SIZE OF THE DOG BRAIN BEFORE AND AFTER PERFUSION OF THE BLOOD VESSELS WITH 10% FORMALIN

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(Presented by Active Member AMN SSSR P. S. Kupalov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 9, pp. 120-122, September, 1963
Original article submitted September 26, 1961

Recently extensive investigations have been made to determine the stereotactic coordinates of different parts of the dog brain [3-5]; in this procedure the required measurements are made on a brain which has been fixed by perfusion of the blood vessels with 10% formalin, but the results are applied to the living animal. Nevertheless the application to the living brain of results obtained on a brain fixed in formalin has not yet been justified, despite numerous investigations into the influence of formalin on various biological specimens [7-13].

The influence of formalin on brain size might be indirectly inferred from results of changes in volume occurring as a result of fixation [1,6,14]. However, these results are contradictory, partly because of the use of different concentrations of formalin. We cannot fail to note that in many cases the brain was fixed by placing it in formalin solution. This method was used also by those authors who have studied the influence of this fixative on brain volume. Therefore even if their results were in agreement they could not be applied to a determination of changes of dimensions due to perfusion through the blood vessels.

In order to carry out seccessful stereotactic studies of the cerebral coordinates it is essential to know precisely the extent of the influence of formalin on brain dimensions.

EXPERIMENTAL METHOD

The head of the anesthetized dog was placed in the stereotactic instrument; then the vault of the skull was freed from soft tissue, and a drill was used to make ten trepanned apertures at strictly defined positions. By means of the stereotactic apparatus an electrode* bare along the whole of its length was introduced through each of the apertures. An electric current was passed through the electrode which caused the formation of a channel in the nervous tissue. These channels represented markers (controls). In each brain eight vertical and two horizontal marks were made (Fig. 1 and Fig. 2, see the table).

After the channels had been marked the stereotactic instrument was removed, the depth of the anesthesia increased, and the thoracic cavity opened. The vessels of the cavity were ligated in such a way that fluid injected through the aorta passed to the head through the carotid and vertebral arteries. The outflow was through the superior vena cava.

By means of an apparatus which was a simplified form of Vyvodtsev's injector [2], the brain was at first irrigated with 5 liters of physiological saline, after which 5 liters of 10% formalin were passed through it.

Next the head of the dog was cut off and maintained at room temperature for 1-50 days. At the end of that time the cranium was freed from soft tissue and then placed in a stereotactic instrument. The cranium was opened with a circular saw, and horizontal sections of the brain were cut at 5 mm intervals with a special microtome. After each section had been made the distance between the control marks made while the dog was alive were measured with the stereotactic instrument to an accuracy of 0.5 mm.

This experiment was carried out on ten dogs.

*A second electrode (an injection needle) was introduced into one of the anterior apertures.

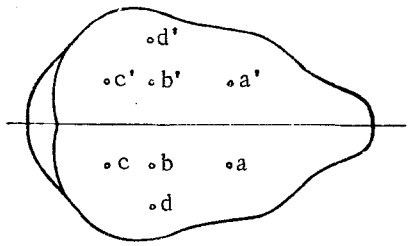

Fig. 1. Distribution of control mark on a horizontal section of a dog brain. Control marks indicated by letters.

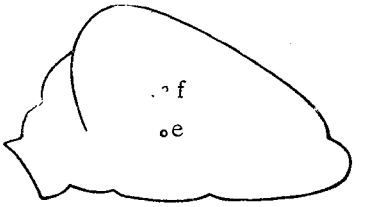

Fig. 2. Distribution of control marks in a saggital section of the dog brain.

EXPERIMENTAL RESULTS

A comparison between the distances of the marks before and after treatment of the brain with formalin showed that in 82.79% of cases there was agreement, and in the remaining cases any difference did not exceed 1 mm. Differences in the main results as large as 1 mm were found in 1.31% of the cases, and differences of 0.5 mm in 15.9% cases.

Measurements between corresponding points at different levels agreed as a rule. The occasional differences of 0.5 mm or more rarely of 1 mm were probably due to causes not related to the action of the fixative (for example, hemorrhage into the cerebral ventricles).

Because the measurements at different levels agreed essentially our analysis of the material may be confined to a review of data obtained on one section, for example the section made at a depth of 10 mm from the apex of the brain.

As can be seen from the table in which material for this section is given (110 measurements), in six heads (No. 1, and 3-7) all measurements agreed with the original value, in two (Nos. 2 and 8) there were two deviations in each, and in one (No. 9) there were three, and in one (No. 10) there were nine differences. The lack of correspondence in measurements obtained after fixation of the brain and those made before fixation were recorded in six cases; in one case the difference was 1 mm, in the remainder 0.5 mm. It should be noted that most of the deviations concerned the width of the brain. This variation may be caused by some very small approach of the cerebral hemispheres to each other as a result of removal of the dura mater.

As has already been pointed out, after fixation in formalin the brain was kept for from 1 to 50 days; (head No. 1 for one day, No. 2 for five days, Nos. 3 and 4 for seven days, No. 5 for ten days, No. 6 for twenty days, Nos. 7 and 8

Distances Between Control Marks Before and After Fixation of the Brain in Formalin

Control marks	Distance in the living brain (mm)	Number of head										Number of deviations from the original val.
		1	2	3	4	5	6	7	8	9	10	
		Distance between control marks after treatment of the brain with formalin (in mm)										
a-b	20	20	20	20	20	20	20	20	20	20	19,5	1
a'-b'	20	20	20	20	20	20	20	20	20	20	19,5	1
a-c	30	30	30	30	30	30	30	30	30	30	29,5	1
a'-c'	30	30	30	30	30	30	30	30	30	30	29	1
b-c	10	10	10	10	10	10	10	10	10	10	10	0
b'-c'	10	10	10	10	10	10	10	10	10	10	9,5	1
a-a'	20	20	19,5	20	20	20	20	20	20	20,5	19,5	3
b-b'	20	20	20	20	20	20	20	20	19,5	19,5	19,5	3
c-c'	20	20	19,5	20	20	20	20	20	20	20	19,5	2
d-d'	40	40	40	40	40	40	40	40	39,5	39,5	39,5	3
e-f	10	10	10	10	10	10	10	10	10	10	10	0
No. of deviations		0	2	0	0	0	0	0	2	3	9	16

for thirty days, and Nos. 9 and 10 for fifty days). It has been shown that keeping a brain treated with formalin for up to 30 days causes no noticeable changes in its dimensions. Also increase up to 50 days of the time for which a perfused head is preserved causes a very small shrinkage. This effect is probably due to loss of moisture.

The results allow us to conclude that fixation of a brain by perfusion of the blood vessels with 10% formalin followed by preservation for up to 30 days has practically no influence on its dimensions. This means that results obtained by measurements made on brains fixed by perfusion of the blood vessels with 10% formalin may be used without any correction for application to various structures in the living brain.

SUMMARY

Control marks related to each other in a definite manner were made in the brain of a living dog by means of a stereotactic apparatus. The brain was then perfused from the thoracic aorta with 5 liters of physiological saline and then with 5 liters of a 10% formalin solution. Subsequently the heads were kept in a room for periods from 1 to 50 days. After preservation the brains were sectioned and the distance between the control marks was measured by means of the stereotactic instrument. It was found that in 82.79% of the brains the initial and final figures agreed; in the remainder there were insignificant deviations of 0.5 or exceptionally of 1 mm.

These results demonstrate that the size of the dog brain remains almost unchanged after fixation with 10% formalin.

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