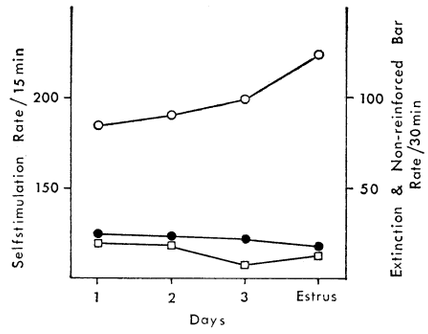

Fig. 1. Mean rates of bar-pressing recorded for all subjects throughout the experiment. Symbols: open circles, self-stimulation (n, 6); solid circles, extinction (n, 6); squares, responses on the no-reinforcement bar (n, 4).

animals were more active at estrus, the changes in self-stimulation behavior cannot be explained as an artifact of increased random activity in the Skinner box, because the extinction-period data, and the rates of response on the no-reinforcement bar, showed no tendency to rise at estrus; furthermore, during observation of behavior in the box, accidental "responses" were rare. My results therefore indicate that the onset of estrus correlates with a change in the reinforcing effects of brain-stimulation.

These results differ from those in an earlier report (*3*) that the hormonal induction of lordosis had no effect on self-stimulation by way of septal-area electrodes. Differences in loci of electrodes might account for this, although in male rats, with electrodes in the septum, self-stimulation rates are affected by changes in androgen levels (*2*).

A further difference between these experiments is that the hormone therapy used to induce lordosis in the ovariectomized rats is unlikely to have restored the levels of activity characteristic of normal estrous rats. Activity cycles and changes in sexual behavior seen during the estrous cycle are probably mediated by separate, though closely integrated, neuroendocrine mechanisms. Thus, in the ovariectomized rat progesterone is important for the restoration of lordosis but not for high levels of wheel-running; and, whereas single injections of estrogen and progesterone restore sexual responses, chronic application of estrogen is necessary to restore high levels of wheel-running (*4*).

Since in ovariectomized rats changes in sexual receptivity, indicated by the appearance of lordosis, can occur in the absence of changes in self-stimulation, it is possible that the effect I report is related more to factors underlying the activity changes accompanying the estrous cycle than to the changes in sexual receptivity. Similar interpretation is possible of reports that deprivation of food and water affect rates of self-stimulation; here again changes in motivation are accompanied by changes in activity (*8*), and it is not clear whether the self-stimulation effects stem directly from the specific drive states or from the activity changes associated with them. If the second alternative is correct, one may expect self-stimulation to be affected by spontaneous changes in gross bodily activity that are not obviously related to states of biological need. Such changes occur within the diurnal cycle of activity and rest in rats, and variations in self-stimulation behavior have been correlated with them (*9*).

A similar effect of cyclic changes in activity has been noted upon the threshold for electroconvulsive shock in rats (*10*); sensitivity to such shock is greatest at estrus and the effect is estrogen-dependent, progesterone having a slightly anticonvulsant effect. Diurnal fluctuation in electroconvulsive-shock threshold, which is qualitatively larger than the estrous-cycle variation, also occurs.

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References and Notes

1. J. V. Brady, J. Boren, D. Conrad, M. Sidman, *J. Comp. Physiol. Psychol.* **50**, 134 (1957); D. L. Margules and J. Olds, *Science* **135**, 374 (1962).
2. J. Olds, *J. Comp. Physiol. Psychol.* **51**, 320 (1958).
3. W. Hodos and E. S. Valenstein, *ibid.* **53**, 502 (1960).
4. W. C. Young and W. R. Fish, *Endocrinology* **36**, 181 (1945).
5. One of the rats had a 5-day estrous cycle. To include the data on this rat in Fig. 1, the mean of the two days following estrus was taken as the score for the first day after.
6. A two-tailed Wilcoxon matched-pairs signed-ranks test was used for all statistical comparisons. See S. Siegel, *Non-Parametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
7. G. H. Wang, *Comp. Psychol. Monogr.* **2**, 1 (1963); J. T. Eayrs, *Brit. J. Anim. Behav.* **2**, 25 (1954).
8. G. Wald and B. Jackson, *Proc. Nat. Acad. Sci. U.S.* **30**, 255 (1944); B. A. Campbell and F. D. Sheffield, *J. Comp. Physiol. Psychol.* **46**, 320 (1953).
9. R. G. W. Prescott, in preparation.
10. D. E. Wooley and P. S. Timiras, *Amer. J. Physiol.* **202**, 379 (1962); *Endocrinology* **70**, 196 (1962).
11. Supported by a studentship from the Science Research Council, London. I thank W. H. Thorpe for advice and encouragement.

