Ionic Basis of Learning-Correlated Excitability Changes in
_Hermissenda_ Type A Photoreceptors

JOSEPH FARLEY AND YUNRU HAN

Programs in Neural Science and Biochemistry, Indiana University, Bloomington, Indiana 47405

Farley, Joseph and Yunru Han. Ionic basis of learning-correlated excitability changes in _Hermissenda_ type A photoreceptors. _J. Neurophysiol._ 77: 1861–1888, 1997. Repeated pairings of light and rotation (conditioning) result in persistent changes in excitability of _Hermissenda_ type B and A photoreceptors, which are correlated with pairing-specific reductions in phototactic behavior. Although considerable attention has been devoted to characterization of conditioning-produced neurophysiological changes that occur in type B cells, less information is available concerning the changes produced in type A cells. Here we recorded from identified, synaptically isolated lateral and medial type A photoreceptors from conditioned, random-control, or untrained animals on retention days following conditioning. Type A photoreceptors from conditioned animals responded to light with a receptor potential that was significantly smaller than those of random-control or untrained animals, which did not differ. The phototactic suppression and type A cell light response magnitudes were negatively correlated for individual conditioned animals. Animals exhibiting strong phototactic suppression also showed small light responses. Expression of the training-associated light response difference was a calcium-dependent phenomenon: reducing extracellular calcium to <1 μM enhanced the generator potential of A cells, regardless of conditioning history, and greatly reduced the differences in generator potential amplitude attributable to training. Voltage-clamp studies revealed that conditioning resulted in a two- to threefold increase in the amplitude of a voltage-dependent, sustained outward K⁺ current (I_{Delayed}). I_{Delayed} magnitudes were positively correlated with phototactic suppression for individual conditioned animals: type A cells of animals exhibiting strong phototactic suppression expressed large values of I_{Delayed}. I_{Delayed} is a composite current, consisting of at least three separable components: 1) residual A current (I_{A}); 2) slow, tetraethylammonium-sensitive calcium-activated K⁺ current (I_{K,Ca}); and 3) a delayed-rectifier-type, voltage-dependent K⁺ current (I_{K,v}). Analysis of these currents failed to reveal significant training-associated changes in I_{A} or I_{K,Ca}. But I_{K,v} was enhanced by ~60–150% in both lateral and medial cells and thus contributes to the conditioning-associated increase in I_{Delayed}.

INTRODUCTION

Persistent excitability changes of _Hermissenda_ type B photoreceptors have previously been reported to occur when animals are exposed to repeated pairings of light and rotation (Crow and Alkon 1980; Farley 1988; Farley and Alkon 1982; Farley and Jin 1997; Farley and Schuman 1991; West et al. 1982). These changes include increases in resting input resistance, enhanced steady-state light responses, and decreases in two somatic K⁺ currents [A current (I_{A}) and slow, tetraethylammonium- (TEA) insensitive calcium-activated K⁺ current (I_{K,Ca})]. The enhanced light response of type B photoreceptors is thought to play an important role in learning-produced reductions in phototaxis (Alkon et al. 1985; Farley et al. 1983), in part through increased synaptic inhibition of type A photoreceptors, one of which (the medial) provides polysynaptic drive to motoneurons involved in phototaxis (Goh et al. 1985).

The lateral and medial type A photoreceptors in each eye have also been shown to undergo training-related excitability changes. Synaptically intact type A cells from conditioned animals were first reported to exhibit reduced light-evoked action potential frequencies (Farley and Alkon 1982) when preparations were oriented vertically. From recordings of horizontally oriented nervous systems, Frysztak and Crow (1993) reported that the lateral (but not medial) type A photoreceptor from conditioned animals showed increased light-elicited spike frequencies. The reason(s) for this apparent discrepancy is not as yet clear, but some plausible suggestions have been made (Frysztak and Crow 1993), including the orientation of the preparation during recording.

In contrast to the reported effects on impulse activity, the effects of training on the light-elicited generator potential are consistent. Conditioning produces persistent reductions in the generator potential amplitude of synthetically intact (Farley and Alkon 1982; Frysztak and Crow 1993) as well as synaptically isolated (Farley et al. 1990) type A cells. Further, the light response amplitudes are correlated with the magnitude of phototactic suppression observed in individual conditioned animals (Farley et al. 1990). In combination with demonstrations that the medial type A cell indirectly excites motoneurons involved in phototaxis (Goh and Alkon 1984), whose activities are correlated with behavioral suppression (Goh et al. 1985), the above findings suggest that medial type A photoreceptors are sites of memory storage that are important for associative modifications of phototaxis.

Because little information is available as to the ionic basis of the learning-correlated changes in type A cell light responses, we studied several somatic voltage- and calcium-dependent K⁺ conductance systems in type A photoreceptors from conditioned and control animals with the use of standard two-microelectrode voltage-clamp methods. We observed that conditioning resulted in persistent enhancement of macroscopic delayed outward K⁺ current (I_{Delayed}). Further analysis of the various components of I_{Delayed} revealed that a sustained, voltage-dependent, calcium-independent current (I_{K,v}) was selectively increased.
by conditioning. Preliminary reports of some of these findings have previously appeared in abstract form (Han and Farley 1991, 1993).

METHODS

Animals

Adult *Hermisenda crassicornis* collected in the wild were provided by Sea Life Supply (Sand City, CA). All animals were housed individually in flow-through cages in artificial seawater (ASW) aquaria (15 ± 1°C, mean ± SE) for ≥3 days before use. Animals were periodically fed with small pieces of mussel (*Mytilus edulis*) and were maintained on a 12-h light/dark cycle.

