PRO EXPERIMENTIS

A new stimulation technique of the crista ampullaris of the lateral canal in the adult cat: Study of the action potential of the vestibular nerve[1]

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Summary. The stimulation of the crista ampullaris of the lateral canal by a short ampullopetal liquid flux produces on the pre-ganglionic vestibular fibres the appearance of a bimodal action potential. The amplitude of this action potential increases with the intensity of the stimulation. This stimulation also provokes the birth of an evoked potential at the level of the vestibular nuclei as well as an ocular jerk.

The vestibular system is the only sensory system which has not as yet been capable of being activated by physiological stimulations of very short duration. This is a major handicap in an electrophysiological study of the functioning of the labyrinthine receptors and of the vestibular pathways.
Also, research tends to center around either the analysis of the spontaneous activity[2,3] of vestibular fibres and second order neurons, or the responses provoked by prolonged rotary[5-8] or caloric[4] stimulations.
The aim of our present research was to perfect a technique that would permit the recording of action potentials on the vestibular nerve after an ampullopetal stimulation of the lateral canal ampullae in the cat. This method was designed to follow a modality that would recall the click and the flash utilized respectively for studying auditive and visual systems.

Materials and methods. The surgical technique was inspired by those of Ewald (cited by Barany[9]) and of Szentagothai[10]. The experiments were performed on 20 adult cats, anesthetized with a 6% solution of sodium pentobarbital. We placed in the membranous lateral canal, oriented towards the corresponding ampullae, a steel needle whose exterior diameter (0.25 mm) was determined from the data of Curthoys et al.[11]. This needle was connected to a Hamilton syringe of 1 µl by the intermediary of a catheter. The entire apparatus was filled with a liquid having the same composition as the endolymphatic liquid. The needle was rigidly affixed to the bone canal with dental cement in order to obtain a hermetically closed system. The plunger of the syringe was set in motion by an electronic device which enabled us to effect reversible excess pressures by injection of liquid in less than 1 msec.
Recording electrodes, formed of 2 coupled silver wires of 50 µm diameter, were placed on the nerve of the lateral canal ampullae. A silver macroelectrode was descended, by stereotaxy, into the vestibular nuclei.

Results and discussion. The stimulation brought about by the injection of 0.01 µl causes the appearance of a threshold response in the form of a bimodal action potential whose 2 components are separated by 1.4 msec. In the course of the stimulations of increasing intensity (0.01–0.08 µl), the voltage of the 1st potential goes from 10 µV to 40 µV, whereas that of the 2nd potential goes from 12 µV to 60 µV. One also ascertains that the time interval which separates the 2 potentials, diminishes so that the average interval is located at 0.94±0.14 msec. The degree of stimulation of the hair receptor cells is therefore proportional, in a certain measure, to the extent of displacement of the sensory hairs[12]. One must take note that the action potential obtained for a stimulation of average intensity (0.05 µl) resembles the action potential recorded on the auditive nerve after a click. In effect, this potential also presents 2 components, called N_1 and N_2[13].

We recorded, moreover, at the vestibular nuclei level evoked potentials that were also bimodal. The 2 peaks are separated by an average time interval of 0.88±0.09 msec. It therefore appears that this interval is the same at the level of the nerve and in the vestibular nuclei. When the animal is coming out of anesthesia, one can confirm that each shock provokes an ocular jerk either ipsilaterally or bilaterally; the rapid phase of the nystagmus is directed to the side opposite the one that received the shock.
Conclusion. The recordings carried out at the level of the nerve of the lateral canal ampullae and of the vestibular nuclei show the efficaciousness of a short physiological ampullopetal stimulation. Many authors[14,15] have shown that, in effect, an ampullopetal flux is the stimulating agent at the level of this ampullae. Our results show that the amplitude of the potential is tied to the surpression created within the ampullae and is therefore tied to the displacement of the cupulae.

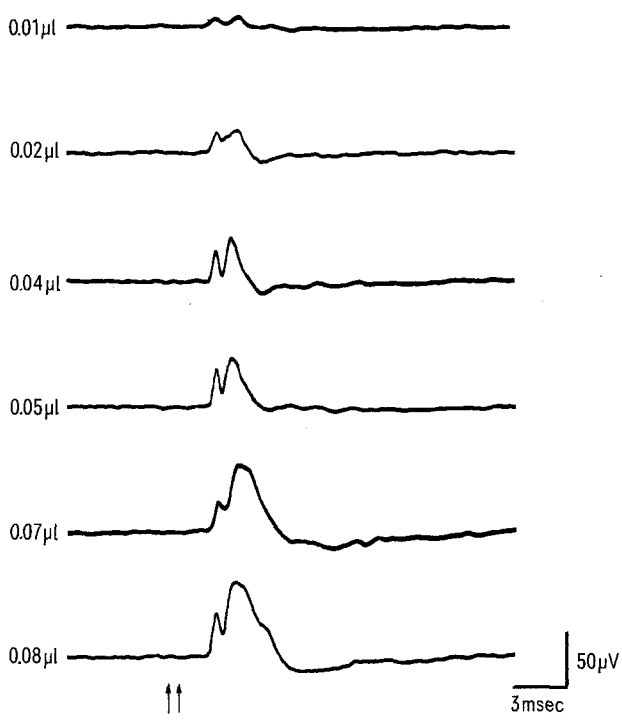

Fig. 1. Bimodal action potentials recorded on the nerve of the lateral canal ampullae in the cat after stimulations of increasing intensity. Notice the especially important increase in the amplitude of the 2nd peak and the appearance of later components after the most intensive stimulations. The 2 arrows indicate the beginning and the end of the injection (duration 0.5 msec). The recording electrode is placed in monopolar derivation.

