causal relationship remains obscure.

The possible role of rhodopsin's diamagnetic anisotropy in this compression effect is addressed by the data in Fig. 2A. A 10-G field was subthreshold, a 50-G field produced a half-maximum compression, and a 100-G field is at the saturation level for this retina (compared with higher field strengths in Fig. 1A). The lowest threshold level seen was 10 to 20 G. These d-c magnetic field strengths are two orders of magnitude lower than those required for outer segment realignment (*3*) and at least one order of magnitude lower than those strengths that produce interaction energies exceeding the thermal energy level (*22*). These data argue that rhodopsin's anisotropy did not cause this response compression. Although experiments with altered magnetic field orientations would be helpful in addressing this issue, they were not technically feasible because of the restraints in positioning our in vitro eyecup preparation within the electromagnet gap. The ERG, however, is a voltage response summed across the entire curvilinear retinal surface. Thus, a considerable photoreceptor orientation already exists relative to the horizontal magnetic field used in these experiments.

Several additional experiments indicate that this effect can be demonstrated in nonhibernating animals only briefly after the offset of ambient lighting (L-D, 6 p.m.). Unlike the responses at low field strengths seen in retinas tested during the L-D phase of the diurnal cycle, strong d-c magnetic fields ($\geq$ 5000 G) were required to elicit any response compression in any retina prepared later than approximately 2 hours after the light offset. This effect is photodependent and not entirely caused by an internal circadian clock, because leaving the lights on at 6 p.m. and carrying out the same experiment did not result in any b-wave response compression. Dark adaptation per se was not involved, because protracted dark adaptation in vivo during any other period of the LD cycle did not bring about the effect. Concurrent light backgrounds did not prevent the magnetic field-induced response compression, but, instead, seemed to add their effects to that of the magnetic field. Our most recent experiments confirm the initial data obtained during the previous winter hibernation season, suggesting a significant seasonal variation in this diurnal effect. During the winter season (November through March), we did not always see a magnetically induced response compression during the L-D period; when this effect was present, the threshold magnetic field strengths required were usually high (> 1000 G). This observation is in line with the reported loss of diurnal retinal melatonin synthesis in turtles during winter (*19*).

These data indicate that d-c magnetic fields have a significant, but brief, suppressive effect on the extracellularly monitored, light-elicited ionic current fluxes in the in vitro turtle retina. These suppressive effects disappear after the field is removed. In vitro measurements of visual sensitivity are not likewise reduced; this suggests that the locus of this effect is not on any gain control mechanism in the retina. Our results do not resolve the question whether this response compression occurs in the photoreceptors themselves or in the synaptic processes involved in ERG b-wave generation. The importance of the diurnal LD cycle implies a hitherto unsuspected aspect of metabolic activity in the retinal outer plexiform layer. The effect on both rod and cone photoreceptor types and the very low threshold magnetic field intensities required suggest that the diamagnetic anisotropy of disk membrane rhodopsin is not crucial for these response compression effects.

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10 June 1982; revised 13 October 1982

Single-Channel Fluctuations in Bimolecular Lipid Membranes Induced by Rat Olfactory Epithelial Homogenates

Abstract. *Chemosensitive single-channel fluctuations were observed to be induced in essentially solvent-free lipid bimolecular membranes by the addition of sonicated homogenates of rat olfactory epithelium. The chemosensitive channels were not observed when respiratory epithelium homogenates were used instead. Ionic selectivity is consistent with potassium ions as the charge carrier. These channels may be associated with the initial events of chemoreception in the rat olfactory epithelium.*

The initial events in olfactory chemoreception are poorly understood compared to analogous processes in bacterial chemotaxis (*1*). We report here a model system for the initial chemosensory events in the mammalian olfactory epithelium, the incorporation of rat olfactory epithelial homogenates into planar, essentially solvent-free bimolecular lipid membranes (BLM's).

Olfactory epithelia from three or four male Sprague-Dawley rats (200 to 225 g; Blue Spruce Farms) were pooled and minced in 10 m*M* 3-*N*-morpholino propanesulfonic acid (MOPS), *p*H 7.40, K^+ counterion, containing 15 μM adenosine triphosphate, 10 μM guanosine triphosphate, and 50 m*M* sucrose. The suspension was processed (4°C) in a Teflon-in-glass homogenizer (one or two up-down strokes, 200 rev/min) with a loose clearance, filtered through four layers of cotton gauze, and then was sedimented at 120,000*g* (30 minutes). The resulting pellet was twice resuspended and twice resedimented at 100,000*g* (20 minutes) in the same buffer. The final pellet was suspended in 250 μl of the buffer and was sonicated (30 to 40 seconds, 4°C). This suspension was sedimented at 12,500*g* (3 minutes), and the supernatant was retained as the homogenate for reconstitution studies. Light microscopic examination revealed vesicles (30 to 50 μm in diameter) which presumably arose from the resealing of ultrasonically disrupted

disrupted ciliary fragments and other material (*2*).

