Does the central human retina stretch during accommodation ?

CLEAR evidence that the human retina stretches during accommodation was presented by Moses[1], but his measurements were all made at the anterior margin of the retina, so they did not show how this stretch is spatially distributed. If it is largely confined to the region of the *ora serrata*, its contribution to the perceptual correlates of accommodation will be much less than if the central retina is distorted as well. A perceptual effect involving accommodation-dependent distortions in the visual field has recently been attributed by its discoverers[2] to such a stretching of the central retina, although non-retinal explanations for this effect are also tenable.

It seemed worthwhile to do an experiment in which the possibility that the central human retina stretches during accommodation could be evaluated, and in which the magnitude of any stretch which was found could be assessed.

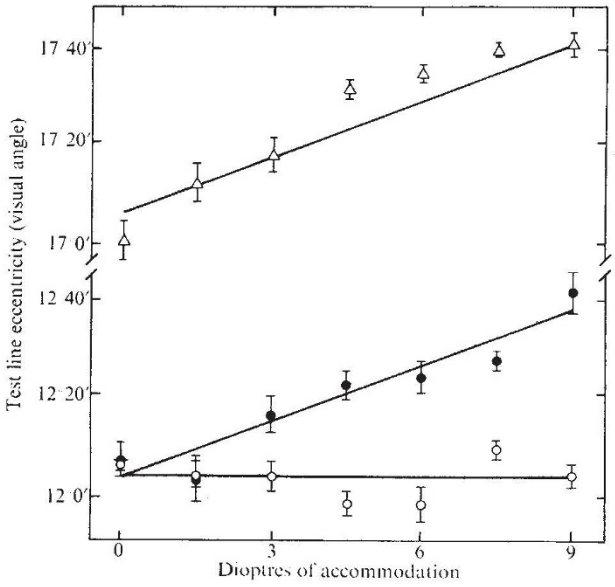

Fig. 1 Test line settings made by the first observer are shown as a function of dioptres of accommodation. Closed filled circles refer to the near edge of the blind spot, the line drawn through them by eye indicating a 4.7% increase in visual angle over the range of accommodation. A line parallel to this has been drawn through the triangles which refer to the far edge of the blind spot. The open circles are control data obtained during cycloplegia: a horizontal line has been drawn through them to indicate that there is no stretching of the retina in these conditions. Error bars show 1 s.e.m. above and below each point, which is the mean of ten determinations. A long test line (2° from top to bottom) and a high retinal illuminance for the background (5.6 log trolands) were used to make the line as conspicuous as possible once it had emerged from the blind spot.

The observer's left eye was patched throughout, and the right provided with a Maxwellian view of a fixation cross and a vertical test line, both seen in silhouette against a background of monochromatic yellow (575 nm) light. The test line was located to the right of the fixation cross; its exact horizontal position was under the observer's control. Because the eye was positioned one focal length behind the Maxwellian lens, the apparatus served as a Badal optometer: that is, movements of the target plane (containing both the fixation cross and the test line) along the optic axis stimulated different degrees of accommodation but did not affect the size of the retinal image.

The observer's task was to fixate the centre of the cross and to keep it in sharp focus, while at the same time moving the test line until it was just visible on the near side of the blind spot (the side closer to the fixation cross). Measurements were made at seven different levels of accommodation (Fig. 1) to plot the distance in visual angle between the fixation cross and the test line. Since the test line marks the near edge of the blind spot, the data imply that the fovea and the optic disk are more widely separated when accommodation is strained than when it is relaxed.

An alternative interpretation of the data would be that threshold criteria changed systematically as a result of accommodative effort, or that the target was focused on the retina more sharply at one level of accommodation than at another. These possibilities were tested, as a class, by measurements analogous to the first set but now localising the far side of the blind spot: if blurring or criterion shifts were involved, the line should have been set closer to the fixation cross as accommodation increased. Just the opposite was found (Fig. 1), thus eliminating these possibilities. The results of these two experiments were confirmed on a second observer.