Training procedure

Training and testing were conducted with the use of methods described in detail previously (Farley 1988; Farley and Schuman 1991; Farley et al. 1990) and will be described only briefly here. The standard conditioning procedure exposed animals to 3 consecutive days of training, 50 trials per day, during which the animals received either paired or random presentations of light and rotation-produced stimulation. During training, animals were confined to the peripheral ends of clear plastic tubes filled with refrigerated seawater secured to the surface of a high-speed turntable that was placed in a refrigerator maintained at 17°C. The duration of each stimulus (light or rotation) presentation was 30 s. For those animals that received pairings of light and rotation, the stimuli were presented in a completely overlapping fashion, with their onsets and offsets occurring synchronously (Grover and Farley 1987). The average intertrial interval (offset to onset) was 2.0 min (range 1.0–4.0 min). For control animals exposed to random presentations of light and rotation, the two stimuli were presented 50 times each per session, randomly and independently of one another at an average interval of 2.0 min. All training was accomplished with the use of dedicated microcomputers, interfaces, and custom software. The light intensity was 300 μW/cm² (broadband), measured at the periphery of the turntable where animals were confined. The rotational stimulation was 99 rpm, which resulted in a centrifugal force vector of ∼2.0 g.

Animals were trained during the intermediate 6 h of the light portion of the 12-h light/dark cycle. After a training session, animals were immediately returned to their home aquaria. We also studied the behavior and electrophysiological characteristics of type A cells from untrained *Hermisenda*. These animals were tested and retested for phototaxis (see below) in the same manner, and at the same times, as those exposed to light and rotational stimulation, but these untrained animals remained in their home aquaria on training days.

Testing procedure

Behavioral tests of phototaxis were conducted as follows. Three to five days after their arrival in the laboratory, an initial baseline measurement of phototaxis for each individual animal was obtained. During these tests, which were confined to the intermediate 6 h of the light/dark cycle, animals were placed in 20-cm clear plastic tubes filled with refrigerated ASW. These tubes were securely fastened to the surface of a “tilt-table” apparatus (Farley and Alkon 1982; Grover et al. 1987) placed in a refrigerator (17°C) and animals were dark adapted for 15 min. The tilt-table was then oriented vertically and a light at the opposite end of the tube was turned on. With the use of a hand-held stop watch, start and finish latencies (to the nearest s) were recorded for each animal as previously described (Farley 1988). All animals used in the present study had initial baseline finish latencies <5.0 min. The intensity of the test light was 180 μW/cm² (broadband) at the peripheral start area of the testing tube and 610 μW/cm² at the finish end. Animals were assigned to the various training conditions in a quasirandom manner, subject to the constraint that the average phototactic latencies for groups be the same (±15 s).

On the day after the third and final training session, animals were retested for phototaxis as during the initial baseline test. All testing of animals was conducted by observers who were blind as to the conditioning history of the animals. Any animal that had not locomoted to the end of the tube within 30 min after the initiation of the test received a 30-min cutoff score. As in previous studies, suppression scores were computed for each individual animal with the use of the ratio \( B/(B + T) \), where \( B \) is the initial baseline finish latency and \( T \) is the retest finish latency. These individual suppression scores were then averaged for the group as a whole. With this metric, a score that is substantially <0.50 indicates suppression of phototaxis. Conversely, scores close to 0.50 (±0.05) indicate unchanged phototactic latencies.

The statistical significance of training-produced suppression was evaluated by means of overall analyses of variance (ANOVAs) in which the data for paired, random, and untrained animals were included. Independent-sample Student’s t-tests were used to compare the results of individual groups with one another. These comparisons were specified a priori, and differences are reported as significant if \( P < 0.05 \) (1-tailed).

Behavioral training and testing of animals was conducted year round with the use of small numbers of animals (\( n = 5–10 \)) in each of the three training conditions (paired, random, and untrained) during each replication. Although training was more effective at some times than others, this seemed to be a function of the particular shipments of animals rather than any obvious seasonal variation.

Electrophysiology

Animals were dissected and prepared for electrophysiological recording as previously described (Farley 1988; Farley and Schuman 1991). The cell bodies of lateral and medial type A photoreceptors were acutely and synaptically isolated in situ with the use of methods described by Farley et al. (1990). Each cell recorded was from a different individual animal (i.e., 1 cell per preparation). Axotomy of type A cells, accomplished by razor lesion of the optic nerve (Alkon and Fuortes 1972), eliminated all spontaneously occurring synaptic potentials, as well as the majority of action potentials during the cell’s sustained response to light in standard ASW. As described previously (Farley et al. 1990), however, many A cells continued to exhibit a few truncated action potentials during the initial limb of the cell’s depolarizing generator potential. In addition, some cells that initially showed no sustained impulse activity during their light response in standard ASW began to spike during illumination after solution changes that increased the resting input resistance. In the majority of these cases, however, the pattern of action potential firing and action potential amplitudes were clearly abnormal (e.g., Fig. 2B).

Impalement of type A cells with microelectrodes was facilitated by proteolytic digestion of overlying tissue that encapsulates the eye. The exact conditions used varied as a function of the particular lot of protease (Sigma Type XXVII, catalogue number 4789). In general, the concentration ranged between 1 and 4 mg/ml of ASW. Exposure times ranged between 8 and 15 min at room temperature (17–18°C). After incubation in protease, the nervous system was washed with a minimum of six volumes of 15°C ASW.

When recordings were obtained from trained preparations, animals were randomly sampled from the paired or random-control treatment conditions. All recordings from trained animals were obtained between 24 and 48 h after the conclusion of training, with no differences in the average time as a function of conditioning.
history, at room temperature (17–18°C), which was the same temperature used in tests of phototactic behavior. Recordings were obtained without knowledge of the specific conditioning history of the animal. Recordings from untrained animals were also obtained during these same recording periods with the use of identical solutions, electrodes, etc.

All recordings were made with an Axoclamp 2A (Axon Instruments) amplifier in current-clamp mode, and appropriate head stage. A silver/silver chloride wire was used to connect the electrode solution to the head stage, and a similar wire was used to ground the bath. Signals were PCM digitized at 44 MHz with the use of a Neurocorder (No. 284) and stored on video tape. Rapid and/or high-frequency signals were displayed for analysis on a digital storage oscilloscope (Kikusui, No. 5020A). Slower signals were displayed on a pen recorder (Gould Instruments, model 220 or 2400). All measurements of resting membrane potentials and light responses were made to the nearest millivolt. Measurements of resting input resistance were calculated from the current-voltage plot to the nearest megohm. The statistical significance of any training-produced differences in cell excitability was assessed by ANOVAs. Independent-sample Student’s t-tests were used to compare the results of individual groups with one another. These comparisons were specified a priori, and differences are reported as significant if \( P < 0.05 \) (1-tailed).

