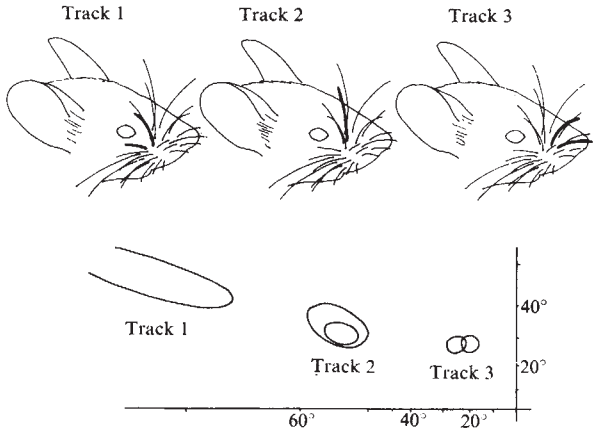

Fig. 1 Correlation between somatosensory and visual receptive fields recorded in three successive electrode penetrations. Lower part of diagram shows visual receptive fields recorded in superficial tectal layers and mapped on tangent screen. Mouse was facing the tangent screen at right angles, 10.5 cm behind it. Origin of coordinate axes marks intersection with the long axis of the mouse. Upper part of the diagram indicates whiskers giving strongest responses in the same electrode tracks at a deeper level; these whiskers are drawn as thicker lines.

to habituate if the same stimulus was repeated, but fired again if the spot or object was moved through a different part of the field. Similar visual receptive field properties in the superior colliculus have been described in other species[5,6,10,12,13].

At a slightly deeper level, in the stratum griseum mediale, cells responding to somatosensory or auditory stimulation were first encountered intermixed with visual cells, and with cells responding to two (or rarely to all three) modalities. Still deeper in the tectum, over a distance of several hundred μm, somatosensory or auditory modalities tended to take over completely until, in some penetrations, the electrode again entered an area of mixed responses. There was no segregation of somatosensory and auditory cells into sub-layers; cells responding to a given modality seemed rather to be arranged in clusters with some intermixing at the boundaries between clusters.

Somatosensory responses were obtained about twice as frequently as auditory. In most penetrations the tactile receptive fields were located on one or a few whiskers. Gently tapping one whisker evoked a short burst of spikes with little difference in response as the direction of hair deflection was varied.

The most striking feature was a very consistent relationship in any one electrode track between the location of visual receptive fields and that of somatosensory receptive fields. A series of three electrode tracks in one experiment is illustrated in Fig. 1. The axes in this illustration represent the horizon and the vertical midline of the mouse, drawn on the tangent screen so as to cross at the extended longitudinal axis of the mouse. Numbers on the axes mark degrees of eccentricity. Visual receptive fields in the three tracks are outlined on the screen. In the upper part of the figure, the whiskers giving the strongest responses from cells deeper in the tectum are drawn more heavily than the others. In the first track there was a clear correlation between temporal visual fields and posterior whiskers; moving nasally in the visual field in tracks 2 and 3 was accompanied by movement to more anterior whiskers. In other series of penetrations a similar correlation was seen between visual receptive-field positions and the whiskers that evoked the best responses at deeper levels in the tectum. If the visual receptive fields were progressively lower in a set of tracks, the whisker fields likewise moved down. By drawing the projections of the whiskers through the eye on to the tangent screen, we could show that for a given perpendicular penetration the visual receptive fields were crossed by just the whiskers that evoked maximal responses from the somatosensory cells deeper in the tectum. This correlation held not only for neighbouring visual and somatosensory cells, but also for single cells driven by both modalities.

In parts of the tectum in which visual receptive fields were far peripheral, and no whiskers lay in the way, somatosensory responses at deep levels were evoked from other parts of the body. Visual fields far down, where the mouse might see its own paw, were associated with tactile fields on the dorsum of the paw. Far temporal visual fields were correlated with somatosensory fields on the flank, shoulder and ear; here an upward movement in the visual field was paralleled by upward movement in the somatosensory map, from the flank to the tip of the ear.

Reactions to auditory stimulation were found less frequently than to tactile stimuli and they tended to be less well localised. Auditory responses could be evoked by complex sounds rich in high frequencies like clicks or crackling noises. Usually only sounds generated from a direction contralateral to the colliculus were effective in driving auditory cells. In eight out of thirteen cells the auditory receptive field in the horizontal plane was restricted to an angle of about 50°–150°, which included the visual and somatosensory receptive field of cells recorded simultaneously or in the close vicinity.

The mouse superior colliculus thus contains three topographical maps, superimposed and roughly in register, of the surroundings as observed through visual, somatosensory and auditory modalities. We assume that this system serves to orient the animal to an interesting stimulus whatever the sense modality.

