

hitherto accepted that the second component represents summated action potentials of the intracortical interneurons¹. The interneurons found in the molecular layer are only the horizontal cells of Cajal which are scattered so sparsely that no appreciable field potentials are expected to be produced by them². Therefore, the second wave cannot be due to the summated action potentials of the intracortical interneurons. The finding that the wave reversed in polarity when the slice was inverted indicates that, like as the first component, the second wave reflects activity developed in the distal portion of the apical dendrites.

This is the first experiment in which electrical response was successfully recorded from the slice prepared from the mammalian neocortex and incubated in the artificial medium. McILWAIN et al. and HILLMAN et al. reported that they were unable to evoke any potential change in the slice in response to a single shock^{4,5}. While in their experiments the slice was 0.35 mm thick, in the present experiments the slices 0.2 mm thick were used. We, too, failed to produce electric activity in the slices 0.35 mm thick. Therefore, their negative results may be accounted for by considering that their slices were too thick to maintain physiological activities in the artificial medium⁶.

Résumé. Pour examiner l'origine de la réponse corticale par stimulation directe, le potentiel produit dans la mince section corticale a été étudié. Dans la tranche qui se compose seulement de la couche moléculaire, la configuration de la réponse a été la même que celle qui a été suscitée dans le cortex intact. La propriété du potentiel indique que non seulement les ondes initiales rapides mais aussi les ondes secondaires retardées ont atteint la portion éloignée des dendrites apicaux.

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Behaviour Research Institute, University of Gunma Medical School, Maebashi (Japan), 24th November 1966.

⁴ H. McILWAIN, S. OCHS and R. W. GERARD, *Am. J. Physiol.* 171, 128 (1952).

⁵ H. H. HILLMAN, W. L. CAMPBELL and H. McILWAIN, *J. Neurochem.* 10, 325 (1963).

⁶ We thank Dr. T. HIRAO for his discussion and encouragement.

Effects of γ -amino-butyric Acid on the Potentials Evoked in vitro in the Superior Colliculus

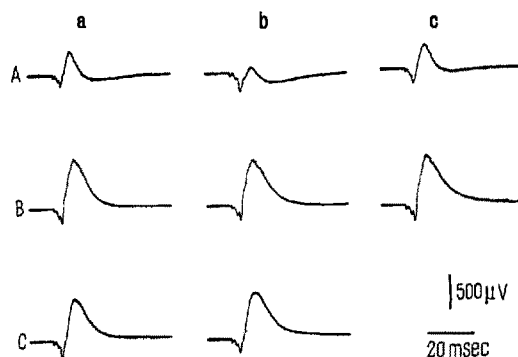
Since BAXTER and ROBERTS reported that the superior colliculus contained a conspicuously large amount of γ -aminobutyric acid (GABA)¹, it has been presumed that GABA may play an important role in impulse transmission in this structure. In the present experiments, we prepared thin slices from the superior colliculus of guinea-pig and studied effects of GABA upon electrical activities produced in the slices in vitro. It was found that GABA mimicked the action of the inhibitory transmitter in the superior colliculus.

The slice consisting of the superior colliculus and the incoming optic tract was prepared in the same manner as the slice from the cerebral cortex was usually made². The thickness of the slice was 0.4–0.5 mm. The slice was incubated in a glucose-saline medium saturated with 95% oxygen and 5% carbon dioxide. Electric stimulation was applied to the optic tract with a pair of ball-tipped silver wires. The recording electrode was also a ball-tipped silver wire resting on the superior colliculus. The normal medium was composed of (final concentration) NaCl (124 mM), KCl (5 mM), KH_2PO_4 (1.24 mM), MgSO_4 (1.3 mM), CaCl_2 (2.6 mM), NaHCO_3 (26 mM) and glucose (10 mM). When the Cl-free medium was prepared, acetate or propionate ion was used to replace chloride ion.

As shown in Figure A-a, the potential induced in vitro in the superior colliculus by optic tract stimulation consisted of an initial small positive deflection and a late negative wave of higher amplitude. Usually, 2 negative spikes were superposed on the descending phase of the initial wave. In contrast to the potential recorded from the superior colliculus, the potential recorded from the optic tract was merely composed of the rapid action potentials of the incoming presynaptic axons and not accompanied by the late negative wave. Therefore, it may be concluded that the late negative wave represents the

potential generated postsynaptically in the superior colliculus and the initial one reflects the action potential of the presynaptic axons in the optic tract.

Figures A–C came from an experiment in which effects of GABA were observed in the normal medium (A), in the Cl-free medium (B) and in the presence of strychnine (C). The stimulus strength and the arrangement of the electrodes were kept constant throughout the experiment.



Effects of GABA on the potentials evoked in vitro in the superior colliculus. All the records were obtained in an experiment in which arrangement of the electrodes and stimulus strength were kept constant. A, recorded in the normal medium, B, in the Cl-free medium and C, in the normal medium containing strychnine ($3 \times 10^{-5} M$). a, before application of GABA, b, 1 min after $2.8 \times 10^{-4} M$ GABA was added to the medium, c, 1 min after washing with normal medium (Figure A) or with Cl-free medium (Figure B). Note GABA was effective only in the normal medium. Upward deflections, negative.

¹ C. F. BAXTER and E. ROBERTS, *Proc. Soc. exp. Biol. Med.* 107, 811 (1958).

² H. McILWAIN and R. RODNIGHT, *Practical Neurochemistry* (Churchill, London 1962).

