monosynaptic reflexes by β-CPG therefore constitutes further evidence in support of the hypothesis that substance P is the transmitter released by primary afferent fibres[8]. Neurons in the rat cerebral cortex can be excited by substance P, acetylcholine (Ach) and L-glutamate[9,10]. In the present experiments the specificity of β-CPG antagonism of substance P has been evaluated on these neurons, utilizing Ach- and L-glutamate-induced excitations for comparisons.

Methods. 6 male Sprague-Dawley rats were anaesthetized with methoxyflurane, nitrous oxide and oxygen and mounted in a stereotaxic frame as described previously[10]. An electrode was placed in the ipsilateral medullary pyramidal tract for identification of corticospinal neurons. Extracellular action potentials of neurons in the sensorimotor cortex were recorded through the centre barrel (2 *M* NaCl) of seven-barrel micropipettes. The remaining barrels were filled by centrifugation with various combinations of the following drug solutions: acetylcholine chloride (0.1 *M*, pH 5.0), L-glutamate (0.2 *M*, pH 8.0), substance P (0.0008 *M*, pH 6.5, Beckman), GABA (0.2 *M*, pH 6.0), β-CPG (0.04 *M*, pH 3.5), NaCl (2 *M*). Antidromic stimulation of the pyramidal tract was used to identify corticospinal neurons by previously established criteria[10].

Results. The majority of these studies were conducted on deep, spontaneously firing cortical neurons, including identified corticospinal neurons. In confirmation of previous observations, Ach and substance P excited cells of this type. Of the 47 unidentified neurons tested with both substances, 31 (66%) were excited by substance P and 39 (83%) by Ach. Substance P excited 25 (93%) and Ach 26 (96%) of the 27 identified corticospinal neurons tested. The potency of substance P as an excitant showed some variability from electrode to electrode and its effects were frequently less pronounced than those of Ach applied from another barrel of the same electrode. Excitations evoked by both substances usually had a longer latency than that observed for L-glutamate-elicited excitation, and that initiated by substance P took one or more minutes to subside.

β-CPG administered by cationic currents (1–30 nA) reversibly reduced the firing rate of spontaneously active or drug-excited neurons. In comparison with GABA, the development of β-CPG's effect was slow (in excess of 1 min) and recovery was prolonged, frequently taking 1 to 3 min. If sufficient time was allowed for the development of a maximal effect, β-CPG appeared to be almost equipotent with GABA as a depressant on the basis of equivalent electrophoretic currents. β-CPG was tested on 28 unidentified neurons and 12 identified corticospinal cells. As is illustrated in Figure A, no selectivity could be demonstrated between the depressant effects of β-CPG on spontaneous, Ach and substance P evoked excitation. In every instance a reduction in substance P-evoked firing was paralleled by that in the Ach-induced response. The recovery curves were also comparable. Excitation evoked by pulses of L-glutamate was less affected by β-CPG (see Figure B), larger application currents being necessary to block the glutamate effect.

Discussion. The present observations confirm previous findings of a depressant action of electrophoretically applied β-CPG on cerebral cortical neurons[4,5]. No evidence was obtained that β-CPG is able to selectively antagonize substance P-induced excitation of cerebral cortical neurons, as Ach excitation was always affected to a comparable extent. Excitation evoked by L-glutamate was relatively resistant to depression by β-CPG, an observation that is consistent with findings on isolated perfused amphibian and mammalian spinal cords[6,8].

The precise mechanism of action of β-CPG remains to be elucidated. Since the GABA antagonist, bicuculline, does not reduce the effect of β-CPG[4,5], activation of GABA receptors seems to be an unlikely explanation for its depressant action. β-CPG depresses excitatory postsynaptic potentials in the absence of any changes in membrane excitability[3], an observation which, taken in conjunction with the present findings, suggests that at least part of its action may be to interfere with the effects of various putative excitatory transmitter agents.

[8] K. SAITO, S. KONISHI and M. OTSUKA, Brain Res. *97*, 177 (1975).
[9] J. W. PHILLIS and J. J. LIMACHER, Brain Res. *69*, 158 (1974).
[10] J. W. PHILLIS and J. J. LIMACHER, Expl Neurol. *43*, 414 (1974).

A new Wave (2nd c-Wave) on Corneoretinal Potential

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Summary. The 2nd c-wave is a new wave of corneoretinal potential which is an on-response with a long latency (65–98 sec), and appears following the end of the c-wave of ERG. It is suggested that the 2nd c-wave is based on the tail of the late receptor potential of the retina.