**Light response protocol**

An axotomized type A photoreceptor was impaled with a single microelectrode (\( \approx 40 \) MΩ when filled with 1.5 M KCl; A-M Systems microcapillary glass No. 6020) and dark adapted for \( \approx 8–10 \) min. The resting membrane potential was then recorded (to the nearest mV) and the resting input resistance was measured by injection of small hyperpolarizing and depolarizing current steps (\( -0.5 \) to \( +0.3 \) nA) into the cell through a balanced bridge circuit. Unless otherwise indicated, all cells satisfied the following criteria: 1) a stable resting potential more negative than \(-45 \) mV and 2) a resting input resistance of \( \approx 30 \) MΩ. Most cells failing to satisfy both criteria were obviously damaged and exhibited markedly abnormal and small light responses.

After \( 10–12 \) min of dark adaptation, the cell was then illuminated for \( 3 \) min (moderate light intensity of \( 110 \) \( \mu \)W/cm², when measured at \( 510 \) nM), and measurements of light response amplitude were noted every \( 30 \) s for up to \( 3 \) min after light onset. In some cases, as described below, cells were also stimulated for \( 3 \) min with a brighter light (\( 300 \) \( \mu \)W/cm²) after an additional \( 8–10 \) min of dark adaptation.

For those cells for which we examined the effects of reducing extracellular Ca²⁺, the bath solution was then changed (\( \approx 3 \) volumes) to low-calcium ASW (see Table 1 and Solutions below) beginning \( \approx 3–4 \) min after the offset of the light step. The cell was then dark adapted an additional \( 5–6 \) min. The resting membrane potential was again recorded and resting input resistance was measured as before. The cell was then illuminated again for \( 3 \) min and light response amplitudes were measured as described above.

It should be noted that when average values for resting membrane potential, input resistance, and light response amplitudes are reported, for cells in low-calcium ASW, the number of observations (\( n \)) that contributed to the averages is sometimes less than the corresponding number for the standard ASW condition. This occurred because 1) some cells were lost during the solution change and 2) clogging of the tip of the microelectrode made accurate measurement of input resistances difficult in some experiments.

**Voltage-clamp protocol**

The electrodes used to measure membrane potential in voltage-clamp experiments were pulled from microcapillary glass (A-M Systems, No. 6020) and had resistances of \( 15–25 \) MΩ when filled with \( 1.5–2.0 \) M KCl. A different microelectrode having a resistance of \( 10–20 \) MΩ when filled with 1.5 M KCl was used for current passage.

Series resistance under typical recording conditions was measured with a step current pulse, and ranged from 50 to 100 kΩ. In the interests of clamp loop stability, we decided not to employ series resistance compensation. For a mean series resistance value of \( 75 \) kΩ, the error in measured membrane potential, without compensation, for a \( 50-nA \) membrane current (which is among the largest we recorded) is \( 3.3 \) mV (\( V_{\text{error}} = i_{\text{m}}R_{\text{s}} \); \( i_{\text{m}} \), membrane current; \( R_{\text{s}} \), series resistance).

All voltage-clamp measurements were obtained from cells that satisfied the following criteria when initially recorded in standard ASW (see Table 1 and Solutions below) unless otherwise noted. When impaled with a single microelectrode, the cell had an initial resting membrane potential more negative than \(-39 \) mV. After impalement with the second electrode, the membrane potential of the cell was more negative than \(-25 \) mV, and the membrane potential as measured through both electrodes differed by \( \leq 2 \) mV. The holding current at \(-60 \) mV was no greater than \(-5.0 \) nA, and changed by \( \pm 2 \) nA over the course of constant recording conditions. Finally, illumination of the dark-adapted cell elicited peak inward currents \( > 5 \) nA when measured under voltage clamp (at \( V_{\text{h}} = -60 \) mV).

Microelectrodes used for intracellular iontophoresis contained 1.0 M ethyleneglyco(bis (aminomethyl)ether) tetra-acetate (EGTA) (pH 7.4, Sigma) plus 0.1 M K⁺ acetate. Iontophoretic application of EGTA was accomplished by switching from voltage-clamp to current-clamp mode and passing appropriate current (about –2 to \(-4 \) nA for 1–3 min) between the EGTA-containing electrode (the current-passing electrode under voltage clamp) and the bath ground. An equal but opposite current was passed through the second intracellular electrode (the voltage-recording electrode) to minimize changes in membrane potential during EGTA injection. Control of membrane potential was again restored by switching back to voltage-clamp mode.

**Solutions**

A variety of different extracellular solutions was used in these experiments to separate different components of ionic current from one another and to assess the roles of extra- and intracellular calcium ions as determinants of membrane conductance and light responses. Table 1 summarizes the composition of the solutions used in these studies, and the names used hereafter to refer to them. Unless otherwise indicated, in all cases in which ions were substituted for one or more of the standard constituents of ASW, osmolality was maintained by compensatory increases or decreases in Na⁺ or Mg²⁺.

All working solutions were prepared fresh from stocks. All salts were reagent grade and were obtained from Sigma Chemical. 4-Aminopyridine (4-AP) ion was also obtained from Sigma and prepared fresh as a stock and stored in a light-tight container at 4°C. TEA ion was obtained from both Sigma and Research Biochemicals International, prepared fresh as a stock, and stored at 4°C. We observed no apparent difference in TEA as a function of the supplier. EGTA was obtained from Sigma. The pH of all solutions was adjusted to 7.6–7.8 with 500 mM NaOH at 18°C.

**Data analysis**

Quantitation and analysis of ionic currents were accomplished with the use of Axon Instruments pClamp version 5.5.1 suite of programs. Unless otherwise indicated, activation time constants (\( \tau_{\text{ON}} \)) for individual current traces were fit assuming a power function where \( n = 3 \). Inactivation time constants (\( \tau_{\text{OFF}} \)) were fit with...
scores and steady-state light response magnitudes and
2
ited strong suppression of phototactic behavior ( suppression tance was smaller for cells from associatively trained prepa-
if training ( Table 2 ) . As judged by ANOVA, the differences
were speci®ed a priori, and differences are reported as signi®cant
f P < 0.05 (1-tailed).
Correlation coefficients [Pearson product-moment correlations
(r)] were also computed between 1) behavioral suppression
scores and steady-state light response magnitudes and 2) behav-
ioral suppression scores and various voltage-dependent ionic cur-
rent amplitudes.

