

ON THE POSSIBILITY OF USING THE SALIVA METHOD IN STUDYING THE HIGHER NERVOUS ACTIVITY OF YOUNG PUPPIES

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The initial problem in our investigation was to trace the state of salivary conditioned reflexes during the growth period of puppies under several influences on their system. Not having obtained stable conditioned reflexes with the puppies, we sought such variants in the methodology of the experiment which would make it possible to obtain the most regular appearance of positive and inhibitory conditioned reflexes in young animals.

The first part of the work was carried out on 7 puppies which underwent operations to produce fistulas of the common duct of the submaxillary and sublingual glands. After the fistulas healed the puppies were accustomed to the laboratory equipment for several days and then, at the age of 2.5-3.5 months, conditioning of their reflexes was begun.

The speed with which the conditioned reflex developed varied (13th, 18th, 29th, 32nd, 45th, and 53rd combination). The conditioned reflex which appeared differed in its extreme instability and often disappeared; even after 100 combinations, null effects were observed not infrequently. Before the age of 5 months, the size of the conditioned reflex was very small in six animals and varied sharply from experiment to experiment. The average size of the conditioned reflex (salivation) of the various animals constituted 0.02-0.1 ml of saliva. After the puppies reached the age of 5 months, the size of the conditioned reflex increased to 0.15-0.3 ml, but it remained very unstable.

We attempted to work out differentiation in 4 puppies. Administration of the differential stimulus increased inhibition by sleep even more. Differentiation could not be developed in two puppies, differentiation appeared in the third puppy at the fifth application of M-120 and in the fourth at the seventh application, but in both cases differentiation was far from complete and extremely unstable.

The size of the unconditioned reflex of puppies up to 5 months old was 1-2ml, after 5 months the unconditioned reflex increased somewhat (1.2-2.3 ml).

Marked passive-defensive reactions predominated in the behavior of the puppies, while several minutes after the beginning of the experiment inhibition by sleep developed in all the puppies.

Thus, the experiments which were carried out made it possible to establish some peculiarities in the behavior of puppies, but investigation of the nature of the salivary conditioned-reflex activity, especially before the age of 5 months, was unsuccessful.

Taking the indications of I. P. Pavlov on the subject of the battle against the inhibition which develops in adult animals during experimentation as a basis, taking into consideration the methodological indications of N. A. Podkopaev and the data of other investigators, and having some personal experience as well, we changed the method of working with the puppies.

The second part of the work was carried out on 17 different puppies. From the age of one month the puppies were systematically accustomed to the laboratory environment; daily the morning feeding was carried out in the laboratory, in the stand, and the puppies received their food from the same feed pan which was used in the experiments. We tried to create the most favorable quiet environment while the puppies were in the laboratory.

At the age of 2 months the puppies were operated upon to create a duct from the submaxillary and sublingual glands. The development of the conditioned reflex began at the age of about $2\frac{1}{2}$ months in 7 puppies, at 3 months in 5, and at 4 months in 2.

In order to accustom the animals to the experiments, only the unconditioned reflex was investigated at first, and the number of tests did not exceed three during the experimental day. After several days the first conditioned stimulus was introduced. As positive conditioned stimuli were used: a metronome—60 beats per minute (M^{60}) and the weak sound of a siren; as the differentiated stimulus—the metronome at 120 beats per minute (M^{120}). A meat-biscuit mixture slightly moistened with water served as the unconditioned stimulus. The development of the conditioned reflexes was carried out with a 3-second delay. The conditioned reflexes were tested after a 20-second delay. Measurement of the amount of saliva secreted in response to the unconditioned reflex continued for 1 minute (30 seconds simultaneously with the action of the conditioned and unconditioned reflex and 30 seconds of the aftereffect). The intervals between stimuli were 5 minutes. The experiments were carried out according to a definite standard, consisting of the fixed order of giving the conditioned stimuli and of equal intervals of time between them. At first a reflex to M^{60} was developed; after the conditioned reflex to this stimulus became more or less constant, development of the conditioned reflex to the siren was begun and after the reinforcement of both reflexes the differentiated stimulus was introduced. The entire experiment lasted for 20-25 minutes. Periodically breaks of several days were made in the work.

Speed of Developing Positive Conditioned Reflexes by Puppies $2\frac{1}{2}$ Months Old

Puppy No.	Conditioned stimulus	At what combination the reflex was developed	Puppy No.	Conditioned stimulus	At what combination the reflex was developed
1	M^{60}	9	9	M^{60}	15
	Siren	5		Siren	7
2	M^{60}	10	10	M^{60}	16
	Siren	12		Siren	5
3	M^{60}	12	11	M^{60}	21
	Siren	9		Siren	13
4	M^{60}	13	12	M^{60}	22
	Siren	8		Siren	—
5	M^{60}	13	13	M^{60}	22
	Siren	—		Siren	10
6	M^{60}	14	14	M^{60}	22
	Siren	10		Siren	12
7	M^{60}	14	15	M^{60}	22
	Siren	13		Siren	14
8	M^{60}	15			
	Siren	7			

With this method of work, not one puppy ever showed inhibition by sleep. The administration of differentiated inhibition did not cause even slight drowsiness. Refusal of food was not observed once. The speed of development of conditioned reflexes was different in different puppies. As is apparent from the data in the table, the conditioned reflex usually was developed faster than with the old method of working. Once developed, the reflex did not disappear, although its extent was subject to large fluctuations, especially until the age of 4 months, but still the fluctuations were less obvious than those of the previous experiments. The size of the conditioned reflex to M^{60} was 0.06-0.2 ml on the average for different dogs up to the age of 5 months, i.e. the reflex was almost twice as high as that of animals in the first group.

Development of differentiation was attempted with 7 puppies, and it was successful in 6 cases. Differentiation was incomplete, but fairly constant.

The success in developing salivary reflexes in young puppies is apparently dependent on the elimination of general inhibition, which leads to sleep.

Thus, when studying the salivary conditioned reflexes in young puppies it is essential: to accustom the puppies systematically from the age of one month to the laboratory environment, feeding them in the stand; to carry out experiments according to a definite standard; to use no less than two positive conditioned stimuli; to develop the conditioned reflex by the almost simultaneous type (3-second delay) with 5 minute intervals between stimuli; to limit the duration of the experiment to 30 minutes; to give periodic breaks of 3-5 days in the work.

All these conditions arise from the attempt to limit the action of the extinction inhibitors which have a tendency to irradiate in young animals.

LITERATURE CITED

- [1] L. M. Barysheva, *Zhur. Vysshei Nerv. Deyatel.* 1, 2, 223-234 (1951).
- [2] A. A. Volokhov, *The Rules of Ontogenesis of Nervous Activity*,* (Moscow-Leningrad, 1951).
- [3] T. F. Komarova and V. A. Troshikhin, *Works of the I. P. Pavlov Institute of Physiology*,* (Moscow-Leningrad, 1953), Vol. 2, pp. 228-251.
- [4] I. L. Laptev, *Problems of Higher Nervous Activity*,* (Moscow, 1949), pp. 540-558.
- [5] F. P. Maizorov, *Arch. Biol. Nauk* 29, 3, 341-354 (1929).
- [6] I. P. Pavlov, *Complete Collected Works*,* (Moscow-Leningrad, 1951), Vol. 4.
- [7] N. A. Podkopaev, *Methodology of Studying Conditioned Reflexes*,* (Moscow-Leningrad, 1934).
- [8] V. A. Troshikhin, *Abstracts of Reports at the XIV Conference on Problems of Higher Nervous Activity*,* (Leningrad, 1951), pp. 42-43.
- [9] A. P. Khudorozheva, *Zhur. Vysshei Nerv. Deyatel.* 4, 1, 93-103 (1954).
- [10] A. P. Chesnokova, *Zhur. Vysshei Nerv. Deyatel.* 1, 4, 555-565 (1951).