion of serotonergic and noradrenergic fibers, in these cases the involvement of the amygdale in these nervous processes will be determined by the formation of emotionally positive behavior by serotonergic systems.

References