When GABA was added to the normal medium in a concentration of $2.8 \times 10^{-4} M$, the postsynaptic potential was markedly depressed (Figure A-b). Though in this record the presynaptic tract potential was slightly increased in height, this was not the consistent observation in the present series of experiments. The concentration of GABA to cause minimum detectable suppression was about $5 \times 10^{-5} M$. Washing the slice with the normal medium resulted in the recovery of the control potential (A-c). The slice was then immersed in the Cl-free medium. In this medium, the postsynaptic potential was substantially augmented (B-a) but application of GABA ($2.8 \times 10^{-4} M$) no longer caused any noticeable suppression in the potential (B-b). Subsequently, in Figure C, the slice was immersed in the normal medium containing strychnine in a concentration of $3 \times 10^{-5} M$. Just as in Figure B, the control response was augmented in this solution and GABA was without effect (C-b). Picrotoxin (up to $1 \times 10^{-3} M$) was found in another experiment to be entirely without effect either on the control response or on the depressing action of GABA.

In the present experiments, it was found that GABA strongly suppressed the potential evoked in the superior colliculus in a substantially low concentration. As reported previously³, in the slices prepared from the cortex, GABA could not cause such strong suppression even in much higher concentration (about 100 times). The fact that GABA was effective in a low concentration in the superior colliculus suggests that GABA exerts a specific inhibitory action on the neurons in this part of the brain.

The natural inhibitory transmitter in the mammalian brain has been shown to exert its depressing effects mainly by increasing membrane permeability to Cl ion⁴. Therefore, it is expected that effects of the inhibitory transmitter are blocked in the Cl-free medium^{5,6}. Strychnine is also a well-known blocker of the action of the

inhibitory transmitter in the spinal cord⁴. The observation made in the present experiments that the depressant action of GABA was blocked by strychnine or in Cl-free medium indicates that in the superior colliculus GABA closely simulates the action of the inhibitory transmitter.

The control response was larger in the Cl-free medium or in the presence of strychnine than in the normal medium. In order to explain this observation, we assume that, in the slice, the inhibitory transmitter was released spontaneously from the nerve terminals. The released inhibitory transmitter might be without effect in the Cl-free medium or in the presence of strychnine but might exert some depressant effects in the normal medium. This could result in the decrease of the control response in the normal medium⁷.

Résumé. L'acide butyrique gamma-amino (GABA) en basse concentration a réduit le potentiel suscité in vitro dans une tranche de *Colliculus superior* chez le cobaye. Cette action suppressive de GABA ne s'est pas produite dans un medium sans chlore ou avec de la strychnine. On en a conclu que GABA simule le transmetteur inhibitoire dans le *C. superior*.

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Behavior Research Institute, University of Gunma Medical School, Maebashi (Japan), 8th December 1966.

³ C. YAMAMOTO and H. McILWAIN, *J. Neurochem.* **13**, 1333 (1966).

⁴ J. C. ECCLES, *The Physiology of Synapses* (Springer-Verlag, Berlin 1964).

⁵ A. TAKEUCHI and N. TAKEUCHI, *J. Physiol.* **183**, 433 (1966).

⁶ C. YAMAMOTO and N. KAWAI, *Science* **155**, 341 (1967).

⁷ We thank Dr. T. HIRAO for his discussion and encouragement.

Responses of the Oculomotor Units to the Labyrinth Stimulation Under Hypothermia

In previous experiments, the influence of the labyrinth on the unitary discharge of the oculomotor nuclei was investigated in curarized guinea-pigs¹⁻⁴. Object of the present research is to analyse the behaviour of the oculomotor unit responses to the stimulation of the labyrinth in curarized guinea-pigs during progressive body cooling and rewarming. It is well known that the body temperature modifies the ocular responses to stimulation of the labyrinth⁵⁻⁷.

Experimental. The experiments were carried out in 36 curarized guinea-pigs (1-2 mg of Intocostrin). All the technical procedures (anaesthesia, surgery, recording, microelectrode preparation) were the same as those employed in our previous papers¹⁻⁴. In several experiments the lower beam of the oscilloscope recorded the action potentials of a few fibres of the ipsilateral oculomotor nerve, picked up by means of a microelectrode. Progressive cooling of the guinea-pigs was accomplished by covering the body of the animals with pieces of ice. The body temperature was recorded from the colon during the entire course of the experiments by a Hg thermometer (sensitivity 0.1°C). The colonic temperature of 18°C was usually attained in 30 min after which the guinea-pigs

were brought back to the initial colonic temperature of 37°C within 30 min by dipping the animal's body in warm water (40°C). The records of the mesencephalic unitary discharge were made both during decrease and increase of the body temperature. In many experiments the temperature of the mesencephalon was also recorded by means of a copper-constantan thermocouple connected to a 4 stage DC amplifier. The mesencephalic temperature variations consistently followed the colonic temperature modifications.

Stimulation of the labyrinth was usually performed by warming a limited point of the osseous ampulla of the superior or lateral semicircular canals exposed by opening an epitympanic recess; the tip of a steel needle heated to a temperature of 60-70°C was applied to the bone of the

¹ E. MANNI, G. B. AZZENA, H. CASEY and R. S. DOW, *Expl. Neurol.* **12**, 9 (1965).

² E. MANNI and C. DESOLE, *Expl. Neurol.* **15**, 206 (1966).

³ G. B. AZZENA, *Brain Res.* **2**, 218 (1966).

⁴ E. MANNI, G. B. AZZENA and R. S. DOW, *Expl. Neurol.* **13**, 252 (1965).

⁵ T. R. MASERA, *Archo Fisiol.* **37**, 217 (1937).

⁶ M. INNOCENTI, *Archo Fisiol.* **41**, 425 (1941).

⁷ L. GIULIO and P. MENZIO, *Archo Fisiol.* **53**, 326 (1953).