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).

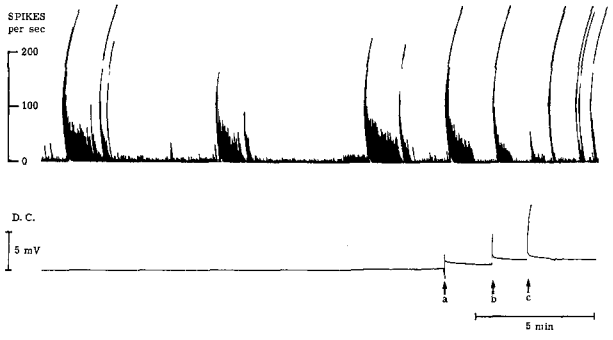

Fig. 2. Cascades of neuronal activity occurring every 6.5 min in an explant from midbrain 16 days of age. The upper trace indicates the quantity of neuronal spikes counted by the digital frequency meter and the lower trace indicates the dc difference between the fluid in the upper and lower chambers. 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.

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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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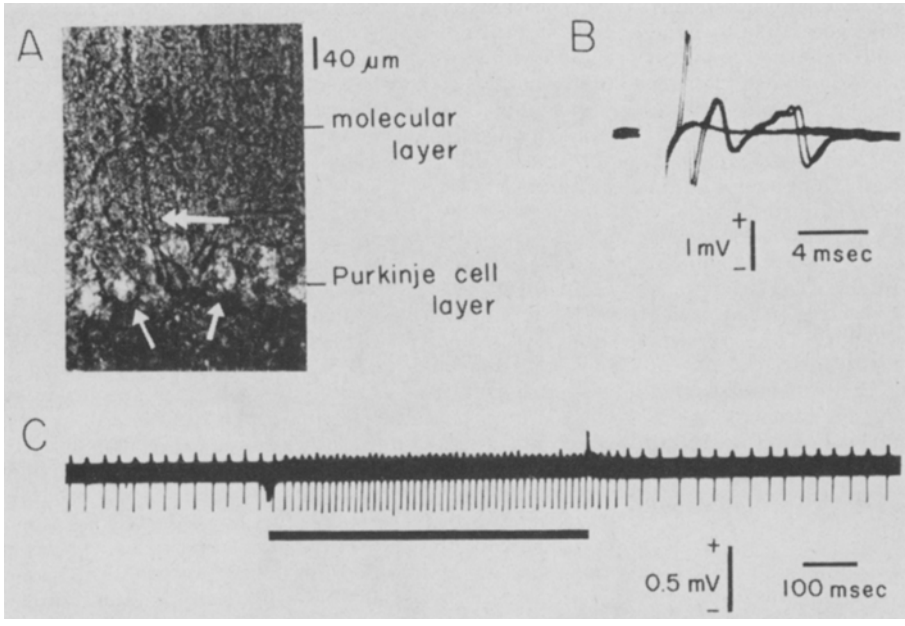

A) Photomicrograph of a cerebellar section of 80 μm thick. Single and double arrows indicate Purkinje cell bodies and dendrite, respectively. B) Climbing fibre response recorded extracellularly from cell body. Superimposed records. C) Increase in discharge rate of a Purkinje cell during application of glutamate to cell soma. Solid line, anionic current (5 nA) was passed through a glutamate-pipette. Retaining current was +10 nA.

until they adhered one by one on to a small metal dish with Aron-Alpha (α-acrylate monomer, Toa Gosei Kagaku, Tokyo). Then the tissue blocks were immersed in the chilled medium and cut to a thickness of 70–80 μm with the Vibrotome (Oxford Laboratories, California) at right angles to the long axis of the folium. The sectioning was performed at a vibration rate of 6.5–7 scale units and a feeding speed of 2–3 scale units with a blade inclined at about 17 degrees. The sections prepared in this way were incubated at 37 °C for more then 45 min and then transferred into the observation vessel, which was similar to that described before[8], and mounted on a microscope stage. Sections were observed from above with an objective lens of long working distance (×40, UD 40/0.65 C, Carl Zeiss, West Germany). Conventional glass microelectrodes of 3 μm tip diameter filled with 4 *M* NaCl were used for extracellular recording of single cell discharges and for electric stimulation. For application of glutamate, single glass pipettes were filled with 1 *M* solution of the amino acid (pH 8.0). The tip of the pipette was positioned in close vicinity of a nerve cell and anionic current was passed through the pipette. The composition of the medium was (m*M*); NaCl, 124; KCl, 5; KH_2PO_4, 1.24; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26 and glucose, 10.

Results and discussion. When the sections were observed microscopically, Purkinje cell bodies were well discernible, especially with the condenser diaphragm partially closed (Figure A, arrows). By adjusting the focus of the microscope finely, it was possible to identify the outline of the cell body. In some cells, contour of the dendrite could be followed in the molecular layer (double arrow). As observed before in thicker slices, most of the Purkinje cells generated spontaneous discharges, which were 5–80 cps in frequency and either almost regular or interrupted by the silent period of various durations[7,9]. Although dendritic branches were thought to be partly damaged during preparation of the sections, only a few cells in sections from the uvula and nodulus showed signs of excessive depolarization, such as the burst discharges with a reversal of spike polarity[7]. For synaptic activation of the Purkinje cells, the tip of a NaCl-filled glass pipette was positioned close to the dendrite of a Purkinje cell, electrical activity of which was recorded from the cell body with another pipette. A negative pulse of 0.2 msec duration passed through the former pipette induced in an all-or-nothing manner a burst of discharges consisting of 2–4 spikes (Figure B). This burst discharge seems to be the climbing fiber (CF) response. The negative pulse probably excited a CF which in turn synaptically activated the near-by dendrite. Already, ECCLES et al.[10] reported that not only stimulation to the inferior olive nucleus and extrafastigial fibres but also direct stimulation to the cerebellar cortex induced the CF response. The CF response and spontaneous discharge were recorded for more than several hours after preparation of the tissues. In another series of experiments, the tip of a glutamate-filled electrode was brought close to the cell body or apical dendrite of a Purkinje cell, the spontaneous discharge of which was picked up with another NaCl-filled pipette. When anionic current was passed through the glutamate-filled electrode, the discharge rate increased with a latency of 20–200 msec and returned to the control level as the current was turned off (Figure C).