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Conductance of channels opened by acetylcholine-like drugs in muscle end-plate

MOST recent views of the way in which acetylcholine (ACh) can cause ion channels to open postulate that the channel can exist only in one or other of two distinct conformations, open or shut[1–4]. In their simplest form, these theories predict that a channel, once it is open, will have the same conductance whichever drug caused it to open. We have estimated average single channel conductances for four cholinomimetic agonists, and find this prediction is not confirmed.

The mean single-channel conductance, and mean lifetime of the open state, have been estimated at the voltage-clamped end-plate of the frog (summer *Rana pipiens*) cutaneus pectoris muscle. Methods similar to those of Anderson and Stevens[5], were used with the addition of Normarski interference optics,

which make the end-plate clearly visible. The drugs shown in Fig. 1 were applied iontophoretically to the end-plate, and samples of the end-plate current fluctuations taken at 1,024 s^{-1}. The mean single-channel conductance, γ, was estimated from the total variance, s_I^2, of the current fluctuations, as $\gamma = s_I^2/m_I(V-V_{eq})$, where m_I = mean end-plate current induced by the drug, V = membrane potential and V_{eq} = equilibrium potential. This method assumes that the fluctuations represent the random opening and closing of channels, and that no appreciable contribution to s_I^2 occurs outside the frequency range examined in our experiments (1–500 Hz).

Measurements in both normal and in glycerol-pretreated muscle fibres showed that V_{eq} was the same (near 0 mV), within a few mV, for all the drugs in Fig. 1. Measurements on normal fibres were obtained by depolarising in small steps of a few mV, spaced by about 10 s, as the contraction threshold (about −55 mV) was approached, until about −30 mV was reached at which point the excitation–contraction mechanism was inactivated. The variance was estimated from the variance of the digitised noise record, and as

$$s_I^2 = \int_{-\infty}^{\infty} S(f)\mathrm{d}f = \int_0^{\infty} G(f)\mathrm{d}f$$

where $S(f)$ is the two-sided, and $G(f) = 2S(f)$ the one-sided spectral density computed from the fluctuations. These methods gave very similar results.

The results are shown in Table 1. The means for all experiments are given, because the results were reproducible from day to day. For example, three separate experiments with suberylcholine (SubCh) gave $\gamma = 28.4 \pm 1.6$, 28.2 ± 2.2, and 29.2 ± 2.4 pmho.

It is clear from these results that the mean single-channel conductances, γ, are not the same for all drugs, but that they vary over an approximately twofold range for the drugs tested. In all seven experiments in which SubCh and ACh were tested on the same cell, it was found that SubCh had a larger γ than ACh, the mean ratio being 1.19 ± 0.04. SubCh also had a longer mean open lifetime than ACh, by a factor of 1.7 (Table 1), the same as the factor found by Katz and Miledi[7]. The agonists 3-phenylpropyltrimethyl ammonium (PPTMA) and 3-(*m*-hydroxyphenyl)propyltrimethyl ammonium (HPTMA), which are full agonists as judged by their ability to cause contraction of the frog rectus abdominis muscle[8], have a substantially smaller γ than either ACh or SubCh.

Katz and Miledi[7] have already shown that the mean length of time for which the ion channel stays open may differ over an approximately tenfold range for different drugs, at the frog muscle end-plate. Contrary to the predictions of the simple two-state theories, it seems that differences in agonist action that can produce a tenfold difference in the mean time that a channel remains open, can also influence the mean conductance of the open channel. For the drugs tested at least, γ varies much less than the mean open-channel lifetime, τ, from drug to drug. It is also intriguing that a large single channel conductance seems to accompany a large open lifetime, but not enough drugs have been tested yet to allow any generalisation to be made.

Table 1 Values of mean single-channel conductance (γ) computed from integrated spectral densities for current fluctuations, and values of mean open-channel lifetime, τ, at 10–15° C and a membrane potential of between −60 and −80 mV, computed as $1/(2\pi f_c)$ where f_c = half-power frequency.

	γ (pmho)	τ (ms)
SubCh	28.6±1.0 (22)	5.6±0.3 (8)
ACh	25.0±0.9 (8)	3.2±0.3 (6)
HPTMA	18.8±0.8 (19)	1.0±0.06 (13)
PPTMA	12.8±1.1 (7)	0.83±0.02 (3)

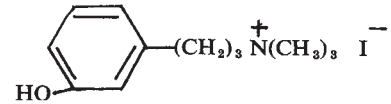
The frog Ringer solution contained tetrodotoxin (100 nM). All results are on normal (not glycerol-treated) fibres. The table gives the mean of values on all cells ± s.e., with the number of determinations in parentheses.