RESULTS

Behavior
In total, electrophysiological recordings were obtained from 57 separate associatively trained, 28 separate random-
control, and 51 separate untrained animals. In agreement with many earlier reports, when tested ~24 h after the con-
clusion of training, the associatively trained animals exhibited
strong suppression of phototactic behavior ( suppression ratio 0.26 ± 0.01, mean ± SE). In contrast, the untrained
animals were not suppressed (0.48 ± 0.01), whereas ran-
dom-control animals exhibited a slight degree of suppression
(0.42 ± 0.02). An ANOVA indicated a significant effect of
training condition [F(2,132) = 64.53, P < 0.0001]. Planned
t-tests con®rmed that the associatively trained animals were
signi®cantly more suppressed than either random controls
(t93 = 8.15, P < 0.001) or untrained animals (t106 = 8.02,
P < 0.001). The random controls were in turn more sup-
pressed than untrained animals (t27 = 3.49, P < 0.05). The
small degree of suppression observed for random-control
animals is consistent with that observed in earlier studies
(Farley 1987, 1988; Farley and Alkon 1980, 1982, 1987;
Grover and Farley 1987; Grover et al. 1987) and pre-
sumeably re®ects the effects of adventitious pairings of light
and rotation, which occurred on ~20% of trials in these
experiments. The results of similar analyses for subsets of
animals contributing to light response and ionic current mea-
surements, as well as correlations between behavioral sup-
pression and excitability measurements in type A photore-
ceptors for these same animals, are reported below where
appropriate.

Neurophysiology: resting membrane potential and input
resistance
In agreement with earlier results (Farley et al. 1990),
no marked differences in resting membrane potential, input
resistance, light responses, or ionic currents were observed
for lateral versus medial type A photoreceptors, regardless
of conditioning history. Thus the results from the two classes
of cells were pooled in statistical analyses unless otherwise
indicated.

Intracellular recordings from axotomized, dark-adapted
type A photoreceptors in standard ASW, obtained 24–48 h
after the conclusion of training, failed to reveal any conspici-
ous differences in resting membrane potential as a function
of training (Table 2). As judged by ANOVA, the differences
among groups in resting membrane potential [F(2,24) =
1.89, P < 0.17, not signi®cant] were not statistically signi-
cant. However, for these same cells the resting input resis-
tance was smaller for cells from associatively trained prepara-
tions (Table 2), although the F value was only marginally
signi®cant [F(2,21) = 2.78, P < 0.09]. This contrasts with
the results of our earlier report (Farley et al. 1990). The
absolute magnitude of the differences in input resistances
produced by the different training conditions was similar in
the present and earlier studies, but for unexplained reasons
the variability was greater for cells from untrained animals
in the present experiments. Collectively, however, the results
are in essential agreement with those reported previously
(Farley et al. 1990).

The switch to low-calcium ASW produced only small
acute changes in membrane potential and input resistance
(Table 2). The slightly more negative membrane potentials
recorded in low-calcium ASW for cells from the associ-
atively trained and random-control conditions were not sig-

TABLE 1. Composition of external electrophysiological recording solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Ca²⁺</th>
<th>Tris</th>
<th>Cl⁻</th>
<th>EGTA</th>
<th>4-AP</th>
<th>TEA</th>
<th>Co²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard ASW</td>
<td>430</td>
<td>10</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>570</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4-AP ASW</td>
<td>425</td>
<td>10</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>570</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TEA ASW</td>
<td>380</td>
<td>10</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>570</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-AP + TEA ASW</td>
<td>375</td>
<td>10</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>570</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-calcium ASW</td>
<td>430</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>570</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-calcium/EGTA ASW</td>
<td>400</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>570</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-calcium/EGTA + TEA ASW</td>
<td>350</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>570</td>
<td>30</td>
<td>50</td>
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</tr>
<tr>
<td>Low-calcium/EGTA + 4-AP ASW</td>
<td>395</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>570</td>
<td>30</td>
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<tr>
<td>High-calcium ASW</td>
<td>375</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>570</td>
<td>5</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-AP + TEA + low-calcium/EGTA ASW</td>
<td>345</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>570</td>
<td>30</td>
<td>5</td>
<td></td>
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<tr>
<td>High-K⁺ ASW</td>
<td>340</td>
<td>100</td>
<td>50</td>
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<td>570</td>
<td></td>
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<tr>
<td>Co²⁺ ASW</td>
<td>430</td>
<td>10</td>
<td>60</td>
<td>0</td>
<td>10</td>
<td>574</td>
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</table>

All concentrations are mM. pH of the solutions was 7.6–7.8. Tris, tris-hydroxymethylaminomethane; EGTA, ethyleneglycolbis(aminoethyl)ether)tetra-
acetate; 4-AP, 4-aminopyridine; TEA, tetraethylammonium ion; ASW, arti®cial seawater.
significantly different from those recorded for the same cells in standard ASW, as judged by paired-sample t-tests (untrained, associatively trained, and random-control t values; all t values < 1.0). The small differences among the different training conditions in resting membrane potential \([F(2,17) < 1.0]\) and input resistance \([F(2,15) < 1.0]\) in low-calcium ASW were not significant.

### Light response of type A photoreceptors

The general characteristics of light responses of axotomized type A cells in standard ASW were as previously described (Farley et al. 1990), and could be easily discriminated from those of type B cells by virtue of the presence of residual impulse activity during the initial depolarizing limb of the receptor potential and the presence of a long-lasting hyperpolarization response (Alkon and Grossman 1978) following the offset of relatively brief \(\leq 30\)s light steps. These long-lasting hyperpolarization responses were not apparent following prolonged \(3\)min light steps, however.

