MENTEN equation according to WOOLF[13]. V_{max} was 31.5 ± SD 1.3 and 32.5 ± SD 1.4 nmol/s·g liver in the presence and in the absence of ICG, respectively. The corresponding *Km* values were 102.8 ± SD 14.4 and 90.6 ± SD 10.6 nmol/g liver. (Difference of slopes $t = 0.69184$; $0.5 > p > 0.4$; difference of regression lines $t = 1.75768$; $0.1 > p > 0.05$). Thus, no significant competition between taurocholate and ICG for their uptake by the hepatocyte could be demonstrated. It may, therefore, be assumed that different pathways exist for the transport of bile acids and anionic dyes from the blood into the hepatocyte. These findings substantiate previous results obtained with a different technique in the intact rat[2,3], and parallel the demonstration of multiplicity of hepatic excretory mechanisms for organic anions by ALPERT et al.[7].

Hepatic transport of organic anions from the blood into the bile may be regarded as serving mainly two purposes: the generation of bile flow, and the elimination of a variety of substances from the blood. It may offer a biological advantage that these two main functions are not dependent on the same transport system. Thus, under certain pathological conditions, the hepatic uptake of some organic anions could be disturbed while the transport of bile acids and, with it, bile flow are maintained[17].

Zusammenfassung. Mittels der Indikatorverdünnungsmethode nach GORESKY[1,11] konnte gezeigt werden, dass die hepatische Aufnahme von Taurocholat und Indocyaningrün (ICG) der MICHAELIS MENTEN Kinetik folgt. Dies ist mit der Annahme eines Carrier-Transportes vereinbar. Zwischen Taurocholat und ICG konnte keine Kompetition um die Aufnahme in die Leber nachgewiesen werden. Dies weist darauf hin, dass für den Transport von Gallensäuren und anionischen Farbstoffen vom Blut in den Hepatozyten verschiedene Transportwege existieren.

G. PAUMGARTNER and J. REICHEN

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3008 Bern (Switzerland),
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Rhythmic Neuronal Activity in Tissue Culture[1]

Rhythmic variations in neuronal spike production have been obtained in explants taken from chick brains following a few hours of incubation[2]. A similar rhythmicity was described in fetal rodent cultures[3] and more recently from cerebellar explants by GÄHWILER[4] and CALVET[5]. During the course of investigating spreading depression in tissue culture[6], the observation of rhythmicity was expanded to several brain areas each having a different, yet characteristic, burst pattern and some having extraordinarily long periodicities measured in minutes[7].

Methods. The technique of culturing and recording is described fully elsewhere[6]. Explants from midbrain, colliculi and cerebellum were taken from newborn rats of more than 24 but less than 72 h of age. These were placed on a glass cover slip and held by a clot over a hole (averaging 200 μm in diameter) drilled through the cover slip. The cultures were maintained by the 'flying cover slip' method in roller tubes for at least 2 weeks by which time the tissue had proliferated and migrated into the hole in the side. The cover slip was placed on a chamber which permitted the addition of Gey's balanced salt solution above and below the tissue with contiguity of the fluid only at the hole. Salt-agar bridges carrying Ag·AgCl electrodes were immersed in the fluid above and below the tissue; thus, no perturbation of the tissue occurred from manipulation of the electrodes. The tissue was grown, so to speak, in the tip of the recording electrode. One electrode was at ground and the other recorded neuronal activity and direct current (dc) difference between upper and lower chambers. The dc signal was amplified and displayed on a penwriter. Neuronal activity was monitored by a digital frequency meter which emitted a pulse for each spike over 20 μV. The quantity of spikes per unit of time (generally 1.0 sec) was displayed on the penwriter. In addition the preparation was made to form one arm of an alternating current (ac) Wheatstone's bridge from which impedance imbalance was recorded and over which shocks to the preparation were delivered.

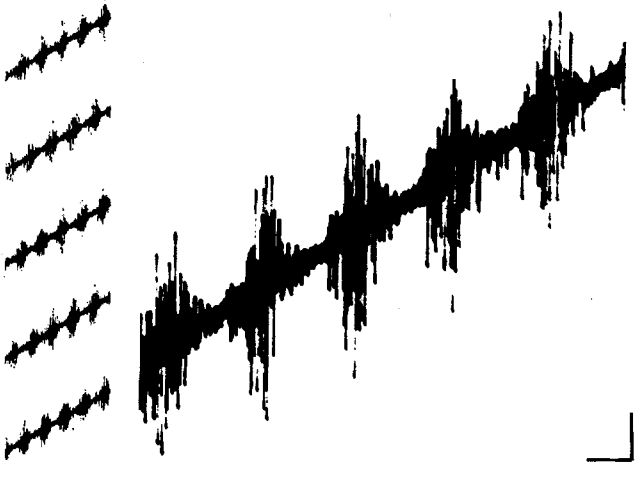

Fig. 1. Bursts of neuronal spikes photographed from the oscilloscope during the recording of neuronal activity in an explant of cerebellum cultured for 26 days. To the left is a print of a strip of film indicating the regularity of these bursts and to the right is an enlargement. Vertical mark indicates 20 μV and the horizontal 50 msec. Hole measured 190 μm in diameter.

