THE INITIAL PHASE OF EXCITATION IN ABSOLUTE DIFFERENTIATION AND THE PRESENCE OF FREE AMMONIA IN THE LARGE CEREBRAL HEMISPHERES OF RATS*

E. A. Vladimirova

Laboratory of the Biochemistry of the Nervous System (Head – the late Active Member AMN SSSR G. E. Vladimirov), I. P. Pavlov Institute of Physiology (Dir. – Academician V. N. Chernigovskii, AN SSSR, Leningrad
(Presented by the late Active Member AMN SSSR G. E. Vladimirov)

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The problem of internal inhibition at the present time attracts the attention not only of physiologists, but of representatives of neighboring disciplines. Continuing an investigation into the biochemistry of cortical inhibition, we observed a characteristic change in the ammonia content in the brain which testifies to the presence of definite stages in the process of development of extinctive and differential inhibition in rats [3, 4]. In particular we discovered a characteristic increase in the level of free ammonia in the brain in the first 5 seconds of activity of a negative conditioned stimulus during absolute differentiation [5, 6].

It was of interest to explain the changes in ammonia content in brain tissue in the first 5 seconds of special stable reinforced absolute differentiation, which was attained by use of a considerably larger number of repeated negative conditioned stimuli than in previous experiments.

EXPERIMENTAL METHOD

All experiments were carried out on white rats weighing 200-280 g, maintained on the same nutritive ration. In a chamber specially constructed by us [1, 2] motor-alimentary and motor-defense conditioned reflexes were developed in the rats. Electrocutaneous stimulation served as an unconditioned stimulus in the latter case.

In our development of the motor-alimentary conditioned reflexes, the isolated action of a positive conditioned stimulation (bell) consisted of 3-5 seconds, accompanied by a reinforcement of 10 seconds. The animal usually ate the portion of food given out in 45-60 seconds. The pause before contact of the bell, as in the previous experiments, was equal to 1 min, and before application of the differential stimulation (buzzer) was 2 min. The buzzer was inserted in the 3rd to the 6th place in the order of stimuli in the experiment. During development of differentiation the buzzer at first was turned on at 30 seconds, and later at 60 seconds.

The brain tissue was fixed at the requisite moment by instantaneous submersion of the animal in liquid air, using the same chamber with automatically falling bottom [1, 2]. This chamber was recently found useful in the work of Japanese authors [8] for investigation of the ammonium content of rat brain at different intervals when stimulated by electric current and by positive conditioned-reflex motor-defense reaction.

Another method of freezing the rat by immersion in a mixture of acetone and dry ice at −80° is described in the literature [7]. However, this method of freezing excludes the photometric determination of ammonia, since even traces of acetone form turbidity on Nesslerization.

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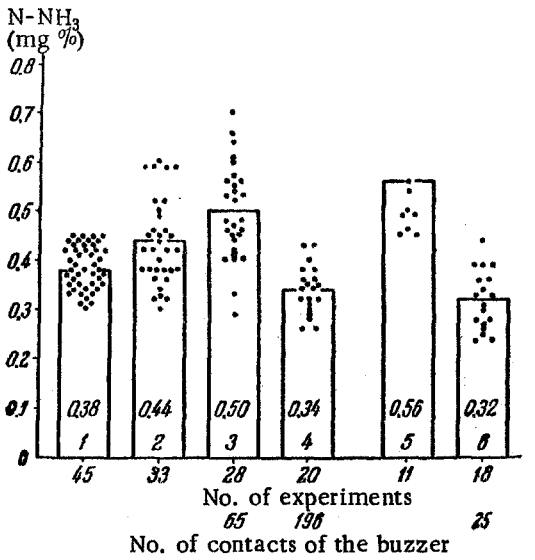
We determined the content of free amonia in the large hemispheres of brain, as in our previous experiments, by vacuum distillation with subsequent photometric determination, the color being developed with the addition of Nessler's reagent.

EXPERIMENTAL RESULTS

Differentiation in rats was sufficiently stable so that the animal in response to differential stimulation did not react either by a positive motor-alimentary reaction or by a noticeable orientation reaction after 65 contacts of the buzzer, on the average.

As was shown in previous investigations, the ammonia content of the brain after the first 5 seconds of positive conditioned-reflex motor-alimentary reaction consists, on the average, of 0.44% (see figure, 2) and after the first 5 seconds from the beginning of negative conditioned stimulus, of 0.50 mg% (see figure, 3).



Change in ammonia content in rat brain in the first 5 seconds of positive motor reaction and in the first 5 seconds of absolute differentiation during different degrees of reinforcement. The numbers on the columns indicate the average ammonia content (in milligram-percent). 1) Normal; 2) positive motor-alimentary reaction; 3) differentiation, 65 contacts of the buzzer; 4) differentiation, 196 contacts of the buzzer; 5) positive motor-defense reaction; 6) differentiation, 25 contacts of the buzzer (motor-defense reaction).

In the first series of experiments with special reinforcement of differentiation the number of contacts of positive conditioned stimulus averaged 472, and differentiation 176; the ammonia content in the large hemispheres of the brain after the first 5 seconds from the beginning of differential stimulation varied within normal limits and was 0.36 mg%, on the average.