Measurement of the light response amplitude in standard ASW (expressed as deviations from resting potential), at 30s intervals following light onset, indicated that cells from associatively trained animals \((n = 8)\) exhibited consistently smaller light responses than those of cells from either the untrained \((n = 14)\) or random-control \((n = 5)\) conditions (Fig. 1). These differences were apparent throughout the light response [main effect of conditioning treatment: \(F(2,24) = 12.1, P < 0.0001\)]. The light response amplitudes declined over the course of the 3-min light step [main effect of time: \(F(5,120) = 9.51, P < 0.0001\)], but the differences produced by the conditioning treatments were preserved [interaction of time with training condition: \(F(10,120) < 1.0, P < 0.0001\)]. Separate ANOVAs were conducted on the light response amplitudes of identified lateral versus medial type A photoreceptors for each of the three conditioning treatments. None of the differences between the two cell types was statistically significant. For associatively trained \([F(1,6) < 1.0]\), untrained \([F(1,11) = 1.25]\), and random-control animals \([F(1,3) < 1.0]\), light response amplitudes for medial and lateral type A cells were approximately equivalent throughout the 3min light step. The values at 0.5 and 3 min are given in Table 3.

The average suppression scores for the animals contributing type A cell light response data, as a function of training condition, were 0.44 ± 0.03 for untrained animals, 0.20 ± 0.04 for associatively trained animals, and 0.39 ± 0.01 for random controls. An ANOVA of these data revealed a significant training effect \([F(2,24) = 9.72, P < 0.001]\), with associatively trained animals showing significantly more suppression than untrained animals \((t_{20} = 4.06, P < 0.05)\) and random controls \((t_{11} = 3.39, P < 0.05)\), which did not differ \((t_{17} = 0.79, P < 0.01)\). Pearson product-moment correlations \((r)\) were computed between the magnitude of behavioral suppression and the type A photoreceptor generator potential amplitude, at both 30s and 3 min after light onset, for individual animals in each of the three conditioning groups. The correlations for the 30s light response values and behavioral suppression are given first. For untrained animals, \(r = 0.66\), which was significant at \(P < 0.01\). For associatively trained animals, \(r = 0.94\), which was significant at \(P < 0.002\). For random-control animals, \(r = 0.67\), which failed to reach conventional significance levels \((P > 0.21)\), probably because of the relatively small number of observations \((n = 5)\). The corresponding \(r\) values for the 3min light response values were \(r = 0.23\) for untrained animals \((P > 0.20)\), \(r = 0.90\) for associatively trained animals \((P < 0.003)\), and \(r = 0.59\) for random-control animals \((P > 0.29)\). Thus behavioral suppression was strongly, significantly, and consistently correlated with the magnitude of the type A photoreceptors’ generator potential for associatively trained animals. Animals with small light responses exhibited strong suppression of phototactic behavior.

There was no indication of systematic differences in the strength of this correlation for lateral versus medial type A cells from associatively trained animals. The correlations between behavioral suppression and the 3min light response magnitudes were \(r = 0.74\) for lateral and \(r = 0.92\) for medial A cells from associatively trained animals, \(r = 0.26\) for lateral and \(r = 0.18\) for medial A cells from untrained animals, and \(r = 0.50\) for medial A cells from random-control animals. Because we recorded from only two lateral A cells from random-control animals, a degenerate \(r\) of 1.0 was calculated.

In low-calcium ASW, the light responses of A cells were larger than those recorded in normal ASW, and differences produced by training were no longer as apparent (Fig. 2). Although light response amplitudes of A cells from trained animals \((n = 7)\) were nominally smaller than those of untrained \((n = 8)\) and random controls \((n = 5)\) at all times, these differences were not statistically significant \([F(2,17) < 1.0]\). Similarly, there were no obvious or statistically significant differences in light responses of lateral versus medial A cells within the associatively trained, untrained, and random-control subsets of animals (Table 3).

### TABLE 2. Training effects on membrane excitability of type A photoreceptors

<table>
<thead>
<tr>
<th>Cell Excitability Measure</th>
<th>Untrained</th>
<th>Paired</th>
<th>Random</th>
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<tbody>
<tr>
<td>Normal ASW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>(-52.8 \pm 1.6 (14))</td>
<td>(-46.9 \pm 3.3 (8))</td>
<td>(-48.8 \pm 2.8 (5))</td>
</tr>
<tr>
<td>Dark input resistance, MΩ</td>
<td>(61.6 \pm 11.2 (11))</td>
<td>(32.4 \pm 3.1 (8))</td>
<td>(51.2 \pm 6.9 (5))</td>
</tr>
<tr>
<td>Low-calcium ASW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>(-52.5 \pm 2.1 (8))</td>
<td>(-52.9 \pm 1.4 (7))</td>
<td>(-51.8 \pm 1.8 (5))</td>
</tr>
<tr>
<td>Dark input resistance, MΩ</td>
<td>(47.7 \pm 6.0 (7))</td>
<td>(49.0 \pm 6.7 (6))</td>
<td>(45.0 \pm 5.5 (5))</td>
</tr>
</tbody>
</table>

Values are means ± SE, with number of cells in parentheses. ASW, artificial seawater.
Light response amplitudes diminished slightly over the course of the 3-min light step [main effect of time: \(F(5,85) = 7.05, P < 0.0001\)], but this did not vary as a function of conditioning history [interaction of time with training: \(F(10,85) < 1.0\)]. In approximately half of the preparations from each training condition (\(n = 4, 5, \) and 4 for untrained, paired, and random conditions, respectively), light responses in low-calcium ASW were also measured to a bright light, following the response to the moderate light intensity and another 10 min of dark adaptation. In every cell studied, the response elicited by the bright light was greater than that elicited by

<table>
<thead>
<tr>
<th>TABLE 3. Training effects on light responses of medial and lateral type A photoreceptors</th>
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<td></td>
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<tr>
<td>Post-light onset, min</td>
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<tr>
<td>Standard ASW</td>
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<tr>
<td>Medial cells</td>
</tr>
<tr>
<td>Lateral cells</td>
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<tr>
<td>Low-calcium ASW</td>
</tr>
<tr>
<td>Medial cells</td>
</tr>
<tr>
<td>Lateral cells</td>
</tr>
</tbody>
</table>

Values are means ± SE, in mV, with number of cells in parentheses. ASW, artificial seawater.
the moderate one. The average increase did not vary as a function of training condition \( F(2,10) < 1.0, \) not significant. The increases were greater during the 1st min or so of light [main effect of time: \( F(5,50) = 14.59, P < 0.0001 \) ]. There were no significant differences due to training in average light responses of this subset of cells to either the bright or moderate light intensities [ \( F(2,10) < 1.0, \) not significant] in low-calcium ASW. Thus, although exposure to reduced concentrations of extracellular calcium increased the light response of type A photoreceptors and abolished training-associated differences in their magnitude, the latter effect did not arise because of saturation in the cells’ responses to light. Regardless of training history, cells in low-calcium ASW exhibited light responses that were a graded function of intensity.

