

# POSSIBILITY OF DEFENSIVE CONDITIONING IN WAKING RABBITS IN ACUTE EXPERIMENTS

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The technique of formation and characteristics of conditioned defensive reflexes are described in waking rabbits fixed in a stereotaxic apparatus and in encéphale isolé preparations. The dynamics of defensive conditioning and the magnitude of the conditioned responses in acute and chronic experiments were practically identical.

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Rabbits have proved to be a very suitable object for the study of electrical activity of the brain during conditioning [2, 4, 8].

Defensive conditioning in rabbits in acute experiments was considered to be interesting because of the much greater technical convenience of this procedure and the possibilities which it offered, especially for the use of a microelectrode technique. The need to record effector behavioral responses ruled out the use of muscle relaxants. In addition, there is some doubt about the possibility of conditioning after administration of curare [7]. For the same reasons, anesthetized animals could not be used, although conditioned reflex formation is probably possible under superficial urethane anesthesia [6].

## EXPERIMENTAL METHOD AND RESULTS

We formed a conditioned defensive reflex in acute experiments on ten encéphale isolé preparations, in 15 waking rabbits fixed in a stereotaxic apparatus, and in two animals without fixation. Conditioning was carried out in chronic experiments, in the usual manner, in three control rabbits with implanted electrodes. All experiments were carried out by V. P. Protocypov's method, i.e., reinforcement was essential for any type of response by the animal.

### Encéphale isolé preparations

The spinal cord was divided under ether anesthesia at the level of C<sub>1</sub>, the rabbits were transferred to artificial respiration, scalped, and fixed in a stereotaxic apparatus. The anesthesia was discontinued, and the fixation points and area of the operation were infiltrated with procaine. Conditioning began 1-2 h after the completion of all manipulations.

The conditioned stimulus was a tone of 100-800 cps and the unconditioned stimulus electrical stimulation of the lower lip by square pulses (1 msec, 3-10/sec) from a "Physiovar" stimulator. Steel needles served as stimulating electrodes. The conditioned stimulus acted alone for 2-5 sec and the unconditioned for 1 sec; intervals between stimuli were 0.5-2 min.

Electrical activity was recorded from various parts of the cortex by needle electrodes using a unipolar technique, the reference electrode being located over the frontal sinus. Movements of the lower jaw were recorded pneumographically by means of a special cuff, and the electromyogram of the muscles of mastication was recorded by needle electrodes inserted under the skin of the cheek. Blood pressure in the femoral artery was recorded by a "Barovar" electromanometer. All indices were recorded on a 17-channel "Alvar" polygraph.

The background electrical activity of the preparations had the characteristic appearance for waking animals. Both acoustic and electrodermal stimulation evoked a well defined arousal reaction in all parts

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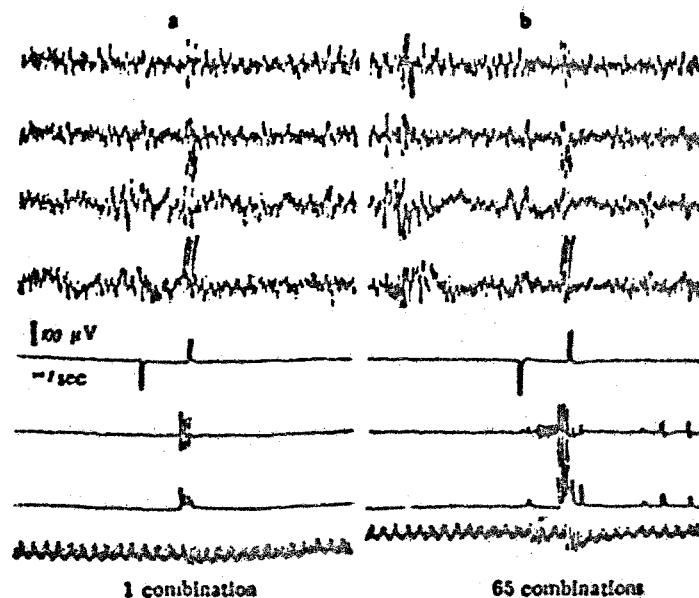