Essentially solvent-free planar BLM's of area ~ 2 mm^2 were prepared by a modification of the Montal-Mueller method (*3*), passing a hydrostatically closed chamber through a lipid monolayer at the air-water interface (*4*). Electrical properties of the membrane under voltage clamp conditions were characterized with an automated computer-based measurement system (*5*). The basic protocol was (i) construction and measurement of BLM capacitance and d-c and a-c conductivity, (ii) addition of homogenate to one side of the membrane, and (iii) delivery of diethyl sulfide, (−)-carvone, or camphor as a dilute aqueous solution of the odorant to the same side of the BLM, concomitant with electrical measurements (*6*).

An initial portion of the record from a typical experiment is shown in Fig. 1. Under voltage clamp conditions (+10 mV) the homogenate was added to the *cis* side of the BLM, and single-channel conductance fluctuations were observed after about 30 seconds. Approximately 20 minutes later the frequency of these fluctuations had decayed nearly to zero. Adding diethyl sulfide (25 n*M*) to the same side of the BLM activated a new set of fluctuations.

The temporal and amplitude distributions of these events were analyzed statistically. The temporal distribution of the single channels fit (correlation coefficient r = .91) a simple exponential distribution of the number of occurrences. The mean time in the open state was 29.3 ± 7.8 seconds [mean ± standard error (S.E.)] for the spontaneously activated channels, and the probability for these channels to be in the open state was 0.66 ± 0.06 [mean ± standard deviation (S.D.)]. The mean time in the open state for the diethyl sulfide–activated channels was 42.3 ± 10.0 (S.E.) seconds, significantly (t-test, $P < .05$) in excess of the value for the spontaneously activated channels. The probability for the open state of the diethyl sulfide–activated channels [0.80 ± 0.06 (S.D.)] is also increased. The mean value for the conductance of the spontaneously activated channels was 58 ± 7 (S.E.) pS, while for the diethyl sulfide–activated channels it was 56 ± 10 pS. The mean value of all data yielded a single-channel conductance of 60 ± 10 pS (N = 23). Some experiments showed single-channel activity which tended to remain "on." Some experiments also evidenced a stepwise increase of conductance with a good resolution of individual steps. The diethyl sulfide–activated single-channel conductance was 62 ± 2 pS.

The final stationary value of the membrane conductance depended on the diethyl sulfide concentration. We observed a linear correlation (r = .84, slope = 2.2, N = 38) between the stationary level of the membrane conductance evoked by the diethyl sulfide and the logarithm of its concentration.

We examined the ionic basis of the diethyl sulfide–induced conductance increase by varying the bathing medium. The activation could be observed without significant reduction in 40 m*M* KCl, 20 m*M* K^+ tartarate, or 20 m*M* K^+-Na^+ tartarate, whereas no activation could be observed in 40 m*M* NaCl. The ionic selectivity was further examined under bi-ionic conditions, where 20 to 100 m*M* KCl was present on the *cis* side and a supporting electrolyte containing 20 m*M* KCl was present on the *trans* side of the BLM. A linear dependence of diethyl sulfide–activated conductance on K^+ concentration was observed under these conditions (r = .95, slope = 0.94). The activation by diethyl sulfide was completely blocked by 0.125 μ*M* 4-aminopyridine, a K^+-channel blocker (*7*).

Homogenates of rat respiratory epithelium, prepared and added to the BLM in the same way as the olfactory epithelium homogenate, showed no influence of diethyl sulfide up to millimolar concentrations. Membranes untreated with either homogenate also showed no detectable change in d-c conductance at millimolar diethyl sulfide concentrations.

Fesenko *et al.* (*8*) described an increase in d-c conductivity elicited by camphor solutions across solvent-containing BLM's sensitized with a fraction from frog olfactory epithelium. It is difficult to compare our observations with their data since millimolar concentrations of odorants were used and a single-channel basis for their phenomenon was not shown. We did not observe effects of saturated camphor solution comparable to that reported by Fesenko *et al.* (*8*). However, an ~ 12 percent decrease in d-c conductance was seen after the addition of saturated camphor solution to the BLM's treated with rat olfactory epithelium. Addition of (−)-carvone (50 n*M*) to the sensitized BLM gave current fluctuations similar to those seen with diethyl sulfide. The channel conductance induced by (−)-carvone was 65 pS; thus, it could correspond to the same channel activated by diethyl sulfide. The fluctuations elicited by (−)-carvone were blocked by 0.125 μ*M* 4-aminopyridine.