**Voltage-clamp results**

In \( \sim 20\% \) of type A cells, particularly those with residual impulse activity, the relatively large outward currents elicited by depolarizing command steps were preceded by small (~2- to 5-nA) transient inward currents. These inward currents appear to arise from activation of voltage-dependent Na\(^+\) channels in remaining axon hillock regions that survived axotomy, because they were blocked by 10 nM bath-applied concentrations of tetrodotoxin, and their kinetics and amplitudes were greatly reduced by holding potentials (\( V_h \)) more positive than \( -50 \) mV (unpublished observations). Because these currents are likely to arise from extrasomatic regions of the cell and their presence obscured and distorted characterization of the somatic outward currents, cells exhibiting transient inward currents \( > 2.0 \) nA were excluded from study.

As was the case for measures of resting membrane potential, input resistance, and light response magnitudes, there were no conspicuous differences between lateral and medial type A photoreceptors in the average amplitudes or kinetics of any of the ionic currents studied here, with one exception. A calcium-dependent K\(^+\) current \( (I_{K_{Ca}}) \) was slightly increased by conditioning in lateral (but not medial) A cells. Thus, for most statistical comparisons, results from both medial and lateral A cells were pooled. However, because

![Figure 2](image_url)
Frysztak and Crow (1993) reported that light-elicited action potential frequencies of medial and lateral type A cells were differentially affected by conditioning, and several of the K⁺ currents that we measured might play a role in controlling spike frequency, we also present the data separately for each of the two cell types, to allow the reader to assess the extent to which training effects occurred for both cell types. In most cases, the relatively small number of observations on each cell type precluded separate statistical analyses with sufficient power to detect significant differences between conditioning treatments, but qualitative trends should nonetheless be apparent.

SEPARATION OF OUTWARD CURRENTS. The outward currents elicited by depolarizing voltage-clamp steps in dark-adapted type A photoreceptors appear to be the sum of at least three separate potassium conductance systems.

$I_A$. Depolarizing command steps from $V_h$ of −60 or −70 mV to potentials of −35 to 0 mV in standard ASW elicited a transient outward current ($I_A$) that peaked ~4–10 ms after the onset of depolarization (Fig. 3A). With steps to potentials more positive than +5 mV, activation of additional outward currents often obscured and distorted the kinetics of the transient current. Thus $I_A$ was primarily studied over the range of −25–0 mV, with the use of brief depolarizations, where contamination by other outward currents was reduced.

Activation of the transient current was moderately voltage dependent (Fig. 3, A and B). Over the range of steepest voltage dependence (from −20 to −5 mV), the current showed e-fold increases in amplitude per ~16 mV. The transient outward current was largely inactivated at $V_h$s more positive than −35 mV, and showed half-maximal inactivation at about −50 mV (Fig. 3, C and D). There were no obvious differences in either the voltage dependence or half-maximal inactivation value for lateral versus medial A cells from untrained animals.

Accurate determination of $I_A$ activation kinetics was not possible for many cells, because the presence of residual voltage-dependent Na⁺ current obscured the initial part of $I_A$ activation. In some cells, however, where the distorting currents were absent (e.g., Fig. 4A), $t_{ON}$ was measured and was found to be only weakly voltage dependent. $t_{ON}$ For one such cell from an untrained specimen appears in Fig. 4B. $t_{ON}$ Declined by only ~1 ms over the range of −30 to −5 mV. Average data

![Figure 3](http://jn.physiology.org/)

**Fig. 3.** Voltage-dependent activation and inactivation of transient K⁺ current [$I_A$ current] in *Hermissenda* type A photoreceptor. A: representative $I_A$ traces elicited by depolarizing command steps to potentials spanning the range of −35 to +0 mV, in 5-mV increments, from a holding potential ($V_h$) of −70 mV. *Inset*: voltage-clamp protocol. These currents were measured in a lateral type A cell from an untrained preparation. At potentials more positive than −30 mV, the currents peak within 10–15 ms after depolarization and thereafter decline to non-0 steady-state levels. B: $I_A$ current-voltage relation for cell whose data appear in A. Peak current values are plotted. In this cell, $I_A$ is first activated at about −30 mV, and increases in a nonlinear manner with additional membrane depolarization. C: steady-state inactivation of $I_A$ by a $V_h$ of −40 mV. Depolarizations to the same potentials as in A from −40 mV elicit rapidly activating outward currents that were ~20-40% of the amplitude of those elicited from −70 mV. These currents fail to inactivate during sustained depolarization. Current traces are from the same cell as in A. D: steady-state inactivation ($H_A$) of $I_A$ vs. membrane potential. Summary of peak current amplitudes measured at 0 mV from the $V_h$s indicated on the abscissa. $I_A$ was half-inactivated at about −60 mV. See **Results** for description of inactivation ratio.
Activation time constants ($\tau_{on}$) for $I_a$ of type A photoreceptors are weakly voltage dependent, whereas inactivation time constants ($\tau_{off}$) are moderately voltage dependent. A: subtracted current traces for $I_a$ of cell in Fig. 3. Current traces elicited from a $V_h$ of $-40$ mV (Fig. 3C) were subtracted from those elicited from $-70$ mV (Fig. 3A), yielding a family of traces that peaked $\sim 10$ ms after depolarization and declined thereafter to 0. Note the more rapid decline of $I_a$ at the more positive membrane potentials. B: voltage dependence of $\tau_{on}$ for net (Fig. 3A) ($\bullet$) and subtracted (Fig. 4A) ($\circ$) current traces. $\tau_{on}$ declined by only $1 \pm 2$ ms from $-30$ to $-5$ mV, and was unaffected by subtraction of the steady-state current. C: voltage dependence of $\tau_{off}$ for net (Fig. 3A) ($\bullet$) and subtracted (Fig. 4A) ($\triangle$) current traces. For the net currents, $\tau_{off}$ declined in a linear fashion by $\sim 125$ ms from $-30$ to $-5$ mV. For the subtracted currents, the decline in $\tau_{off}$ with membrane depolarization was steeper and nonlinear. The greatest decline occurred from $-25$ to $-15$ mV. At more positive potentials, the declines were comparable with those of the net current, although the time constants were larger.