Fig. 1. Conditioned defensive reflex in encéphale isolé preparation (rabbit). From top to bottom: EEG of motor, sensory, auditory, and limbic areas of cortex; acoustic stimulation marker; electromyogram of muscles of mastication and artefacts showing electrical stimulation of lower lip; mechanogram of lower jaw; blood pressure in femoral artery. Explanation in text.

of the cortex. We were unable to extinguish the EEG response to acoustic stimulation, even when applied 100 times without reinforcement. Changes in blood pressure and tone of the muscles of mastication were observed only at the first presentation of the acoustic stimulus, and as a rule they were easily extinguished. Movements of the lower jaw in response to acoustic stimulation were generally absent before conditioning.

The unconditioned responses to stimulation of the lower lip (10-30 V) consisted of contraction of the muscles of mastication, movement of the jaw, and a fall of blood pressure (Fig. 1, a).

Since the EEG response to nociceptive electrical stimulation and the arousal response to acoustic stimulation were practically indistinguishable, it was difficult to judge formation of a conditioned reflex by this index. The first signs of a conditioned response usually appeared on the electromyogram after 20-30 combinations; a conditioned-reflex decrease in arterial pressure quickly became apparent. Contraction of the muscles at first was tonic in character, but later, as the number of combinations increased, movements of the jaw appeared (Fig. 1, b).

Transection of the spinal cord often caused a progressive fall in blood pressure. When its level was below 50 mm Hg, all conditioned responses disappeared. If, however, the condition of the preparation remained satisfactory, after 70-100 combinations conditioned responses were observed in 8-9 cases out of 10.

#### Waking rabbits

The rabbit was tied to a frame under open ether anesthesia which lasted not more than 3-5 min, fixed in a stereotaxic apparatus, and scalped. The place of fixation of the skull and the area of operation were infiltrated with procaine, and 2-3 ml of 4% amidopyrin solution was injected intramuscularly.

As well as the global electrical activity which was recorded as in the preceding series of experiments, in the present series the electromyogram of the limbs and respiration recorded by a piezoelectric pickup were recorded on the polygraph. The conditioned-stimulus consisted of flashes from a 'Soneclat' stimulator, frequency 2-4/sec, and the unconditioned stimulus consisted of square pulses (1 msec, 10-30/sec) applied to the skin of the hind limb. Photic stimulation was applied without reinforcement for 2-7 sec and the unconditioned electrodermal stimulation for 1-2 sec; intervals between stimuli were 0.5-2 min. The parameters of the conditioned and unconditioned stimuli were the same for the two rabbits whose head was not fixed and also for the chronic experiments on three control animals with implanted electrodes.

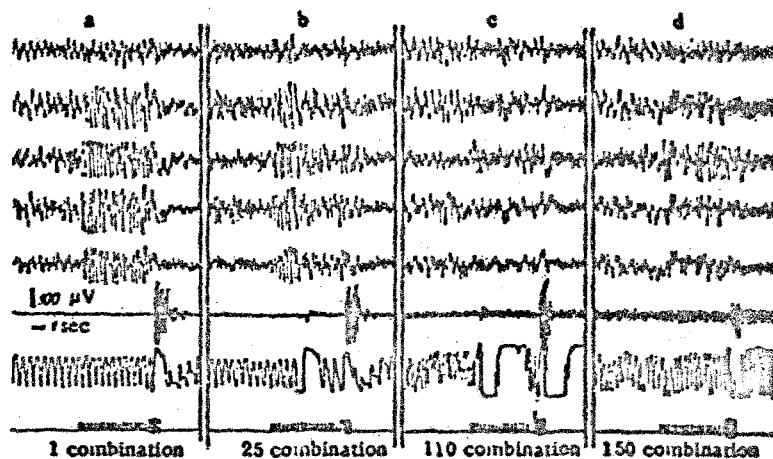


Fig. 2. Conditioned defensive reflex in waking rabbit fixed in stereotaxic apparatus. From top to bottom: EEG of left motor, sensory, visual, auditory, and limbic areas of cortex; EMG of right forelimb; respiration; marker of flashes (3/sec) and electrical stimulation of right hind limb (20/sec). Explanation in text.