We describe here a new wave of the corneoretinal potential which is an on-response and appears within a certain period, ranging from the end of the c-wave of the electroretinogram (ERG) to the light peak of the corneoretinal standing potential. The wave was tentatively termed by us '2nd c-wave', for the following reason: – the amplitude of the 2nd c-wave increases frequently with increase in that of the c-wave and vice versa[1].

Twenty adult cats were used. Before recordings, the cat was kept in darkness for 30 min. The corneoretinal potential was picked up by 2 Zn-$ZnSO_4$ nonpolarizing electrodes with cotton wicks between the cornea and the incized skin of the upper lid. The potential was then led to a pen-oscillograph through a high impedance DC amplifier. Anesthesia was maintained with nitrous oxide in oxygen (80%/20%) after initial preparation with halothane (2%). Paralysis of the eye muscles and the maintenance of ventilation and temperature have been described elsewhere[2]. The pupil was dilated with 1% atropine sulfate. The cornea was protected by a contact lens from drying out. The light from a xenon arc lamp was directed to the eye by means of Maxwellian view. The intensity of the light was 1000 lx at the corneal surface and varied by use of neutral density filtres. The light duration was provided by an electromagnetic shutter.

Four traces of the corneoretinal potential shown in Figure 1 differ from each other as to the c-wave. The 1st trace (1) illustrates the most common shape. The 3rd and

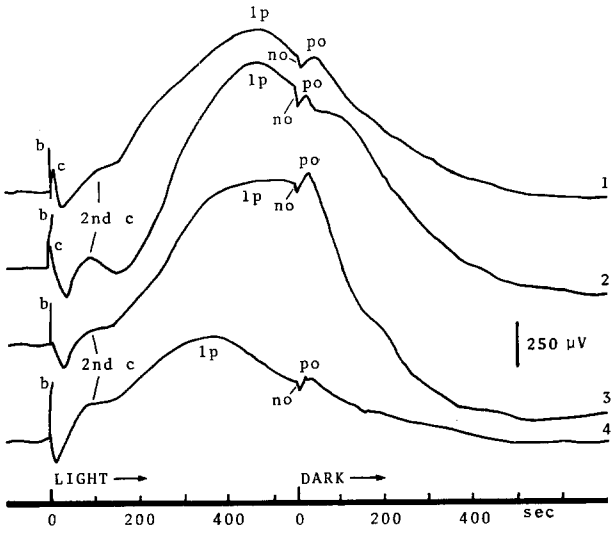

Fig. 1. The corneoretinal potential changes evoked light and dark stimuli in cats with or without the c-wave of ERG. b, b-wave of ERG; c, c-wave of ERG; lp, light peak of the corneoretinal standing potential; no, negative off response; po, positive off response. Onset and offset of the light are shown at zero sec.

4th traces are very rare shapes because of the lack of the c-wave. There were only two cats (10%) without the c-wave in the present study.

The 2nd c-wave develops after the end of the c-wave and superimposed on the rising arm of the light peak, as shown on the 1st trace in Figure 1. Sometimes the 2nd c-wave is clearly isolated from the light peak potential, as shown by the 2nd trace in Figure 1. But in most cases, the 2nd c-wave lies as a small bend on the ascending arm of the light peak (traces 1, 3 and 4). The 3rd and 4th traces show that the 2nd c-wave is not always coexistent with the c-wave. The peak time of the 2nd c-wave which is measured from the onset of the stimulus light, falls within the range from 65 sec to 98 sec and the average value was 73 sec.

Figure 2 shows some response form of the 2nd c-wave under various conditions of the stimulus duration and intensity. The amplitude of the 2nd c-wave increases markedly with increase in the duration of the stimulus light. Under 2 conditions of stimulus duration of 10 sec and 80 sec, the falling phase of the 2nd c-wave is clearly shown and this character is much distinguished, in comparison with the cases for stimulus durations of 160 sec and 640 sec (left column of Figure 2). It is shown in the right column of Figure 2 that the 2nd c-wave increases in

