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## Electroencephalographic Evidence of Osmosensitive Elements in Olfactory Bulb of Dog Brain.* (25004)

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Verney and his associates(1,2) presented physiological evidence of the presence, within the bed of internal carotid artery of the dog brain, of elements sensitive to changes in osmotic pressure of blood. Activation of these "osmoreceptors" by hypertonic solutions stimulated release of neurohypophysial antidiuretic hormone (ADH). The precise location of the receptors was unknown, though it was suggested that they might lie within the supraoptic nucleus of the hypothalamus. Studies which employed electroencephalographic (EEG) recording technics in rabbits and in cats(3,4,5,6) have indicated that osmosensitive elements may lie rostral to the hypothalamus in the olfactory system. Characteristic EEG changes were observed in olfactory projection areas following intracarotid injection of hypertonic saline which activated release of oxytocin(4) as well as ADH(5). In a related investigation(6) hypertonic solutions were shown to act independently on hind brain to induce consistent changes in arterial pressure. The vast amount of information assembled by Verney *et al.* in the dog made it desirable to investigate osmoreceptor function in this species with electrophysiological methods.

*Methods.* A total of 24 mongrel dogs of both sexes were prepared under ether or sodium pentothal (20 mg/kg) anesthesia. Subsequently only a local anesthetic (4% procaine) was employed while animals were immobilized with gallamine triethiodide (Flaxedil) administered intravenously, and maintained with respirator pump. Blood pressure was recorded from femoral artery with a Statham transducer and a Varian inkwriter. Intracarotid injections (1-5 ml) of the following solutions were given through a polyethylene cannula: 0.15 M NaCl (isotonic), 1-1.5 M NaCl (hypertonic), 2 M sucrose and glucose. The scalp was incised and a skull flap removed. Using a stereotaxic instrument

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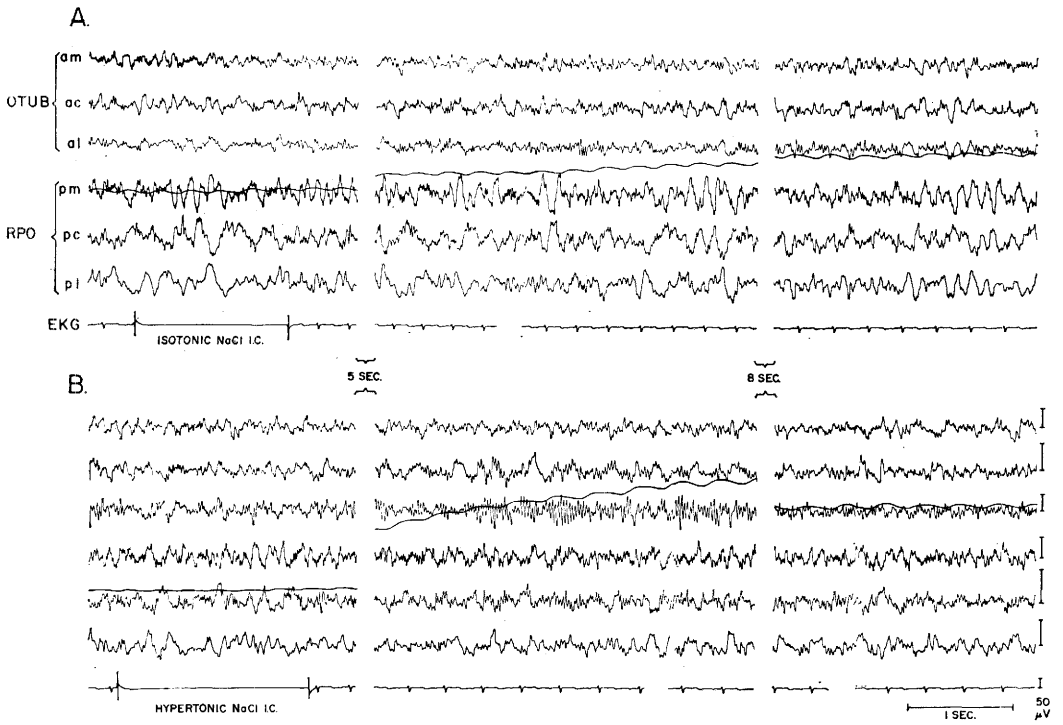