for four cells from four different untrained preparations (including the cell of Fig. 4, A and B), appear in Fig. 5E and support the conclusion that the activation rate was only weakly voltage dependent. For the average data, $\tau_{on}$ declined from $\sim 6$ ms at $-30$ mV to $\sim 1$ ms at $-5$ mV. However, the variability among cells in activation rates was great, particularly at threshold, and most of the decline in $\tau_{on}$ occurred at potentials at which the current was small and estimation of $\tau_{on}$ was most prone to error ($-30$ to $-20$ mV). $\tau_{on}$ values for each cell were averaged for potentials of $-20$ mV or greater. There were no obvious differences in these values for medial ($2.56 \pm 0.90$ ms, $n = 4$) versus lateral ($2.70 \pm 1.02$ ms, $n = 3$) A cells ($t_1 = 0.10$).

When elicited by depolarization to potentials spanning the range from $-20$ to $-5$ mV, from $V_h$s equal to or more nega-
tive than −60 mV, the transient current declined rapidly after reaching its peak (Fig. 3A). Two approaches to estimating $\tau_{\text{OFF}}$ were tried, with somewhat different results. When estimated from the decline of the net outward current (i.e., the traces of Fig. 3A), without subtraction of the steady-state component of current, $\tau_{\text{OFF}}$ decreased linearly with membrane depolarization (Fig. 4C). When the contributions of delayed outward currents to $I_A$ inactivation were minimized by subtracting the currents elicited from a $V_h$ of −40 mV from the net current (Fig. 4A), $\tau_{\text{OFF}}$ declined in a nonlinear manner with depolarization, with the values at −25 and −20 mV being notably larger with this method of estimation (Fig. 4C). In contrast, estimates of $\tau_{\text{ON}}$ were relatively unaffected by correction for the steady-state components of current (Fig. 4B). Because of the clear contribution of other delayed components of outward current at potentials more positive than 0 mV, it is likely that they also contribute to the net outward current over the range of −20 to −5 mV as well. Therefore the time constants estimated from steady-state corrected currents are likely to represent less contaminated measures of $I_A$ inactivation, and $\tau_{\text{OFF}}$ probably declines in a nonlinear way with depolarization. In contrast to activation rates, inactivation rates were moderately voltage dependent.

The transient outward current was blocked by a 5 mM concentration of 4-AP (not shown) (see Farley et al. 1990). The peak amplitude of the transient current also varied in the manner that would be expected if $K^+$ were the main charge carrier when the extracellular $K^+$ concentration was increased (high-$K^+$ ASW). In these experiments, currents were elicited by depolarizing command steps to +10 mV, to activate the transient conductance, from a $V_h$ of −70 mV, and at the peak of the current the membrane potential was stepped back to various test potentials (from −90 to −15 mV). The ionic current records were obscured during the capacity transient caused by the test step but could be seen after ~5 ms to show a simple exponential decline to some steady-state level that was dependent on the test voltage. An exponential curve was used to back-extrapolate these tail currents to the beginning of the step, and their amplitudes were measured and then leak-corrected. Measurements were first obtained in standard ASW, followed by measurements in high-$K^+$ ASW. Plots of the extrapolated ‘instantaneous’ current versus test voltage relation for individual cells in standard ASW showed that the currents were outward for potentials more positive than −62 mV and inward at more negative voltages. When the extracellular $K^+$ concentration was raised from 10 to 100 mM, the apparent reversal potential changed by ≈47 mV. For three cells, the average reversal potential in 10 mM-$K^+$ ASW was −65 ± 2.1 mV, whereas in 100 mM-$K^+$ ASW it was −18 ± 2.5 mV. The 10-fold increase in external $K^+$ concentration resulted in a change in reversal potential of ≈47 mV, rather than the value of 58 mV expected if the $A$ conductance equilibrium potential obeyed the Nernst relation. This deviation may be due to contamination of $A$ conductance by other conductances carrying inward current (e.g., residual $Na^+$ conductance), or by other ions than $K^+$ carrying charge through $A$-type channels. Our results do not allow us to choose between these. However, the slight deviation from the Nernst relation should not obscure the main point, which is that the transient current is carried predominantly by $K^+$.

Measurements of the peak amplitude of $I_A$ elicited by brief (~50-ms) depolarizations from a $V_h$ of −70 mV in standard ASW (no blockers present) failed to reveal any systematic differences due to training. Representative $I_A$ traces for cells from untrained, associatively trained (Paired), and random-control conditions appear in Fig. 5A. An ANOVA of the average $I_A$ amplitudes over the potential range of −35 to −5 mV (Fig. 5B) indicated no statistically significant effect of training [$F(2,9) < 1.0$]. Nor was the interaction of training condition with membrane potential significant [$F(12,54) < 1.0$]. For those cells in which it could be measured accurately, there were no significant differences in $\tau_{\text{ON}}$ [$F(2,7) < 1.0$] that were associated with training (Fig. 5E). Similarly, $\tau_{\text{OFF}}$ values (corrected for steady-state currents) [$F(2,4) < 1.0$] also failed to differ with conditioning treatment (Fig. 5F).