The two-state behavior described here is reminiscent of the discrete fluctuations observed on chemical activation of BLM's containing electroplax acetylcholine (ACh) receptor (*9, 10*) as well as in glutamate-gated single ion channels in locust muscle (*11*) and in patch-clamped studies of the ACh receptor in frog muscle (*12*). The increase in the mean open

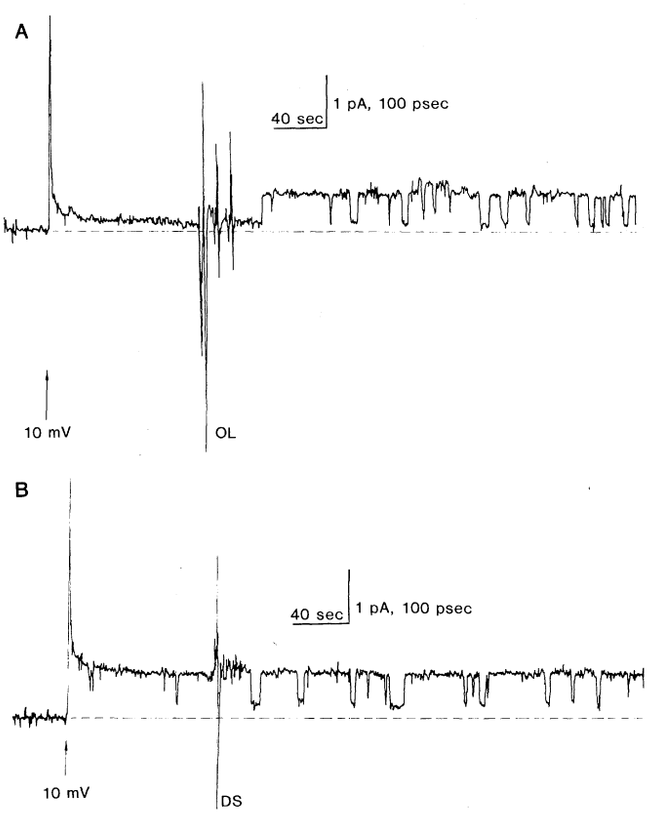

Fig. 1. Single-channel conductance fluctuations. Only the initial portion of the record is illustrated. Lipid bimolecular membranes were prepared from egg phosphatidylcholine (Sigma type 3) in 20 m*M* NaCl, 20 m*M* KCl, and 2 m*M* $CaCl_2$, *p*H 7.40. The initial conductivity was 0.19 × 10^{-8} mho/cm^2; initial capacitance was 0.76 μF/cm^2. (A) Rat olfactory homogenate (protein concentration, 30 μg/mL) was added in 100-μl portions (denoted by *OL*) under voltage clamp (+10 mV). The dashed line marks zero current. (B) Effect of diethyl sulfide (final concentration, ~ 25 n*M*) on single-channel conductance fluctuations in the experiment illustrated in (A). Addition of diethyl sulfide is denoted by *DS*.

ciliary fragments and other material (*2*). time of the open state in the present system after treatment with diethyl sulfide is very similar to the behavior of the glutamate-gated channel (*11*).

Although channel open times are long in comparison with those from patch-clamped living cells (*12*), they are in general accord with results of others (*13, 14*) for reconstituted single ion channels in planar bilayers. Benz *et al.* (*13*) reported analogous behavior of open channel states from the functional reconstitution of a porin from *Salmonella typhimurium*. Channels formed from a sea anemone neurotoxin (*15*) have a tendency to remain open, although bistable fluctuations have been reported for the same material (*16*).

Our data suggest the presence of a discrete chemosensitive ion channel in rat olfactory epithelium which can be functionally transferred into an artificial, essentially solvent-free lipid bimolecular membrane. This channel is activated by odorous compounds such as diethyl sulfide and (−)-carvone. We suggest that occupancy of this channel is associated with the initial events of chemoreception.