FIG. 1. Typical EEG response to intracarotid hypertonic saline. A. Control record after inj. of isotonic saline (3 ml 0.15 M NaCl). B. EEG response to hypertonic saline (3 ml 1.0 M NaCl). Arterial pressure is indicated by line running through EEG records. In both cases it starts from base line of 70 mm Hg and after hypertonic saline it reaches a value of 130 mm Hg. Heart rate is indicated by electrocardiogram (EKG). Small letters inside brackets correspond to electrodes from which EEG tracings were taken. The first 3 channels reflect activity in olfactory tubercle (OTUB) recorded from bipolar electrodes 3 mm apart and in the same transverse plane; the most lateral (al) is just medial to lateral olfactory tract; the central (ac) is in anterior commissure; most medial (am) is in nucleus accumbens. In a similar manner, a row of 3 electrodes is placed 3 mm posteriorly in the pre-optic region (RPO); the EEG tracings are thus labeled correspondingly from medial to lateral (pm, pc, pl).

patterned after that of Hume and Ganong (7), a movable bracket of 6 concentric bipolar electrodes, oriented by use of x-rays, was lowered into deep brain structures. Electroencephalographic recordings were made with use of 8-channel Grass inkwriter. In 2 cases the response to hypertonic saline was also monitored oscilloscopically.

*Results.* Intracarotid injections of hypertonic saline in the dog induced characteristic EEG changes (Fig. 1). Control injections of isotonic saline (A) caused no appreciable alterations in either electrical activity of brain or blood pressure. The latter was recorded to serve as indication that hypertonic saline was reaching the brain(6). The typical EEG response to hypertonic saline (Fig. 1, B) is most marked in channel 3. In general, 5 to 8 seconds after intracarotid injection, a high amplitude burst of synchronous activity appears and lasts for approximately 5 seconds. Its frequency lies in the 40-70 cycles/sec range and recorded with the use of oscilloscope as well as electroencephalograph.

The most dramatic response to hypertonic saline (Fig. 1, B, channel 3) is discretely localized just medial to lateral olfactory tract. It is also present to a lesser degree in the anterior commissure (channel 2). In 18 animals it has been recorded from the lateral part of olfactory tubercle, including lateral olfactory tract, and extending anteriorly into the olfactory peduncle. Only a relatively slight change of a synchronous nature can be recognized in the preoptic region (Fig. 1, B, channel 5). When seen at this antero-postero level (3 out