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Unit Responses from Commissural Fibers of Optic Lobes of Fish

Abstract. *The paired optic lobes of teleost fish are connected by two commissures. One of these, the tectal commissure, was studied with metal microelectrodes. Fibers are rhythmically active for prolonged periods in the dark and respond to light by a decrease in the rate of discharge. There is a rebound acceleration when the light is turned off. Each fiber is influenced by light in one eye only, and there is no response when light is projected into the opposite eye. This behavior resembles the "off" response recorded from the optic lobes and the optic nerve of fish. Unlike most units from the visual pathways of lower animals, single commissural fibers do not seem to give any recognizable response to patterned input such as small light or dark objects or small light sources stationary or moving anywhere in the visual field, nor do they respond to a vertical black bar moved over a white background.*

In fish, the left and right optic nerves cross completely, and each optic lobe of the brain receives direct information from the visual field of only one eye. Nevertheless, the perceptual processes of the two eyes are not independent, for behavior patterns based on visual cues learned through one eye are frequently found when the opposite eye is used alone (*1*). The physiological mechanism which maintains the perceptual unity behind such interocular transfer of learning is quite unknown. Behavioral experiments, paralleling those done on mammals, indicate that the commissural fibers joining the paired optic lobes are somehow involved (*2*).

The participation of cortical and midbrain commissures in the highest levels of mental activity in man and animals is of special interest because it raises the possibility that analysis of the messages carried by the commissures might give a clue to the way in which perceptual and mnemonic functions are handled in the brain. We have therefore studied the activity of fibers of one of the two commissural systems that connect the paired optic lobes of fish. Both commissures appear to be involved in interocular transfer of learned behavior (*2*). We now describe electrophysiological experiments on the tectal commissure; we have not yet been able to make satisfactory re-

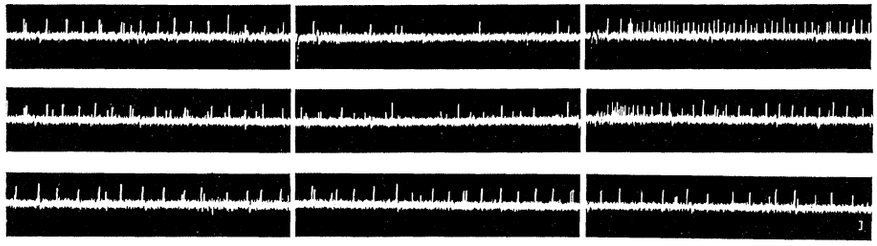

Fig. 1. Activity recorded from a unit of the intertectal commissure of *Astronotus ocellatus*. Each trace is 2 seconds long, calibration is 20 μv; all spikes have been retouched. The top line shows the modification of background discharge when a spotlight directly above the fish was turned on and off. The other two sequences show the effect of shining a small spot of light into the right eye (middle line) or the left eye (lower line). Note the inhibition of discharge by light to both eyes or to the right eye alone and the lack of effect when the light is presented to the left eye alone.

cordings from the posterior commissure.

Goldfish were used for preliminary experiments, but the best recordings were obtained from nine "Oscars" or "velvet fish" (*Astronotus ocellatus*). The fish, 5 to 10 cm long, were immobilized with gallamine triethiodide (Flaxedil) and held in a Plexiglas tank between polyurethane sponges so that their eyes were under water. A water-filled Plexiglas bubble with one flat and one convex spherical surface, just less than a hemisphere of radius 25.4 cm, formed one side of the fish tank, the convex side facing outward. The fish was positioned so that the eye next to the flat side of the bubble was at the center of the spherical convex surface. Small light sources or small dark objects could be moved over the outside of the hemisphere to cover most of the visual field of one eye (*3*). In addition, a white cardboard screen could be hung just behind the hemisphere and a black stripe 1 cm wide could be moved by hand over the white surface. Experiments were generally carried out in a darkened room, although the light level could be increased either by turning on the room lights, which gave background illumination of 7.5 millilamberts, or by turning on a small spotlight, containing a 15-watt bulb powered by a 6-volt battery, placed directly above the head of the fish. This gave a background illumination of 4.3 mlam. Background illuminations were determined by measuring with an exposure photometer (Salford Electrical Instruments Ltd., Ilford, Essex, England) the amount of light reflected from a metal sheet painted white placed at the position which the fish's head occupied during the experiments. Either eye could also be illuminated with a pencil of light from an ophthalmoscope set so the brightness of the light source (diameter 1 mm) was 540 mlam, as measured with the same instrument. Action potentials were recorded from the intertectal commissural fibers, through a hole in the skull, by means of a glass-insulated tungsten microelectrode with an exposed tip 3 to 5 microns across, plated with a layer of gold and a layer of platinum black (*4*). A cathode follower, a Tektronix 122 amplifier, an audio amplifier, and a Tektronix 502 oscilloscope with a Grass camera completed the recording equipment.