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15 April 1982; revised 24 February 1983

Formation in vitro of Sperm Pronuclei and Mitotic Chromosomes Induced by Amphibian Ooplasmic Components

Abstract. *A cell-free preparation of the cytoplasm from activated eggs of* Rana pipiens *induces, in demembranated sperm nuclei of* Xenopus laevis, *formation of a nuclear envelope, chromatin decondensation, initiation of DNA synthesis, and chromosome condensation. Both soluble and particulate cytoplasmic constituents are required to initiate these processes in vitro. The observed changes resemble processes occurring during fertilization and the mitotic cycle in early amphibian embryos. Therefore, this cell-free system may be useful in biochemical analysis of the interactions of nucleus and cytoplasm that control nuclear behavior.*

In the cytoplasm of the fertilized egg, the sperm nucleus undergoes changes; its nuclear envelope breaks down, its chromatin disperses, and a new nuclear envelope is assembled around it to form the male pronucleus, which enlarges, synthesizes DNA, and enters mitosis (*1*). Similarly, sperm or somatic cell nuclei injected into the cytoplasm of activated amphibian eggs decondense, swell, synthesize DNA, and enter mitosis (*2–3*). Cytoplasmic preparations from *Xenopus laevis* eggs have been shown to induce decondensation of the chromatin of red blood cell nuclei (*4*) and DNA synthesis in liver nuclei (*5*). Swelling of sperm nuclei in cytoplasmic preparations from eggs has been observed in sea urchins (*6*). However, only certain aspects of the process of pronuclear formation appeared to be reproduced in these earlier cytoplasmic preparations. We report that when demembranated sperm nuclei from *X. laevis* were incubated in a cytoplasmic preparation from activated *Rana pipiens* eggs, the sperm chromatin underwent changes similar to those in activated eggs.

Lysolecithin-treated sperm (*7*) from *X. laevis* were incubated with a cytoplasmic fraction prepared from activated *R. pipiens* eggs with the buffer and procedures described (*8*). Sperm incubated in this buffer did not show any morphological change. Instead, the cytoplasmic fraction prepared in this buffer induced a series of changes in sperm nuclear morphology (Fig. 1). The sperm nuclei were highly condensed when added to the cytoplasmic preparations (Fig. 1A), but during incubation they became partially decondensed (Fig. 1B). These nuclei continued to decondense to form small pronuclei, which later enlarged (Fig. 1C). The decondensing sperm nuclei synthesized DNA, as evidenced by the incorporation of [methyl-^{3}H]thymidine 5′-triphosphate (*9*) ([^{3}H]dTTP; Fig. 1D). This incorporation was inhibited by aphidicolin (*9*), a specific inhibitor of DNA

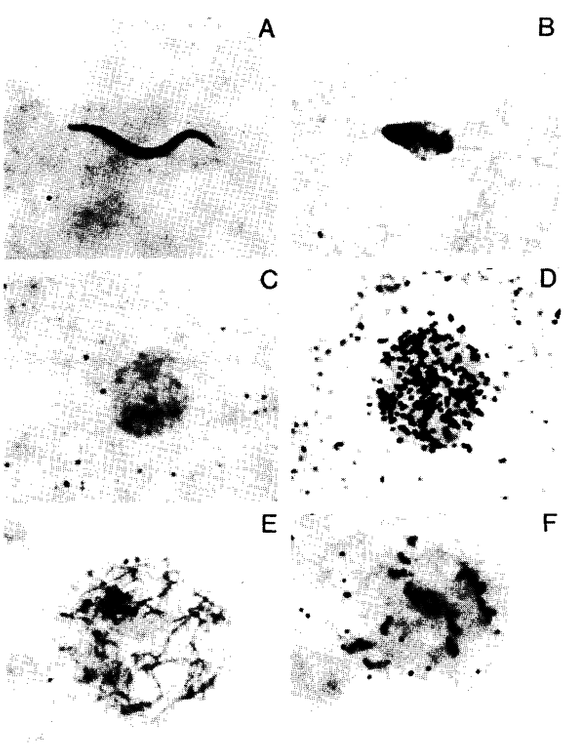

Fig. 1. Morphology of *Xenopus laevis* sperm nuclei during incubation in a cell-free cytoplasmic preparation (*14*). (A) Intact sperm nucleus. (B) Partially decondensed sperm nucleus. (C) Completely decondensed sperm nucleus. (D) Autoradiograph of decondensed sperm nucleus that was incubated for 3 hours in the cytoplasmic preparation containing [^{3}H]dTTP (40 μCi/ml). (E) Chromosomes with a prophase-like appearance. (F) Chromosomes with a metaphase-like appearance. Magnification, ×1100.