FIDONE et al. already succeeded in recording action potentials from neurons in slices of the frog sympathetic ganglion which were made so thin that each neuron was microscopically observed[11]. The findings of the present experiments show that essentially the same technique is applicable to the mammalian brain. Generation of the CF response by electric stimulation indicates that the synaptic transmission is maintained in such a thin section. Since the CFs were mutilated in the sections, no CF discharges occurred spontaneously. Although the mossy fibres were also sectioned, Purkinje cells still generated spontaneous discharges. It remains to be clarified whether the excitation originated in the Purkinje cells or granule cells. Glutamate ejected with weak currents induced strong excitation which was quick in onset and termination.

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This was in agreement with previous observation made in vivo[12]. Since recording and stimulating electrodes can be applied independently to portions of a neuron identified microscopically, thin sections as used in the present experiments are expected to serve as excellent preparations for studies on chemosensitivity of the neuronal membrane as well as on neuron-neuron and neuron-glia interaction in the brain.

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Zusammenfassung. In Dünnschnitten vom Kleinhirn des Meerschweinchens wurden in künstlichem Medium Purkinjezellen identifiziert und Spontanentladungen der Zellkörper registriert. Elektrophoretischer Antransport von Glutaminsäure zu Zellkörper oder Dentriten rief jeweils starke Erregung hervor.

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Neurosecretory Cells in the Hypocerebral Ganglion of *Gryllus bimaculatus* de Geer (Orthoptera: Gryllidae)

The occurrence of various types of neurosecretory cells (NSC) in the brain and the ventral ganglia, which control all the major physiological events in the life cycle of insects, is well known. There is no previous report of the presence of neurosecretory cells in the hypocerebral ganglion beyond the recording of B cells in *Schizodactylus*[1].

The Bouin-fixed paraffin embedded material was serially cut at 6 µm and stained with Chrome Haematoxylin-Phloxine (CHP), Paraldehyde-Fuchsin (PF) and Heidenhain's Azan (Azan).

The hypocerebral ganglion has 2 types of neurosecretory cells which can be classified as A and B cells on the basis of their tinctorial affinities with PF, CHP and Azan (Figures 1–3). The first type, designated as 'A' cells, is characterized by cytoplasmic inclusions staining deep purple with PF, blue-black with CHP and red with Azan. The fine homogenous inclusions of the second type of cells, the 'B' cells stain greenish with light green-orange G, red with phloxine of CHP and faint blue with the aniline blue-orange G counterstains of Azan.

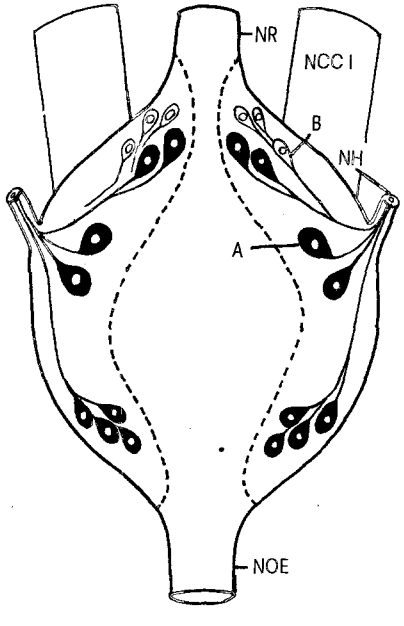

Fig. 1. Diagrammatic representation of the distribution of neurosecretory cells in the hypocerebral ganglion of *Gryllus bimaculatus*. A and B, neurosecretory cell types; NCCI, nervus corporis cardiaci; NH, nervus hypocerebrii; NOE, nervus oesophagei; NR, nervus recurrentus.

Each half of the hypocerebral ganglion has 8 to 10 A cells and 4 to 6 B cells found along with a large number of nerve cells. The anterior part of the ganglion has 2 cells in the median and midventral position, 2 cells in the anterior region and 4 to 6 cells in its posterolateral part (Figure 1). The axons arizing from the A cells run towards the lateral border of the ganglion (Figure 3) from where they enter the corpora cardiaca through the nervi hypocerebrii (NH, Figure 1). It is easy to trace the axonal pathways (AX) due to the presence of stained neurosecretory material in them which stains deep purple with PF, red with Azan and blue-black with CHP.

The mid-dorsal region of the anterior half of the hypocerebral ganglion has 4 to 6 B cells which stain greenish with PF, red with CHP and faint blue with Azan. The axons of these cells can be traced only for a short distance due to lack of staining colloids.

A large amount of neurosecretory material (NSM) has been observed laterally in the hypocerebral ganglion of males as the two NCC I pass through its lateral margins, whereas the amount of NSM is comparatively less in females as the NCC. I are free and have no connection with hypocerebral ganglion. The small amount of NSM, which is particularly seen before copulation and oviposition in females, is the product of the neurosecretory cells of this ganglion. Thus the greater amount of NSM in males is the product of the neurosecretory cells of the hypocerebral ganglion as well as the pars intercerebralis of the brain.

Thus the present work records for the first time in insects the presence of both A and B cell types and neurosecretory pathways up to the neurohaemal organs (corpora cardiaca).

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