There remained the chance that some misalignment of the apparatus, rather than a stretching of the retina, produced the effect. To rule out this possibility a control was run with accommodation paralysed with 1% Cyclogyl, but with the target plane moved along the optic axis as before. A smaller aperture stop was now used to reduce the diameter of the pencil of light passing through the pupil centre to less than 0.5 mm, thereby ensuring that the stimuli would always be seen in sharp focus despite the lack of accommodation. The effect found earlier was now clearly absent (Fig. 1).

A final experiment was undertaken to determine how great a contribution to the effect is made by changes in the magnifying or other properties of the crystalline lens. It is known[3] that convergence can bring about accommodation; and it seems likely that in a presbyope, devoid of accommodative ability, convergence would still be accompanied by a central command for accommodation. The lens would not respond to such a command, but if the ciliary muscle contracted, then the retina might be stretched just as in a younger subject.

This possibility was investigated using a 66-yr-old presbyopic observer. A different apparatus (with slightly different stimulus dimensions) was employed so that the observer could be presented with the fixation cross binocularly, while the test line was as usual seen by the right eye only. Natural viewing at a distance of 3 m was used, and the observer moved the test line with a pulley so that it fell on the near margin of the blind spot. While the right eye remained in the primary position throughout, mirrors were introduced in front of the left eye to make it converge a total of 25°. Measurements made both with and without the mirrors showed that the visual angle between the fixation cross and the blind spot increased some 4.6% when convergence was brought into play; this is comparable to the figures obtained during actual accommodation in the initial experiments: 4.7% for one subject (age 28) and 4.4% for the other (age 23). This similarity of results for the presbyopic and non-presbyopic observers suggests that changes in the lens do not play a primary role in producing the perceptual effect under study.

The results of the present study, taken together, argue persuasively that the central region of the human retina stretches substantially—some 4½%—during marked (9 dioptre) accommodation. This is much larger than the figure of 2% stretch, which is obtained by dividing Moses' measured 0.5 mm advance of the *ora serrata* by the distance from the *ora* to the optic disk[4]; the reason for this difference remains unknown. The stretching reported here has implications for our understanding of accommodative micropsia[5] and other perceptual phenomena: in particular the stretch (if it is non-uniform) may underlie the spatial distortions discovered by Blank and Enoch[2], as in fact they propose, but this remains to be established. The stretching of the central human retina may also be of interest to those who study the eye from a clinical point of view.

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Acetylcholine as an excitatory neuromuscular transmitter in the stomatogastric system of the lobster

THERE is much physiological evidence that L-glutamate acts as an excitatory transmitter at many arthropod neuromuscular synapses[1–3]. The only suggestion that acetylcholine (ACh) has such a function is due to Futamachi[4]. He showed that the nerve terminal region innervated by the largest crayfish slow flexor excitatory motor neurone is depolarised by iontophoretic applications of ACh, but not L-glutamate. I now have biochemical and physiological evidence that ACh is the neurotransmitter at some, but not all, of the excitatory neuromuscular synapses of the lobster stomatogastric system.

The stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, contains about 30 neurones, 23 of which are motor neurones and make excitatory neuromuscular synapses with 38 pairs of striated muscles which move the lobster's stomach[5]. These muscles are typical of crustacea, with fibres approximately 100 μm in diameter and up to several centimetres long. According to present knowledge, their sole motor innervation is from the stomatogastric ganglion[5]. The motor neurone somata are 40–100 μm in diameter, show both attenuated action potentials and considerable sub-threshold synaptic activity[6], and are identified physiologically by the motor nerve in which their axons are found[7].

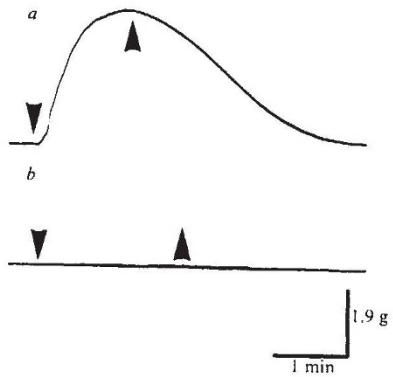

Fig. 1 Effects of bath application of ACh and L-glutamate on a dorsal dilator muscle. The muscle was connected to a Grass FT03 force displacement (tension) transducer, and the output was displayed on an oscilloscope and photographed with a kymograph camera. The muscle was placed in a 1.5 ml chamber through which saline flowed continuously at 6–8 ml min^{-1}. *a*, 2×10^{-5}M ACh with 0.01 mg ml^{-1}. Tensilon was introduced into the chamber at the downward arrow. The muscle contracted, and the upward arrow indicates the start of the wash in normal saline. *b*, 1×10^{-3}M L-glutamate was introduced to the chamber at the downward arrow, causing no response, and the wash was started at the upward arrow. The muscle continued to contract in the presence of ACh for 6 h after the test with L-glutamate.