Results and discussion. Using these techniques spontaneous rhythms were observed in explants from the several brain areas yielding distinctly different patterns of

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activity. Cerebellar explants (Figure 1) characteristically gave rapid bursts of spikes occurring every 100 msec and having a duration of 50 msec. Some cerebellar cultures were observed to have periodicities of a longer nature: every minute with bursts of 30 sec. The longest periods were observed in explants of the midbrain area (Figure 2) having intervals of generally 4 to 7 min but as long as 10 min yielding a cascading firing pattern of over 1.0 min duration while the rest of the period remained essentially quiet. These cascades could be driven but evidenced a relative refractoriness to successive electric shocks. Explants from the colliculi gave peaks of activity having a serrated appearance to the spike discharge pattern with periods of approximately 4 min in length.

Not every explant produced a rhythmic pattern of neuronal activity but when present the type and pattern of spike discharge was sufficiently consistent to be a reliable characteristic of explants taken from a particular area of brain. Explants from the midbrain area produced rhythmical discharges 20% of the time, cerebellum 10%, and colliculus 5%. Explants not initially showing rhythmic neuronal firing would occasionally do so later either spontaneously or would be induced to do so by small, brief shocks of the order of 10^{-9} Amp/μm^2 given for a duration of 1.0 msec. The rhythmic pattern once initiated would continue for over 1 h with little variation. Eventually the spike production failed, indicated by gradual diminution of the number of spikes generated during each episode until finally none was produced. The pattern could be revived briefly by exchanging the fluid in the recording chamber. Investigations were made at room temperature (27 °C).

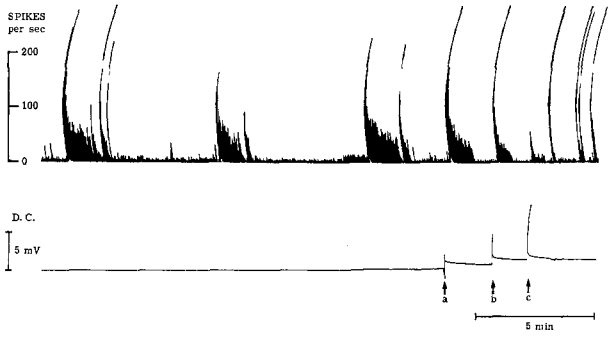

Fig. 2. Cascades of neuronal activity occurring every 6.5 min in an explant from midbrain 16 days of age. The upper trace indicates the quantity of neuronal spikes counted by the digital frequency meter and the lower trace indicates the dc difference between the fluid in the upper and lower chambers. This pattern of activity has been continuous for more than 45 min. It was only altered by shocks at a, b and c where a current density of 9.5×10^{-9} Amps per μm^2 was delivered for 0.05, 0.5 and 5 msec. It can be seen that successive shocks with lengthened duration had less affect indicating refractoriness of the tissue. Hole measured 200 μm in diameter.

Of the brain areas studied cerebral cortical tissue alone did not demonstrate a regular pattern of activity; however, there was observed irregular slow wave activity having a duration of 100 msec and an amplitude of 500 μV (approximately 5 times the amplitude of spikes) which appeared to initiate neuronal spikes. The activity has been reported by CALVET[5] who suggests that it may result from the simultaneous activation of post synaptic potentials. In any case, there must be a genetic component to account for the uniformity of activity in a given brain area that is maintained in tissue culture. The rapid rhythms reported could be explained by interneuronal circuitry. More likely these rhythms are driven by pacemaker cells not unlike the pacemaking Purkinje cells described by GÄHWILER in cerebellar explants[4]. Small alterations in measured impedance have occasionally been observed reflecting transient changes in extracellular space probably resulting from neuroglial interaction.

Résumé. Les enregistrements électriques non-perturbants des neurones maintenus en culture, ont montré des décharges spontanées prototypes des aires du système nerveux central desquelles les groupes de cellules cultivées avaient été obtenues. Les groupes de neurones dont les enregistrements figurent ci-dessus, ont été extraits du télencéphale, mésencéphale, cervelet, et des tubercules quadrijumeaux de souris nouveau-nées. Seuls les neurones provenant du télencéphale n'ont pas produit de décharge électrique rhythmique.

F. D. WALKER

Institute of Psychiatric Research, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis (Indiana 46202, USA), 19 August 1974.

Recording of Electrical Activity from Microscopically Identified Neurons of the Mammalian Brain

In dissected preparations of the peripheral and invertebrate nervous tissue, a single neuron or a synaptic site can be visualized microscopically with its functions intact. Impulse transmission in these synapses was, therefore, intensively studied and unequivocal results were obtained[1–3]. In the mammalian brain, however, recording of electrical activity from a microscopically identified neuron has not been successful. Although brain sections of 0.3–0.4 mm thick have been found to exhibit spontaneous and evoked potential activities in the artificial media[4–6], they are still too thick for observation of neurons therein. In the present experiments, I have attempted to prepare much thinner sections of the guinea-pig cerebellum and record action potentials from the Purkinje cells while observing them with the aid of a microscope.

Material and methods. Procedures for preparation of the tissue were identical with those described previously except for the thickness of sections[7]. Guinea-pigs were stunned and killed by blows on the back of the neck and thorax. The brain was taken out of the skull and the vermis of the cerebellum was isolated on a piece of filter paper covered with the medium. From the nodulus and uvula, blocks of the cortex of about 1 mm thick were prepared and kept in the medium at 0–4 °C for 5–20 min

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