As an electrode was driven down onto the commissure, the noise level increased by about 20 to 40 μv from the resting level of 10 to 20 μv recorded when the microelectrode first made contact with the fluid covering the brain. The noise level would go down abruptly when the room lights were turned on but did not change appreciably when light was projected into one eye. It was usually obvious that we were recording the asynchronous activity of many commissural nerve fibers. Regular discharges, apparently coming from single commissural units, could be heard over the loudspeaker; we could frequently pick out a corresponding action potential on the oscilloscope trace. The continuous activity made identification of units quite difficult, but single spikes 40 to 60 μv in amplitude would sometimes emerge from the increased background noise; then they could be held without change in the shape of the action potential for periods up to 1 hour. In 48 such cases, where single units were clearly identified on the oscilloscope by a characteristic size and configuration of the action potential, their behavior was remarkably similar. They were spontaneously active in the dark, discharging at a regular rate which varied widely from unit to unit, the maximum recorded being about 30 spikes per second. If the fish was left alone in the dark with no additional visual stimulus the frequency of discharge of a unit would fluctuate and slowly decrease. One fiber which was held without visual stimulation for 1 hour became quite quiet but began steady rhythmic activity again after the room lights had been on for a few seconds. We have not yet seen any response, from the commissural fibers we sampled, to a small discrete object, light or dark, or to a small light source, moving in the visual field represented by the surface of the water-filled hemisphere. Neither have we seen any response to movement of the vertical black bar over the white background.

These results contrast sharply with those we were able to record, during the same experiments, from the optic nerve fibers, which are sensitive to small spots of light in a limited visual field or to movement of a small dark or light object in and out of the receptive field (*5*). However, commissural fibers were influenced by a sudden change in the general level of illumination. The effect was an inhibition of discharge rate when the light level was increased by turning on the room light or the spotlight above the fish; there was a rebound acceleration when the light was turned off. Inhibition followed by rebound could be reproduced by shining the ophthalmoscope light into only one eye, either the right or left, depending on the fiber sampled, and suddenly switching the beam away. When precautions were taken to exclude stray light there was no discernible change in discharge pattern when the light was directed into the opposite eye (Fig. 1). We have not determined either the minimal change in background light or the minimal duration of stimulus required to give this response.

This kind of behavior is, however, quite similar to that described by Cronly-Dillon and by Jacobsen and Gaze (*5*) for the slowly adapting units giving prolonged "off" responses, which are found in the inner plexiform layer of the goldfish tectum. Every commissural fiber we sampled was of this type. We found no fibers which were not affected by light and no fibers which corresponded to the slowly adapting "on" units that are also common in the inner plexiform layer of the goldfish tectum. However, units giving prolonged "off" responses are not peculiar to the tectum but are also found in the

optic nerve of the goldfish. Indeed, Jacobsen and Gaze have expressed surprise that in neither the frog nor in the optic lobe of the fish are there any units with different types of function from those in the optic nerve. While the commissural fibers from which we record do behave in a manner which is apparently very similar to this one class of optic nerve unit, we can at least be sure in our experiments that we dealt with the activity of tectal cells and not with the terminals of the primary optic nerve fibers. It seems quite probable that many "off" units of the tectum come from this class of tectal cells too. Are these cells all given over then to merely spreading across the brain one kind of apparently crude visual coding which has already been carried out by the retina? Or is the similarity between the "off" units of the optic nerve and commissural or tectal "off" units coincidental and are the commissural fibers carrying, perhaps in subtle variations of frequency or in the spatial distribution of discharge between different cells, some form of higher coding involved in visual perception?

Behavioral studies that demonstrate the importance of the tectal commissure for interocular transfer indicate that intertectal exchange over this pathway is somehow involved in perceptual mechanisms related to fine pattern vision. Therefore, in spite of the apparent insensitivity of isolated commissural fibers to localized or patterned visual input under the conditions of our experiments, one cannot discount the possibility that the cells which give rise to the tectal commissure may be involved in something much more subtle than might be supposed from the simple responses to light levels described here.