Choline acetyltransferase catalyses the enzymatic biosynthesis of ACh and correlates well with the presence of ACh in *Aplysia* neurones[8]. I have assayed this enzyme in individual somata of physiologically identified stomatogastric neurones. First, neurone somata were penetrated with microelectrodes for identification of the cells, a drawing was made of their relative positions, and then the cells were dissected individually into small glass tubes by the method of Giller and Schwartz[9], after being frozen in a mixture of 70% ethylene glycol and 30% *Panulirus* saline. The tubes containing the cells were kept frozen on dry ice until the assay was performed, usually within 2 d of dissection.

Each ganglion contains 2 PD (pyloric dilator) motor neurones and one LP (lateral pyloric) motor neurone[6]. Table 1 shows a summary of choline acetyltransferase assays on single PD and LP somata. The PD somata contained choline acetyltransferase activity in 16 out of 18 cells assayed. The actual values obtained showed a large variation which may reflect damage caused during the identification or dissection, or actual variation in the amount of the enzyme present in different cells. The mean of these measurements is 12.6 ± 8.1 pmol ACh per cell per h which may be somewhat low because I made no attempt to discard values from cells which may have been damaged. I estimate that the amount of enzyme is about 125 pmol ACh produced per nl cell volume per hour. This value is comparable with those found in cholinergic neurones in Aplysia[9].

Table 1 Acetylcholine transferase in single PD and LP somata

	pmol ACh, per cell per h*	No. of somata with activity / No. of somata assayed
PD cells	12.6 ± 8.1	16/18
LP cells	$< 2.7 \pm 0.8$	1/10
Limit of sensitivity	2.4 ± 0.7	

* Means plus standard deviations of values from 10 experiments.

PD cells showed activity in all but two cells assayed, with a range between 4.0 and 29.9 pmol ACh per cell per hour. The LP cells were below the limit of sensitivity in all cases but one, where a low level of activity was found. Assays were performed as by Coggeshall *et al.*[10]. 10 μl of buffer-substrate (final concentrations, 0.1 M $NaPO_4$, *p*H 7.9; 0.25 M NaCl; 10 mM choline chloride; 1 mM EDTA; 0.1 mM eserine; 16 mM $MgCl_2$ and 0.4 mM ^{14}C acetyl CoA, specific activity 5–10 μCi μmol^{-1} (New England Nuclear Corp.)) was added to the tubes containing cell somata and to empty tubes (blanks). Tubes were incubated at 38° C for 30 min. The reaction was stopped with 100 μl of 50 mg ml^{-1} sodium tetraphenylboron in 3-heptanone. Tubes were mixed, 100 μl of 0.2 M $NaPO_4$, *p*H 7.0, was added, mixed and spun in a table-top centrifuge for 2.5 min. 50 μl of the organic phase was counted in 10 ml of scintillation fluid. Ten to twenty blanks were run with each assay, and the limit of sensitivity was estimated on the basis of the variation in the blanks and the specific radioactivity of the reaction mixture

In all experiments but one, the LP cell showed no acetylcholine transferase activity. As the LP cell is frequently the largest cell in the ganglion, and is always one of the largest, this difference in enzyme activity is not a trivial consequence of differences in the size of the PD cells and LP cell within a ganglion.

The PD neurones innervate the dorsal dilator muscle (muscle cpv 1a,b according to Maynard and Dando's terminology[5]). Figure 1 shows the effects of bath application of ACh and L-glutamate on the dorsal dilator muscle. Figure 1*a* shows the tension produced by 2×10^{-5}M ACh with 0.01 mg ml^{-1} Tensilon (an acetylcholinesterase inhibitor). Dose-response curves show that the threshold for