In the second series of experiments the number of repetitions of the bell was increased to 528 and the buzzer to 207 times on the average. The ammonia content in the brain was somewhat less than in the previous series of experiments, being 0.33 mg% on the average. The average ammonia content of rat brain in the 2 following series of experiments with 509 contacts of the bell and 196 contacts of the buzzer consisted of 0.34 mg% (see figure, 4), that is, 32% less than the ammonia we found by 5 seconds from the beginning of buzzer application, but with the usual reinforcement of differentiation (see figure, 3). Decrease in the level of ammonia was statistically verified both with respect to normal level ($P < 0.01$) and even more to the amount of ammonia during positive conditioned stimulation ($P < 0.01$) (see figure, 2).

It must be noted that the appearance of absolute differentiation, reinforced either with 196 or 65 repetitions of the buzzer, was completely identical; the animals did not manifest even an orientation reaction when differential stimulation was incorporated. However, associated change in the level of free ammonia in the brain indicates a difference in the functional condition of the central nervous system in both cases.

It seems to us that the absence of specific increase in the level of ammonia in the brain in the first 5 seconds of special stable reinforced differentiation was a result of the appreciable shortening of the excitation phase and the increased concentration of inhibitory processes.

It must be noted that the ammonia content in the brain was still lower in the first 5 seconds of differentiation produced by the motor-defense method, after the use of a comparatively insignificant number of buzzer contacts (25 on the average). The ammonia in the brain equalled 0.32 mg% on the average (see figure, 6), which is 16% lower than normal (see figure, 1). This decrease in ammonia was statistically verified ($P < 0.01$) with respect to the normal and even more with respect to the amount of ammonia found in the first 5 seconds of positive conditioned reflex motor-defense reaction (0.56 mg%) (see figure, 5). An increase in the level of

ammonia to an average of 0.54 mg% occurred in only 7 cases out of 25. These were animals with insufficiently established differentiation (18 buzzer contacts with nonreinforced electrocutaneous excitation).

When the order of distribution of ammonia values presented in the figure is examined, it is not difficult to see that during the first 5 seconds of the usual stable differentiation by means of the motor-alimentary method, the ammonia in the majority of cases was higher than normal, while with special stable reinforced differentiation it often lies within normal limits of ammonia or lower, as in the first 5 seconds of differentiation developed by means of the motor-defense method.

At the same time the ammonia levels in the brain in the first 5 seconds of positive conditioned-reflex motor-defense reaction were significantly higher (on the average of 0.56 mg%; see figure, 5) than with the positive motor-alimentary reaction (0.44 mg% on the average; see figure, 2).

The varying picture of change in the ammonia level in the brain took place in the first seconds of differential inhibition developed, on the one hand, by means of the motor-defense, on the other hand, by means of the motor-alimentary method. In the first case the level of ammonia decreased (0.32 mg %) and in the second became higher (0.50 mg %). And only when differentiation was specially reinforced was the same decrease in the ammonia level (0.34 mg %) found as in the first case.

On what depended the absence or non-appearance of the beginning stages of excitation during differentiation developed by means of the motor-defense method, in spite of the small number of buzzer contacts with nonreinforced electrocutaneous stimulation ?

We consider it probable that extinction occurred more rapidly on electrocutaneous (painful) stimulation of the initial intermittent reaction related to the positive conditioned-reflex reaction than when differentiation is developed with the motor-alimentary method. In this case the absence of increase in the ammonia level in the brain in the first 5 seconds of differentiation developed with the motor-defense method can also be attributed to appreciable shortening of the initial stages of excitation. In addition to this, apparently, the nature of the unconditioned stimulation employed has a significant effect on ammonia metabolism in the brain and on the character of the development of differential inhibition.

The latter circumstance, probably, can explain the well-known difficulties arising from attempts to create a condition of neurosis in animals by the use of "knockdown" stimulatory and inhibitory processes by means of the motor-defense method.

Our data have a direct relationship to the problem of the mechanism of the emergence of inhibition in the brain cortex, since some moments of development of threshold, extinction, and differential inhibition are reflected in changes in the free ammonia content of the brain.

On the basis of results of the present investigation it was possible to reach the following conclusion.

Biochemical investigation of the brain facilitates discovery of differences in the metabolism of the central nervous system during the first seconds of absolute differentiation with different degrees of reinforcement employing common well-defined differential reactions. The absence of specific increase in the ammonia level in rat brain in the first 5 seconds of special reinforced absolute differentiation is dependent, apparently, on appreciable shortening of the initial phase of excitation and increased concentration of the inhibitory processes.

The degree of reinforcement of differentiation, and also the nature of the unconditioned stimulus used, have a significant effect on the process of formation of differential inhibition and ammonia metabolism in the brain.

SUMMARY

The use of Pavlov's conditioned reflex method combined with rapid fixation of intravital changes occurring in the free ammonia level of the large cerebral hemispheres of rats enabled us to discover variations in the brain metabolism already within the first 5 seconds of the absolute differentiation with various degrees of stability, the differentiation reactions being visually absolutely uniform in character. The absence of specific rise in the ammonia level in the brain during the first seconds of a particularly stable differentiation is evidently caused by a considerable shortening of the initial phase of excitation and the intensification of the inhibitory processes concentration.

It is not only the degree of stabilization of the differentiation but also the origin of the unconditioned stimulus used that exercise a considerable effect upon the process of differential inhibition and ammonia metabolism in the brain.

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