It might be expected that fixation in the stereotaxic apparatus would evoke a stress reaction in the animals, manifested by desynchronization of the background corticogram and quickening of respiration. However, in the absence of special stimulation, desynchronization was observed only during the animal's feeble and rare attempts to set itself free, and respiration also remained quiet. If no stimuli were applied for 20-30 min, typical sleep spindles appeared on the EEG and respiration became quiet and regular.

The first flashes usually evoked an orienting response in the form of EEG activation, a change in respiration, and restless movements. However, all these phenomena quickly disappeared (after 5-10 presentations), and in response to flashes of light a rhythm-driving response to the flashes was recorded in all parts of the cortex including the somatosensory area (Fig. 2, a). Respiration remained quiet at this time and no contractions of the muscles were observed. In some rabbits the rhythm-driving response was recorded immediately, without the stage of the orienting reflex. Electrical stimulation of the skin of the hind limb (20-70 V) always evoked an EEG arousal response, changes in respiration, and a marked motor response.

The first EEG manifestations of formation of a defensive conditioned reflex were usually found after 12-18 combinations, when in response to rhythmic flashes, a rhythm-driving response and also a stress response occurred (Fig. 2, b). After 50-70 combinations the background electrical activity became desynchronized and the repeated flashes evoked only a stress response, which spread over the whole cortex and was indistinguishable from the unconditioned response (Fig. 2, c). In the next stage a rhythm-driving response again began to appear in the visual cortex, while the stress response was concentrated in the motor and sensory areas of the cortex (Fig. 2, d). Parallel with the EEG indices, respiratory and motor components of the conditioned reflex appeared.

All the above remarks apply equally to experiments in which the animals were not fixed in the stereotaxic apparatus and to the chronic experiments.

The dynamics of conditioning in an acute experiment using stereotaxic apparatus is shown in Fig. 3.

The conditioned respiratory and EEG responses were well marked in nearly all rabbits, but the motor component of the conditioned reflex was observed in only half of the experiments.

During extinction, in the course of 5-10 presentations of flashes without reinforcement, all the conditioned responses became very well marked. Even if no conditioned motor responses had been produced in this animal, it could still appear during extinction. To produce more or less noticeable extinction, usually about 100-150 applications without reinforcement were needed.



Fig. 3. Dynamics of formation of conditioned defensive reflex in acute experiments on waking rabbit. Abscissa, number of combinations; ordinate, number of conditioned responses in ten combinations; 1) conditioned-reflex EEG arousal response in somatosensory cortex; 2) conditioned-reflex respiratory response; 3) conditioned-reflex motor responses.

In some experiments we attempted to produce differentiation. The differential stimulus was a metronome (encéphale isolé) or rhythmic flashes of 10-20/sec (waking rabbits). In both series of experiments differentiation was produced with difficulty, and it was unstable and inconstant. The same picture was observed in the chronic experiment.

In the experiment of M. N. Livanov [4] and M. N. Livanov and co-workers [3], in which defensive conditioning was carried out in rabbits tied to a frame in a period of two weeks, from 70-200 combinations of regular flashes with electrical stimulation were needed.

In our rabbits in the chronic experiment, when conditioning took place in three sessions, about 100-150 combinations also were required (up to level of eight conditioned responses out of ten combinations). Defensive conditioning took place at approximately the same rate in response to regular flashes under acute experimental conditions also, but in this case all stages of conditioning were rather more prominent.

It is interesting to note that cats, when fixed in a stereotaxic apparatus, behave completely differently. We twice attempted to carry out experiments on cats but the animals' unceasing attempts to set themselves free compelled us to abandon the experiment.

The impression has been obtained that rabbits, when tied to a frame, fall into a state resembling hypnosis, a state probably similar to that observed in fixed sheep and baboons [5]. Fixation in a stereotaxic apparatus aggravates this state although the conditioned reflex not only is not abolished but may actually be produced rather more rapidly. This may be associated with the fact that the immobilization becomes almost absolute, and "... the blocking of movements ... causes compensatory activation of other ... somatic and autonomic components of conditioned and unconditioned responses. ..." [1].

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