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References and Notes

1. R. W. Sperry and E. Clark, *Physiol. Zool.* **22**, 372 (1949); A. Schulte, *Z. Vergleich. Physiol.* **39**, 432 (1957); R. A. McCleary, *J. Comp. Physiol. Psychol.* **53**, 311 (1960); J. J. Bernstein, *Exp. Neurol.* **6**, 173 (1962); D. J. Ingle, *Science* **149**, 1000 (1965).
2. D. J. Ingle, *Perspectives Biol. Med.* **8**, 241 (1965); R. F. Mark, in preparation.
3. H. O. Schwassmann and L. Kruger, *J. Comp. Neurol.* **124**, 113 (1965).
4. H. A. Baldwin, S. Frenk, J. Y. Lettvin, *Science* **148**, 1462 (1965).
5. J. R. Cronly-Dillon, *Nature* **203**, 214 (1964); M. Jacobsen and R. Gaze, *Quart. J. Exp. Physiol.* **49**, 109 (1964).
6. We thank R. W. Sperry for facilities used in this research and for help with the manuscript; one of us (T.M.D.) is an NSF undergraduate research participant.

7 February 1966

Active Transport of 5,5-Dimethyl-2,4-Oxazolidinedione

T. C. Butler (*1*), commenting on our recent report (*2*), has suggested an explanation other than active transport for the movement of 5,5-dimethyl-2,4-oxazolidinedione (DMO) across the intestinal wall. As he points out, Hogben *et al.* (*3*) have postulated that a narrow zone of fluid adjacent to the mucosal cell surface has a higher hydrogen ion concentration than the remainder of the bulk mucosal perfusing solution. Such a microlayer of fluid with a low *p*H would lead to a relatively high concentration of un-ionized DMO next to the mucosa and, subsequently, to augmented mucosa-to-serosa movement of DMO by means of nonionic diffusion. By this mechanism, values for the ratio of $[DMO]_{serosa}$ to $[DMO]_{mucosa}$ greater than 1 could be obtained without postulating active DMO transport.

While this is a reasonable possibility, we have evidence which strongly suggests that this is not the explanation for the net movement of DMO we reported (*2*). For example, it can be shown that the net movement of another weak acid, cholic acid, which has a pK_a value (6.4) nearly identical to that of DMO, is from the serosal to the mucosal solution in everted gut sacs prepared from the jejunum. The *p*H of the bulk serosal fluid becomes acid relative to the bulk mucosal solution during the incubation of these sacs (*2*, *4*). Hence, cholic acid, which can cross the intestinal wall via passive nonionic diffusion (*4*), moves from the serosal media to the mucosal media in response to the hydrogen ion gradient which is measurable between the two bulk solutions.

Thus, it seems highly improbable that a hypothetical *p*H gradient adjacent to the mucosal cell surface could account for net mucosal-to-serosal movement to DMO while, at the same time, a second weak acid with a nearly identical pK_a value moves in the opposite direction, from the serosal to the mucosal solution, as would be expected from the relative *p*H values of the bulk perfusing solutions. In point of fact, we consider such data to support even more strongly the concept that DMO is actively transported by the small intestine of the rat, since this substance is moving not only against an electrochemical gradient but, as these data would indicate, against a *p*H gradient.

Furthermore, the distribution of DMO transport activity down the length of the small bowel (*2*, Fig. 1) is remarkably similar to that of α-aminoisobutyric acid, which clearly is actively transported and the movement of which, theroretically, is independent of any *p*H gradient effect. In addition, the active transport of DMO appears to be stimulated by the presence of glucose in the perfusing media—a finding which has been described in association with the active transport of numerous other substances.

Although we recognize the difficulties of extrapolating from the small intestine to the muscle cell, it seems reasonable to question the validity of the DMO muscle *p*H method on the basis that this substance may be transported into or out of the cell. The apparent "close agreement" of the HCO_3^- and DMO methods for intracellular *p*H determination commented on by Butler cannot be used as an independent validation of the DMO method for at least two reasons: (i) There is considerable scatter in the intracellular *p*H values given by either method (varying from 6.85 to 7.10); and (ii) the validity of the HCO_3^- method has been seriously challenged, and this challenge has not been adequately answered (*5*).

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References

1. T. C. Butler, *Science* **151**, 1564 (1966).
2. J. M. Dietschy and N. W. Carter, *ibid.* **150**, 1294 (1965).
3. C. A. M. Hogben, D. J. Tocco, B. B. Brodie, L. S. Schanker, *J. Pharmacol. Exp. Therap.* **125**, 275 (1959).
4. J. M. Dietschy, H. S. Soloman, M. D. Siperstein, *J. Clin. Invest.*, in press.
5. E. J. Conway and P. J. Fearon, *J. Physiol.* **103**, 274 (1944).

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A Piston Extractor for the Hughes Press

Simplified forms of the Hughes press (*1*) have been described which permit rapid and efficient breakage of microbial cells (*2*). Such a press consists essentially of a cylinder block in which the cells are placed and then frozen with dry ice. A piston is inserted into the cylinder, which is then mounted on a second block containing a suitable receiving vessel, and the frozen cells are forced through a small hole in the bottom of the cylinder by use of a hydraulic ram. A disadvantage of this technique is that the cylinder