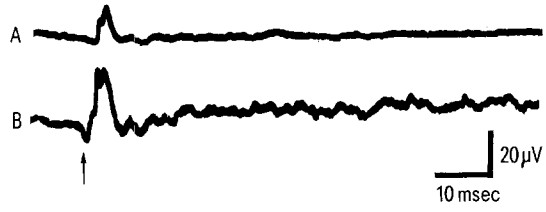

Fig. 2. Simultaneous recordings of the action potential on the nerve of the lateral canal ampullae (A) and of the evoked potential in the lateral ventral vestibular nucleus (B). The time interval which separates the 2 components of the potentials does not increase. The arrow indicates the beginning and the end of the injection.

The bimodal form of the potential poses a problem of interpretation that anatomico-functional correlations by histo-physiological methods will perhaps enable us to solve. Indeed, we know that the epithelium of the crista ampullaris contains 2 populations of sensory cells (HC I and HC II) afferented in a non-problematical way[3] by fibres of different calibers (1–12 μm)[16]. The 2 peaks of the action potential are therefore perhaps due to the mode of functioning of these cells.

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A method for the determination of glutamate oxaloacetate transaminase in sperm plasma from boars and bulls[1]

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Summary. A method is described for the determination of glutamate oxaloacetate transaminase (GOT) activity in sperm plasma from boars and bulls as a test for fertilizing ability.

GOT (E.C.2.6.1.1) activity in sperm plasma can be used as a criterion for semen quality and fertility[2–6]. Because existing colorimetric methods lack selectivity[7–9], and the coupled reaction assay for determination in blood serum (ultimately a measurement for NADH oxidation at 340 nm)[8,10] proved to be unsuitable for use in sperm plasma, we modified the Boehringer UV method[8].

Material and methods. 0.1 M Na/K phosphate buffer, pH 7.6; 0.58 M Tris, pH 10.85; trichloroacetic (TCA) solutions of 25- and 2.5% (w/v) in aqua dest.; solutions of 40 mM L-aspartate, 125 mM α-oxoglutarate and 2.4 mM NADH in phosphate buffer; malate dehydrogenase (MDH), diluted just before use to 250 μg/ml in phosphate buffer. All chemicals were of reagent grade. Absorbances were measured on a Shimadzu UV 200 spectrophotometer in 1-cm path length cells. Aqua dest. was used as a blank.

Sperm plasma was prepared by centrifugation of undiluted or with current buffers diluted[10] sperm for 15 min at 3500 × g. The supernatant was centrifuged once more and the resulting sperm-free supernatant diluted with phosphate buffer, 1:50 for bulls and 1:4 for boars, based on the original sperm volume. Then 1-ml samples of these dilutions were mixed with 2 ml aspartate and 0.2 ml oxoglutarate and incubated in stoppered polyethylene tubes for 30 min at 37 °C (L-aspartate + α-oxoglutarate $\overset{\text{GOT}}{\rightleftharpoons}$ L-glutamate + oxaloacetate). Next 0.5 ml 25% TCA was added to the incubation mixture under cooling at 0 °C. Centrifugation for 10 min at 8000 × g and, if necessary, filtration through 'Blaubandfilter no. 589[3] from Schleicher & Schüll' gave a clear and colourless solution for the subsequent MDH reaction (Oxaloacetate + NADH + $H^+ \overset{\text{MDH}}{\rightleftharpoons}$ L-malate + NAD^+).

For this purpose 2 ml of this solution was mixed with 0.8 ml Tris and 0.1 ml NADH (final pH 7.4–7.6). The absorbance at 340 nm and 25 °C was measured (A_1), 0.1 ml MDH was added and after 5 min the absorbance was measured again (A_2).

In case $A_2 < 0.15$ (due to a relatively high initial concentration of oxaloacetate) another 0.1-ml portion of NADH was added and after 5 min the new absorbance was read (A_2^1). In this case also a reference solution of 0.9 ml Tris, 2 ml 2.5% TCA and 0.1 ml NADH was measured (A_1^1). Absorbance values A_1 and A_2^1 were corrected to a reaction volume of 3 ml. Defining the GOT unit as the amount that catalyses the formation of 1 μmole of oxaloacetate per min at 37 °C under our conditions, it follows that GOT activity = $A \times d \times 185/\varepsilon$ units per ml of undiluted sperm plasma, in which $\Delta A = A_1 - A_2$ (or $A_1 + A_1^1 - A_2^1$), d = dilution factor of the original sperm plasma (50 or 4) and ε = molar absorption coefficient for NADH[7] at 340 nm = 6.22×10^3. If desired, the results may be related to the number of spermatozoa per ml of undiluted sperm, determined e.g. by Coulter Counter measurements.

Results and discussion. With diluted sperm plasma, linearity of the progression-with-time curve for the GOT reaction was established. Moreover, the relationship between ΔA and standard quantities of GOT and also between ΔA and oxaloacetic acid quantities, having been subjected to the whole and 2nd half of the reaction procedure respectively, proved to be linear. The reproducibility of measurements of 1 ejaculate is shown in the following table: