insulating micropipette. A micropipette was pulled from borosilicate 'theta' capillary (Hilgenberg Glas, D–3509 Malsfeld, Federal Republic of Germany) with a horizontal puller (Narishige) using low heat and a strong pull in order to obtain a tip of about 1.5 µm and a short taper. A few small pieces of elephant wax (m.p. 100 °C) were dropped down one barrel so as to lodge at the beginning of the taper (figure 1A). The micropipette was placed on the stage of a microscope and a spiral heating element (Pt 50% + Ir 50%) was positioned over the tip as shown in figure 1C. With a minimum of heat, the wax was melted so that it flowed to the tip of the barrel but did not enter the other barrel. Using, as a guide, a small funnel made from a Pasteur pipette, the Pt-wire was advanced down the barrel with a micromanipulator. The wax was then resoftened and the Pt-wire pushed to the extreme tip of the micropipette. Next, the electrode tip was bevelled on a plate of 0.3 µm rugosity (Stähli, CH–2542 Pieterlen, Switzerland) with the Pt-containing barrel lowermost to make a bevel 2–3 µm wide (figure 1D). The back of the other barrel was broken for 2–3 mm backwards, filled with Ringer's solution and a Ag–AgCl-wire introduced. The wires were sealed in with wax, and the electrode was then ready for use. Experiments showed that it is not necessary to cover the tip with a membrane, although technically this is feasible. The electrical set-up used for the microamperometric measurement of PO_2 and the simultaneous recording of the bioelectrical activity reference barrel has been described[3].

Results and discussion. When immersed in an air-equilibrated Ringer's solution and polarized by −600 mV, these PO_2-microelectrodes passed currents of 20–160 pA (20 microelectrodes). The current was fairly stable over several days (± 10 pA maximum variation) even if the microelectrode was kept immersed in Ringer or used repeatedly in experiments. We attribute this stability to: a) the hydrophobic properties of the wax insulation in the tip; b) the well-defined geometry of the exposed surface of the Pt, produced by the bevelling; c) the absence of a membrane whose alteration during experiments or over days of storage in water could cause a change in O_2-current.

Figure 2 shows examples of the application of electrodes in 2 different excitable tissues, the retina of the honeybee drone and the cortex of the rat brain. The bioelectrical activity was recorded simultaneously by the reference barrel. In conclusion, a double barrelled PO_2-microelectrode has been developed that permits accurate and reproducible measurements of PO_2-variations, and simultaneous recording of bioelectrical activity in excitable tissues.

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Visual responses in the central nervous system of the scallop *Pecten ziczac*[1]

L. A. Wilkens and B. W. Ache

Department of Biology, University of Missouri–St. Louis, 8001 Natural Bridge Road, St. Louis (Missouri 63121, USA), and Department of Biological Sciences, Florida Atlantic University, Boca Raton (Florida 33431, USA), 23 March 1977

Summary. Electrical activity recorded from the lateral lobe of the visceroparietal ganglion in the scallop Pecten ziczac reflects predominantly the visual response to onset of light stimulation, suggesting that shadow reflex mechanisms likely occur elsewhere within the central nervous system.

Action potentials recorded from the optic (pallial) nerves of the scallop and related lamellibranch molluscs appear in response to both the onset and termination of a light stimulus[2–4]. These functionally distinct responses arise from separate retinal layers of the eye[2–6]. Depolarizing receptors in the proximal retina produce the 'on' response whereas hyperpolarizing receptors in the distal retina are inhibited by light and produce the 'off' response[7]. A mechanism in which primary visual receptors independently trigger activity signaling a decrease in light intensity, i.e., an 'off' response not dependent on peripheral synaptic interactions, appears to be unique to bivalve molluscs[3,8].

Shadow response reflex behavior has evolved to varying degrees in nearly all bivalves. In the scallop, this response is believed to be the direct result of 'off' fibre discharges[3,9] since images are formed only on the distal retina and since 'off' fibre activity corresponds with the shadow reflex. In the present experiments, visual responses have been recorded in the visceroparietal ganglion as well as in the optic nerves.

Methods and materials. Sand scallops, Pecten ziczac, were collected from Harrington Sound, Bermuda, and kept in circulating natural seawater. A semi-intact scallop preparation was used in which only the lower (right) valve was removed. In P. ziczac, most of the pallial eyes are contained in the upper mantle. The visceroparietal ganglion and pallial nerves were exposed on the ventral surface of the adductor muscle. Ganglionic and pallial nerve activity was recorded extracellularly, using either suction electrodes or wire hooks insulated with oil[11], amplified, and filmed directly from an oscilloscope. The light stimulus (maximum intensity 19.5 W/m² at 3 cm) was delivered through a fibre optic light pipe and monitored by a photocell. Animals were initially dark-adapted for 10 min and then subjected to sequences of on–off light stimuli. Following 30 sec of light adaptation, sequences of off–on stimuli were then presented. All experiments were conducted at 20 °C.

Results and discussion. The following data are representative of the results obtained from experiments on 33 animals. Simultaneous recordings from the pallial nerve and the lateral lobe of the ganglion are illustrated in figure 1. Following dark adaptation (A), both 'on' and 'off' discharges appear in the nerve. Activity in the ganglion, however, is associated exclusively with 'on' responses in the nerve. After light adaptation (B), the 'on' response in the optic nerve is diminished along with an increase in latency whereas the 'off' response is potentiated: response frequency and duration increase, accompanied by a marked decrease in latency from 170–215 msec to 65–85 msec. Both 'on' and 'off' responses appear in the ganglion after light adaptation. The initially small ganglionic 'off' response decrements rapidly however (cf. activity marked by asterisks). 'Off' activity was not observed centrally in most preparations and, when present, was always small compared to 'on' activity and occurred only after light adaptation at high intensities. Photic responses from another animal (figure 2A) are illustrated in traces 1–3 and 5–7 in response to stimuli of relative intensities 2, 31 and 100%, following dark and light adaptation, respectively. Both 'on' and 'off' discharges are elevated in the nerve (with decreased latency) at higher intensities, as was also the case with P. maximus[3]. In the ganglion, 'off' discharges appeared again only after high intensity light adaptation. In traces 4 and 8, 'on' and 'off' responses were elicited at a stimulus intensity equal to that of 3 and 7, but in the presence of a conditioning background light. As a result, the nerve responses and the ganglionic 'on' response were reduced

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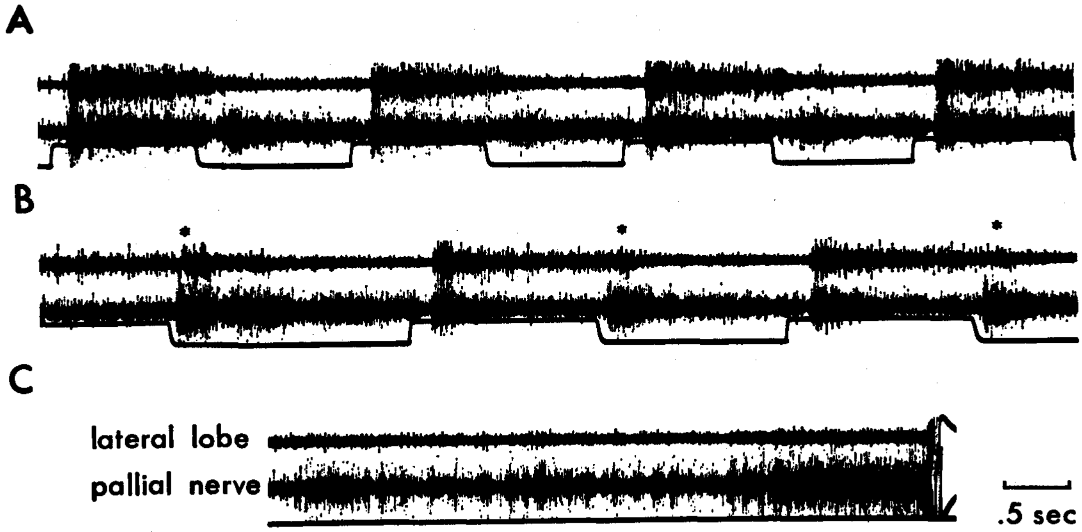

Fig. 1. *A*, *B* Extracellular records of light-evoked neural activity in Pecten ziczac following 1 min of dark and light adaptation, respectively. *C* Similar records, but of tactile-evoked activity (light adapted). Shown in each record, beginning with the top trace, is activity from the surface of the visceroparietal ganglion (lateral lobe) and the pallial nerve. The bottom trace is the stimulus monitor, an upward deflection indicating onset ofi llumination.

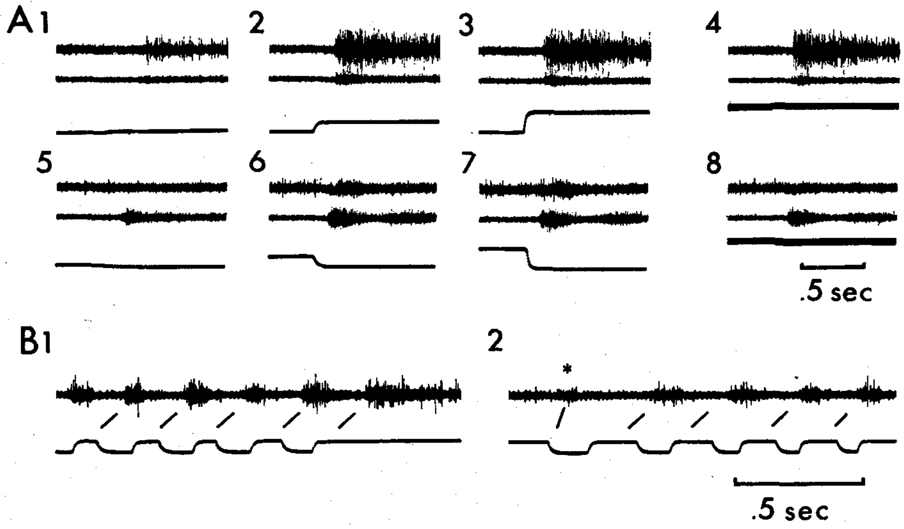

Fig. 2. *A* Neural activity in response to light flashes of varying intensity following 1 m periods of dark adaptation (1–4) or 30-sec-periods of light adaptation (5–8). Identity of traces in *A* are the same as in figure 1. *B* Ganglionic responses to repetitive light flashes at constant intensity following dark (1) and light (2) adaptation. The slanted lines indicative activity associated with stimulus onset and, in one instance (*), the stimulus termination.

slightly (cf. 3–4 and 7–8), whereas ganglionic 'off' activity disappeared completely. Thus, a small decrease in light, regardless of the initial absolute intensity, is apparently not a sufficient stimulus for the ganglionic 'off' response although behavioral shadow reflexes occur in response to step decreases of as low as 0.5%[10].

'On' response activity appears to be predominant in the lateral lobe of the ganglion, a functional optic lobe[12], and responds without decrement at frequencies of 5–6 Hz (figure 2B). Mechanically-evoked activity from the mantle also appears not to enter the lateral lobe (figure 1C). If shadow response behavior relies on activity in 'off' fibre tracts[3], our results suggest that these fibres synapse at sites within the ganglion other than in the lateral lobes, perhaps directly in the dorso-central (motor) lobes. The function of the lateral lobe, suggested in part by its intricate glomerular structure[12], is apparently concerned with more complex behaviors such as orientation to boundaries in the visual field[10].

An alternative interpretation of the present results would suggest that 'on' activity, incorporating integration of both spatial and temporal features of the stimulus, may trigger the shadow reflex. Some evidence for this hypothesis exists since, in our experience and from other experiments[10], shadows produced by uniform darkening of the visual field are typically ineffectual. Rather, an effective stimulus for valve closure always involves movement, a situation in which the more predominant, rapidly responding 'on' receptors would be re-illuminated following transient shadow periods. Further experimentation is needed to resolve the central mechanism of the shadow response.

The effect of a low protein diet on the adipocytes of rat perigenital fat

A. Wise

Department of Nutrition, University of Isfahan, Isfahan (Iran), 28 January 1977

Summary. Weanling rats were fed a low protein diet for 6 weeks and their weights were 50% less than controls. There were significantly fewer adipocytes per µg adipose tissue, but estimates of the number of adipocytes per rat indicated that the diet had much less effect on adipocyte number than on b.wt.

The physiological control of adipocyte production is not well understood, but evidence points to the involvement of the hypothalamus[1], possibly related to growth hormone[2]. By sampling at various ages it has been demonstrated that adipocyte production continues for some time after weaning[1,3–5], and during this time rats may be susceptible to the influence of nutrition on the endogenous control of adipocyte production. For example, a high fat diet causes increased adipocyte number[6], and starvation has been shown to inhibit[5] or to have no effect[4] on adipocyte production, and one report showed no change in adipocyte number after underfeeding[7]. Adipocyte number is also affected by preweaning nutrition and adult rats which were suckled in small litters had more adipocytes than those suckled in large litters[3], and this phenomenon was also demonstrated in genetically obese rats[8]. When rats are fed a low protein diet, their growth is stunted, but they have the capacity to grow to nearly normal weight when given a good diet later in life[9,10]. The purpose of the present work was to investigate the response of rat adipose tissue to a low protein diet, with particular reference to the number of adipocytes in the tissue. Rats were also suckled in different size litters to confirm whether the adipocyte counting method was sensitive enough to detect a well established difference in adipocyte number.

Materials and methods. Sprague-Dawley rats were suckled in litters of either 4 or 16, during which time the dams were fed a nutritionally adequate diet[11]. For 6 weeks after weaning, the small-litter rats were fed a high protein diet and half of the large-litter rats were fed the high protein diet (controls) and the rest were fed a low protein diet. Both diets contained (g/kg) 40 corn oil, 40 mineral mix and 20 of vitamin mix, with either 100 or 250 casein and

Weights, fat contents, adipocyte concentration and estimates of number of adipocytes in 9-week-old rats

		Small litter* HP Diet	Large litter** HP Diet	LP Diet
Number of rats	(male)	4	5	5
	(female)	4	6	6
Weight (g)	(male)	313 ± 5	212 ± 4	98 ± 4
	(female)	201 ± 9	169 ± 6	95 ± 10
Fat (g)	(male)	29 ± 3	12 ± 1	11 ± 0.5
	(female)	16 ± 1	10 ± 1	12 ± 1.5
Adipocytes μg^{-1}	(male)	6.13 ± 0.88	9.19 ± 0.97	6.55 ± 0.61
	(female)	7.39 ± 0.42	8.94 ± 0.82	7.41 ± 1.24
	(both sexes)	6.76 ± 0.49	9.05 ± 0.63	7.02 ± 0.71
Adipocytes $\times 10^6$ per rat	(male)	200 ± 27	142 ± 17	102 ± 10
	(female)	147 ± 30	107 ± 9	102 ± 8

Mean values ± SE. * Litter of 4 pups; ** litter of 16 pups; fed at high protein (HP) or low protein (LP) diets after weaning.