Acetylcholine (ACh) $CH_3-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3\ Cl^-$

Suberylcholine (SubCh)

$$(CH_3)_3\overset{+}{N}CH_2CH_2-O-\overset{\overset{O}{\|}}{C}-(CH_2)_6-\overset{\overset{O}{\|}}{C}-O-CH_2CH_2\overset{+}{N}(CH_3)_3\ 2I^-$$

3-(*m*-hydroxyphenyl) propyltrimethyl ammonium (HPTMA)



3-phenylpropyltrimethyl ammonium (PPTMA)

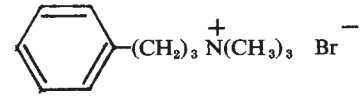


Fig. 1 Drugs applied iontophoretically to *Rana* muscle end-plate.

The spectra of current fluctuations produced by ACh and SubCh could be fitted well with a single time constant '$1/f^2$' model[1,5]. The mean single channel conductance, estimated from the zero frequency asymptote of spectral density[5], as $\gamma = S(0)/2m_I(V-V_{eq})\tau$, agreed well with estimates of γ from the noise variance in the case of these drugs. The spectra of the other two agonists, however, had a component at low frequency, in addition to the major high frequency component. The sum of two single time-constant spectra was found to fit these results. The 'mean open lifetimes' in Table 1 were derived from the high frequency component, but whether or not they estimate accurately the lifetime of an actual species depends on the explanation for the two components that is adopted. It is possible that several species of channel exist with different mean open times, corresponding, conceivably, with the binding of different numbers of agonist molecules. But it can be shown quite simply that if the channels all have the same conductance when they are open, then the value of γ calculated from the noise variance should be this conductance, regardless of how the channels are distributed among the species of different mean open lifetimes[6].

Two classes of explanation can be imagined for the observation that mean single channel conductances are not the same for all drugs. It could be that the channel can exist in several distinct open conformations, each of which has a different conductance, and that different drugs favour different conformations. On the other hand, it could be that the open channel has essentially the same conformation for all drugs, but individual drugs have, for example, somewhat different effects on local field strength or local *p*H so that each drug affects the *p*K of ionisable groups, or access of ions to the channel, in a slightly different way. It has been reported[9] that reduction of the ACh receptor with dithiothreitol changes ACh voltage fluctuations in a way which suggests that the primary change is a lowered single-channel conductance.

Our conclusions depend, of course, on the correct estimation of γ. It is conceivable, for example, that current fluctuations actually reflect receptor occupancy by the drug[5], and that the channel oscillates more rapidly than we can observe between open and shut states while it is occupied. In this case our estimates of γ would be only a lower limit of the true open channel conductance.

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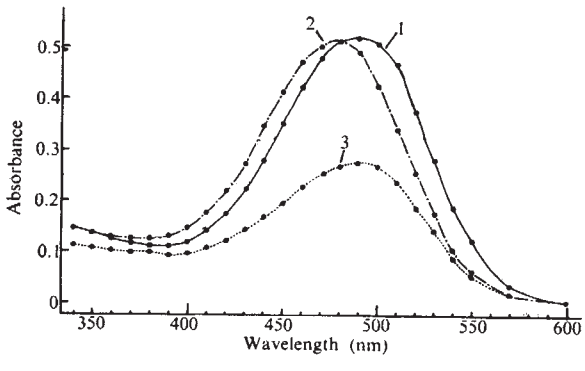
Reversible spectral change of squid retinochrome by salts

THE cephalopod retina contains two kinds of photosensitive pigments, rhodopsin and retinochrome. Retinochrome was first found in the inner segment of squid visual cells[1]. More recently it was reported that the outer segment might also contain retinochrome together with rhodopsin[2]. The chromophore of retinochrome, all-*trans* retinal, is predominantly converted into the 11-*cis* form by illumination. It has been suggested that retinochrome supplies 11-*cis* retinal for the regeneration of cephalopod rhodopsin in the dark, by acting as an isomerase[1]. The validity of the mechanism, however has not yet been established *in vitro* or *in situ*.

The chemical behaviour of retinochrome has been studied and compared with that of rhodopsin[1,3]. Retinochrome has an absorption spectrum that depends on *p*H, and is sensitive to hydroxylamine and $NaBH_4$, while rhodopsin has an absorption spectrum independent of *p*H and is not affected by those reagents. This suggests that the link between chromophore and protein in retinochrome is more susceptible to the environment than in rhodopsin. On the other hand, evidence from circular dichroism measurements indicates that the chromophore of retinochrome is fixed to the protein part so that its rotation is restricted as in rhodopsin[2,3]. Here we show that the absorption maximum of retinochrome is shifted to the shorter wavelength with increasing salt concentration.