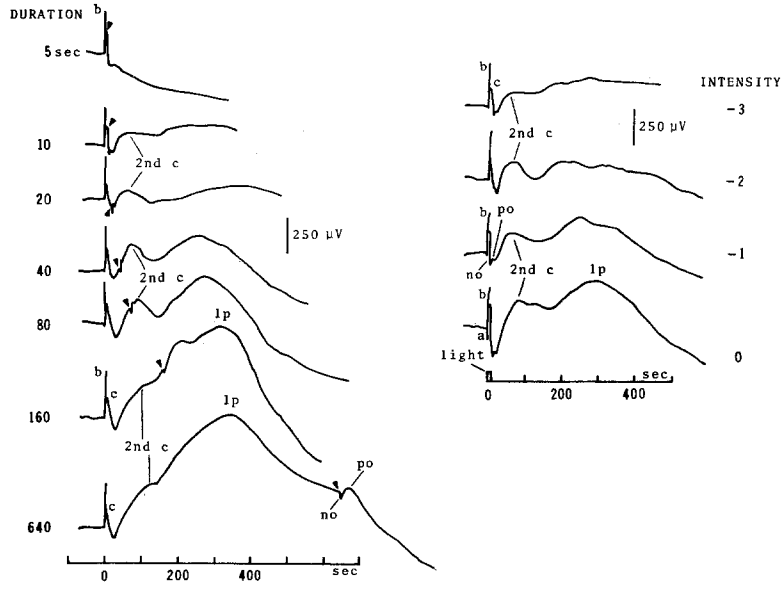

Fig. 2. Configuration of the 2nd c-wave under various light stimuli. Left column shows the response to the various stimulus durations ranging from 5 sec to 640 sec, where the light intensity is fixed at the relative intensity of −3 log unit. Cessation of the light stimulus is marked by a small arrowhead in each trace. Right column shows responses to the various stimulus intensities, where the stimulus duration is fixed at 10 sec. Conventions as for Figure 1.

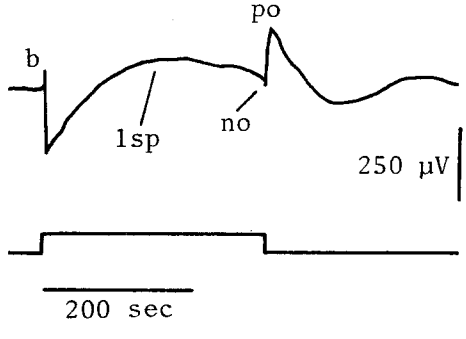

Fig. 3. The 2nd c-wave modified under ether anesthesia. The record was obtained after abolishing the c-wave and the light peak. lsp, large show positive potential following the enhanced fall of the b-wave. Light intensity is fixed at the relative intensity of −3 log unit. Conventions as for Figures 1 and 2.

amplitude with increase in stimulus intensity, and it becomes most marked at the relative log intensity −2. Thus, an adequate stimulus intensity of light has been found for inducing the typical form of the 2nd c-wave.

The obscurity of the falling phase in the 2nd c-wave for stimuli of higher intensity and longer duration is caused by accelerating effect on the growth rate of the light peak. This effect makes it difficult to perceive the 2nd c-wave on the light peak.

The 2nd c-wave is undoubtedly an on-response as illustrated in Figure 1. Hardly any interaction between the 2nd c-wave and the off-responses can be seen as demonstrated in the traces of 40 sec and 80 sec of Figure 2. However, attention must be paid to the evidence that

[1] S. Sato, J. Iwate med. Ass. *26*, 301 (1974), abstract in English.
[2] T. Nikara, P. O. Bishop and J. D. Pettigrew, Expl Brain Res. *6*, 353 (1968).

the 2nd c-wave seemingly behaves just like a positive off-response under some stimulus conditions, such as all traces of the right column in Figure 2 as well as the traces of 10 sec and 20 sec of the left column.

It is well known that appropriate applications of ether are effective for isolation of P_I, P_{II} and P_{III} components in ERG[3]. Therefore, we studied on the movements of the 2nd c-wave under the ether anesthesia sufficient to abolish the c-wave (P_I) and the light peak. Figure 3 shows that the 2nd c-wave was replaced by a large slow positive potential following the enhanced fall of the b-wave (P_{II}), and its peak time was between 140 sec and 180 sec. This value was far longer than that of the 2nd c-wave.

Attention must be paid to the shape of the potential variation produced during a period from the beginning of the light stimulus to the peak of the large slow positive potential. The shape of the b-wave, which shows a reduced rise and an enhanced fall, infers that activity of P_{III} component predominates over that of P_{II} component. If we ignore the small rise of the b-wave, the remaining potential variation seems to be similar in shape to an isolated late receptor potential (P_{III}) of retina, picked up by microelectrode techniques. Thus the 2nd c-wave seems to correspond in configuration to the tail of the isolated late receptor potential.