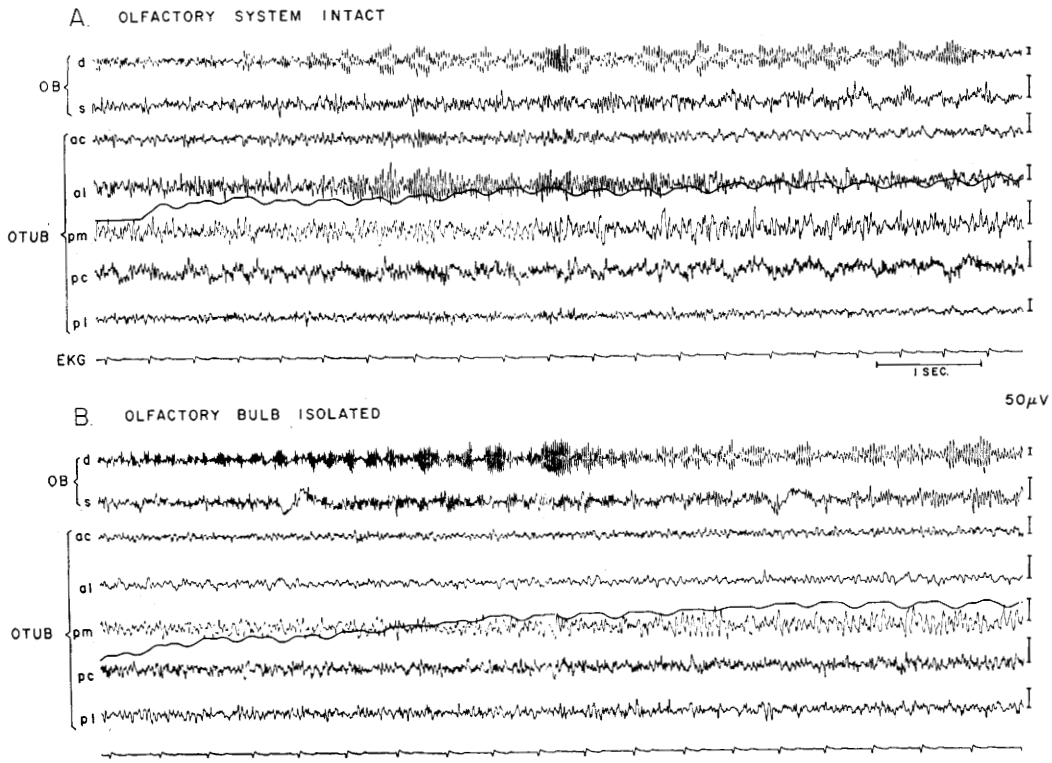


FIG. 2. EEG responses in olfactory bulb before and after isolation from basal forebrain. Both EEG records begin 3 sec. after intracarotid inj. of hypertonic saline (1.5 ml, 1.5 M NaCl). A. Synchronous response appears in both OTUB and OB before sectioning between them. B. Synchronous response appears only in OB after isolating it from rest of brain. In both cases arterial pressure increased approximately 50 mm Hg from 120 mm Hg baseline. EEG trace in channel 1 is taken from electrode (d) in the olfactory bulb (OB) whose tip rests in mitral cell layer; another (s) rests on bulb's surface. Two additional rows of bipolar electrodes are placed deep in the olfactory tubercle (OTUB). The anterior lateral electrode (al) is in the lateral olfactory tract and another (ac) is placed 3 mm medial to this at same depth. The other row is 2 mm posterior in region of diagonal band. Three electrodes are positioned 3 mm apart; the most lateral (pl) lies just medial to lateral olfactory tract; the most medial (pm) and the one in between (pc) are at same depth as lateral electrode.

of 9 animals whose anterior electrodes were showing positive changes), the response has been located in the lateral preoptic area not far from the nucleus of lateral olfactory tract.

Since the induced activity appeared to be stronger in the more anterior regions, additional electrodes were placed in the olfactory bulbs (OB) of 5 dogs. Typical EEG changes in response to hypertonic saline have been observed in the OB in each of these cases (Fig. 2, A). The OB record in channel 1 shows the high amplitude synchrony, which also appears in the olfactory tubercle, quite marked in channel 4. Other areas close by can be seen to pick up this change of activity only to a slight degree. To determine from which of these regions the activity was emanating, a complete section between the bulb and the tubercle was made with a blunt instrument. Following this the response to hypertonic saline was no longer obtained in the OTUB, while the OB was still reactive (Fig. 2, B). In addition, the activity induced in the olfactory bulb is independent of the olfactory epithelium: a procaine spray blown into the nostril, which will block the bulb's response to a blast of odor-laden air, does not interrupt the response to hypertonic saline. The activity, therefore, has characteristics of Adrian's "intrinsic olfactory activity"(8).

These responses are not dependent on specific receptors for sodium or chloride, but

rather on osmosensitive elements, since they can be evoked by hypertonic glucose and hypertonic sucrose. Whatever influence they may have on the activation of the supraoptico-hypophysial system is a question for further study.

*Conclusion.* Intracarotid injections of hypertonic saline and sugar solutions in the dog induce characteristic synchronous EEG changes in the olfactory bulb which are independent of influences from the olfactory epithelium or the rest of brain. In the intact brain, these osmosensitive elements in the bulb transmit their nervous impulses caudally along olfactory pathways, but their influence on the supraoptico-neurohypophysial system is unknown.

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## Infectivity of Ribonucleic Acid (RNA) from Type I Poliovirus in Embryonated Egg.* (25005)

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Multiplication of MEFI strain of Type II poliovirus in the chick embryo after prolonged propagation in the suckling hamster is now well established(1,2,3,4). No reports of confirmation of reproduction of Type I or III poliovirus in chick embryo have appeared (see (5)). However, it has been reported(6) that *in vitro* cell cultures from chorio-allantoic membrane of chick embryo can support 10 to 20 transfers of all 3 types of poliovirus, to an extent indicative of multiplication. The present report describes synthesis of Type I poliovirus in embryonated eggs after inoculation by amniotic route with ribonucleic acid (RNA) derived from Type I (Mahoney) poliovirus. Polio-RNA previously exposed to ribonuclease (RNAase) failed to yield the plaque-forming agent.

*Materials and methods.* Materials and procedures leading to this study have been published(8); additional or modified technics will be described. Mahoney Type I poliovirus grown on human amnion cell culture was obtained from Cutter Labs. (Berkeley, Cal.) as 100-fold concentrate designated $E_1$. Ribonucleic acid (RNA) preparations were made by phenol method of Gierer and Schramm(9), which consists of 3 extractions with phenol, followed by removal of phenol by ether extraction and subsequent removal of the ether by nitrogen. For our preparations, freshly distilled phenol was used; pH of preparation was adjusted finally by addition of a tenth volume of 0.9 M NaCl containing 0.04 M sodium phosphate at pH 7.6. Aliquots were used which, without additional dilution, had been frozen in a $CO_2$ box at $-70°C$, and thawed once. Occasionally fresh preparations were used. The plaque forming capacity of polio-RNA was usually tested simultaneously with experimental test fluids. Most viral assay tests were carried out in monolayers of a line of human amnion cells(10), although these were supplemented occasionally with small tests using monolayers of HeLa L5, a clonal line used previously(8). When comparative tests were done, no significant dif-

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