Eyes were enucleated from fresh squids (*Todarodes pacificus*) under dim white light, frozen immediately, and stored at −20° C until used. For each preparation, we used about 40 eyes. They were thawed gradually at room temperature and bisected, after which the hemispheres containing the retinae were shaken in phosphate buffer (1/15 M, *p*H 6.8) in order to detach the outer segments of the visual cells and free the retinae

Fig. 1 Absorption spectra of retinochrome at *p*H 5.4 and 13° C. Curve 1 is the absorption spectrum of desalted retinochrome—digitonin solution. Next, solid NaCl (60 mg) was added to the solution (1 ml) and the *p*H was adjusted to 5.4 by addition of 0.6 μl 1 N NaOH (curve 2). Curve 3 is the spectrum of the sample after desalting by dialysis.



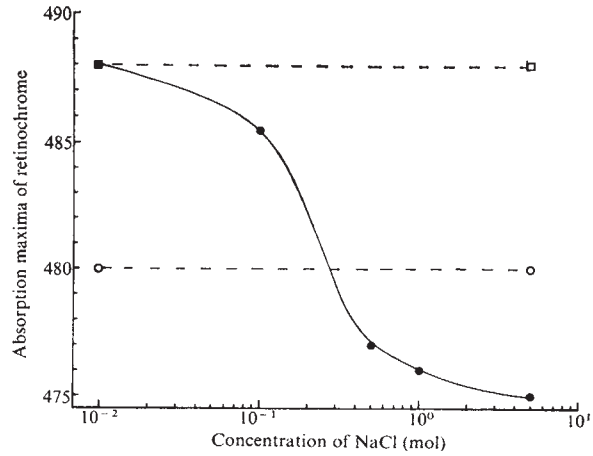


Fig. 2 Absorption maxima of retinochrome (●), rhodopsin (○) and acid-metarhodopsin (□) in various concentrations of NaCl at *p*H 5.4. The salt concentration of the samples was increased by addition of solid NaCl. When the sample became turbid by addition of NaCl, the absorption maximum was determined after centrifugation for 1 h at 28,000*g* at 4° C. Acid-metarhodopsin was prepared by converting rhodopsin to alkaline metarhodopsin by exposure to yellow light (λ > 520 nm) at *p*H 10, and then lowering the *p*H to 5.4.

containing the retinochrome. From the outer segment we extracted rhodopsin according to a method described previously[2]. When retinochrome had been extracted from the outer segments together with rhodopsin, the two were separated by the chromatographic method described previously[2]. Retinochrome was prepared from the outer segment-free retinae[1].

Ommochrome was eliminated from the extracts by passing them through a DEAE cellulose column (1 cm in diameter and 5 cm in height). The extract was placed on top of a column equilibrated with 0.02 M phosphate buffer (*p*H 6.8) and eluted with 0.02 M phosphate buffer containing 0.1% digitonin. As ommochrome was strongly adsorbed at the top of the column, eluates were free from ommochrome. The eluates were gathered, concentrated by a Sartorius membrane filter and then desalted by passage through a Sephadex G-25 column (1 cm in diameter and 20 cm in height), which was equilibrated with non-ionic 0.1% aqueous digitonin. We used the desalted retinochrome and rhodopsin samples to investigate the effect of salt on retinochrome and rhodopsin. Absorption spectra were measured with a 124 type Hitachi spectrophotometer at 13° C. Ionic strength was monitored with a conductivity meter (CD-35 M II, MS KiKi).

Figure 1 shows the absorption spectrum of retinochrome from 340–600 nm at *p*H 5.4. Retinochrome has an absorption peak at 488 nm in desalted digitonin solution (curve 1). When solid sodium chloride was added to this sample to a final concentration of 1 M, the absorption maximum was shifted to 478 nm (curve 2). At this salt concentration, no further change of the absorption spectrum was observed for at least 8 h at 13° C. When the sample was desalted by dialysis with a Sartorius membrane filter against distilled water for 5 h at 5° C, it again exhibited an absorption spectrum with the peak at 488 nm (curve 3). The absorbance at 488 nm was reduced to about half the initial value, because of the dilution of the sample during dialysis. This result shows that the change in the λ_{max} of retinochrome by salt is reversible.

Figure 2 shows the effects of salt concentration on the λ_{max} of retinochrome, rhodopsin and acid-metarhodopsin at *p*H 5.4. The λ_{max} of retinochrome shifted considerably at concentrations between 0.1 M and 0.5 M NaCl, and was at 475 nm in 5 M NaCl, while no shift of λ_{max} of rhodopsin and acid-metarhodopsin was observed. We further investigated the effect of various salts (NaCl, KCl, LiCl, $MgCl_2$ and $CaCl_2$) on the λ_{max} of retinochrome. All the salts so far examined caused the shift of the λ_{max} from 488 nm to a limit of 475 nm at *p*H 5.4