This working hypothesis may be supported by the report of KNAVE et al.[4,5]. They recorded a slow cornea-negative potential in sheep by conventional electroretinography, and they considered it to be a late receptor potential. Although their recording period was not long enough, their potential closely resembles in movements our remaining potential. Therefore, we have a strong impression that the 2nd c-wave is based on the tail of the late receptor potential of the retina.

We have confirmed the 2nd c-wave also in the human and chicken eye as well as in the cat, but not in the frog.

[3] R. GRANIT, J. Physiol., Lond. *77*, 207 (1933).
[4] B. KNAVE, A. MOLLER and H. E. PERSSON, Vision Res. *12*, 1669 (1972).
[5] B. KNAVE and H. E. PERSSON, Acta physiol. scand. *91*, 187 (1974).

Interactions Between Two Identified Cells in the Visceral Ganglion of the Snail, *Helix pomatia*

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Summary. An interneurone, making excitatory synaptic connections with a second neurone has been identified in the brain of *Helix pomatia*. The results suggest that the connection is monosynaptic.

Interneurones mediating a variety of synaptic actions to several postsynaptic cells have been identified in *Aplysia*[2,3], *Helix*[4], and *Planorbis*[5]. These systems are an advantage in the study of synaptic transmission as well as for detailed pharmacological studies once the transmitter compounds have been identified. For this reason, attempts were made to identify an interneurone in the suboesophageal ganglia of the snail, making monosynaptic connections with other cells. This report describes the interaction between 2 identified cells in the visceral ganglion of *Helix pomatia*[6].

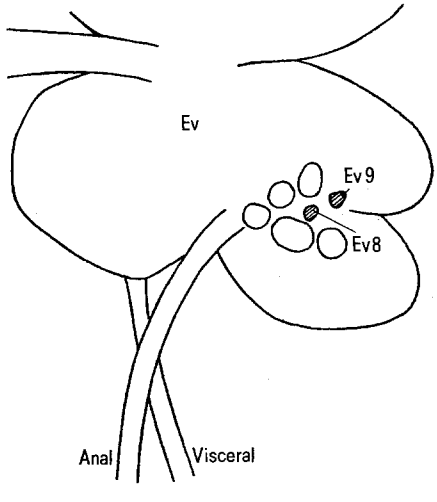

Fig. 1. Diagram of the ventral surface of the visceral ganglion showing the positions of the 2 cells postulated to be in monosynaptic connection. Ev denotes the ventral surface (v) of the visceral ganglion (E).

Materials and methods. Standard electrophysiological methods were used. The snail brain was removed from the animal together with the attached nerve trunks and pinned onto a 'Silastin' block. The preparation was placed in an organ bath containing 10 ml of Ringer (NaCl: 80 m*M*; KCl: 4 m*M*; $CaCl_2$: 8 m*M*; MgC_2: 5 m*M*; *Tris* buffer: 5 m*M*; pH: 7.4). The neurones to be impaled were exposed by dissecting away the outer connective tissue. Simultaneous recordings were made from cell pairs using glass microelectrodes containing 1 *M* KAc. A Tetronix 502A oscilloscope was used and permanent recordings were obtained on a Watanabe pen recorder. Each cell could be depolarized by the bridge or stimulated intracellularly with a stimulator independently of the second cell.

Results and discussion. Chemically mediated synaptic pathways were demonstrated in approximately 4% of tested different cell pairs in the central ganglia. Over 300 cell pairs were tested. One consistent interaction was demonstrated which showed the characteristics of a monosynaptic-like connection. A strong 'one for one' connection was demonstrated between 2 small cells on the ventral surface of the visceral ganglion. The location of the cells within the ganglion is indicated in Figure 1. Ev denotes the ventral surface (v) of the visceral ganglion (E)[7]. The presynaptic cell was designated Ev9. The properties of the postsynaptic cell, Ev8, have been studied previously[6].

[1] We thank the M.R.C. for a Training Grant to S.E.J.
[2] E. R. KANDEL, W. T. FRAZIER, R. WAZIRI and R. E. COGGESHALL, J. Neurophysiol. *30*, 1352 (1967).
[3] D. GARDNER and E. R. KANDEL, Science *176*, 675 (1972).
[4] G. A. COTTRELL, Nature, Lond. *225*, 1060 (1970).
[5] M. S. BERRY and G. A. COTTRELL, J. Physiol. Lond. *244*, 589 (1975).
[6] S. E. JUDGE, Ph. D. Thesis, University of Southampton (1976).