The failure to measure training-related differences in $I_A$ amplitude or kinetics cannot be attributed to weak behavioral conditioning effects for the subsets of animals that contributed $I_A$ measurements. The average suppression scores for these untrained, associatively trained, and ran-
dom-control animals were 0.51 ± 0.02, 0.27 ± 0.05, and 0.46 ± 0.02, respectively. An ANOVA indicated a significant training effect \( F(2,13) = 11.07, P < 0.002 \). Individual \( t \)-tests indicated that the associatively trained animals were significantly more suppressed than untrained \( (t_p = 10.57, P < 0.001) \) and random-control \( (t_l = 8.96, P < 0.001) \) animals. The latter two groups did not differ. None of the correlations between \( I_h \) amplitudes (measured at 0 mV) and behavioral suppression were significant, within any treatment condition \( (r = 0.21, 0.10, \) and 0.09 for untrained, associatively trained, and random-control conditions; all \( P \) values > 0.30).

\( I_{\text{Delayed}} \): When the contribution of \( I_h \) to the total outward current in standard ASW was reduced by holding the cell at \(-30\) mV, a second, sustained component of outward current \( I_{\text{Delayed}} \) was activated by depolarizations to potentials equal or more positive than \(-20\) mV. This second component of current could also be seen during sustained depolarizations to the same potentials when the cell was held at more negative potentials \((-60\) mV\), although its activation kinetics was obscured by relaxation of \( I_h \) under these conditions. The characteristics of \( I_{\text{Delayed}} \) in cells from untrained preparations were as follows (see also Farley et al. 1990). When elicited from a \( V_h \) of \(-30\) mV, \( I_{\text{Delayed}} \) was apparent as a sustained outward current (Fig. 6A) that developed with a time constant of 1–2 ms (Fig. 6C) and showed little inactivation during maintained depolarizations \((400–2,000\) ms\) to potentials equal or more negative than \(+5\) mV (Fig. 6A). At potentials more positive than \(+5\) mV, \( I_{\text{Delayed}} \) declined slowly in most cells, with \( r_{\text{OFF}} \) values in the range of several hundred milliseconds. Activation of \( I_{\text{Delayed}} \) was voltage dependent. Over the region of steepest voltage dependence \((0 \text{ to } +10\) mV\), the current showed \( e \)-fold changes in amplitude per 11 mV. When elicited from a \( V_h \) of \(-30\) mV, the current was undetectable in the majority of cells at potentials more negative than \(-20\) mV (Fig. 6B). However, as shown ahead, \( I_{\text{Delayed}} \) was partially inactivated at \( V_h = -30\) mV and measurements made from this \( V_h \) probably underestimate the contribution of \( I_{\text{Delayed}} \) to the net outward current in the unclamped cell.

The results of changing the external K\(^+\) concentration indicated that \( I_{\text{Delayed}} \) is carried largely by K\(^+\) ions (not shown). Tenfold increases in external K\(^+\) \((\text{high-K}^+ \text{ ASW})\) decreased the steady-state current amplitude at 10 mV by \( \sim 70\% \) and shifted the reversal potential of the tail currents by \( \sim 53\) mV in the depolarizing direction, as expected for a K\(^+\) current. For three cells, the average reversal potential in 10 mM external K\(^+\) was \(-72 \pm 1.9\) mV; that in 100 mM external K\(^+\) was \(-19 \pm 2.1\) mV.

The amplitude of \( I_{\text{Delayed}} \), when measured \( \sim 398 \) ms after the command depolarization from \( V_h = -30\) mV, was relatively unaffected by addition of 5 mM 4-AP to the bath (not shown) (see Farley et al. 1990). In some cells, however, a residual transient current occurring within \( \sim 40\) ms after depolarization at potentials more positive than \(+5\) mV was reduced (not shown). It is likely that the occasional appearance of the transient component of current, and its block by 4-AP, reflect residual \( I_h \) that was not completely inactivated at the \( V_h \) of \(-30\) mV, or activation of other high-threshold, rapidly inactivating K\(^+\) channels. Measurements of \( I_{\text{Delayed}} \) in 4-AP from different \( V_h \)s indicated an average half-maximal, steady-state inactivation value of about \(-18\) mV (Fig. 7).

Thus, as noted earlier, measurements of \( I_{\text{Delayed}} \) in standard ASW from \( V_h = -30\) mV, although relatively free of residual \( I_h \), are measurements of partially inactivated currents (\( \sim 75\% \) of full amplitude). When elicited from \( V_h = -60\) mV (close to the resting membrane potential of an undamaged synaptically intact cell), \( I_{\text{Delayed}} \) could be seen to first activate at about \(-30\) mV.

Although \( I_{\text{Delayed}} \) was little affected by 4-AP, it was reduced by 50 mM bath-applied TEA (TEA ASW) (Fig. 8, A–C). This reduction was variable, however, ranging between 50% and 95% in different cells. As shown below, \( I_{\text{Delayed}} \) is a composite current, consisting of a delayed-rectifier-type K\(^+\) current and TEA-resistant calcium-activated K\(^+\) current in addition to residual \( I_h \). The variable block of \( I_{\text{Delayed}} \) by TEA in different cells probably reflects cell-to-cell variability in the relative contribution of these currents to \( I_{\text{Delayed}} \). In unclamped type A cells, TEA greatly enhanced the steady-state light response (Fig. 8, D and E). In five cells an average enhancement of 29 ± 4% was observed. Thus, consistent with the results of activation curves measured from \( V_h \)s close to the resting potential (e.g., Fig. 7A), activation of \( I_{\text{Delayed}} \) is sufficiently great at the potentials reached during the steady-state light responses of A cells (about \(-30 \text{ to } -15\) mV for lights of moderate intensity) for it to contribute significantly to light response magnitude.

When the steady-state outward current \( I_{\text{Delayed}} \) amplitudes were measured in type A photoreceptors from a \( V_h \) of \(-60\) mV in standard ASW (no channel blockers present), we found the current to be \( \sim 200–300\% \) greater in cells from associatively trained animals (Fig. 9). An ANOVA indicated that the training-associated differences were significant [main effect of training condition: \( F(2,14) = 6.38, P < 0.05 \)]. The results from the two control conditions did not differ significantly from one another [\( F(1,8) < 1.0 \)]. The conditioning-produced differences in amplitude were first apparent at \(-20\) mV \((t_l = 2.38, P < 0.05; \text{comparison between associatively trained and random-control animals})\), as well as more positive potentials.

Separate analyses of the results from lateral \((n = 2, 5)\) and medial \((n = 4, 6)\) type A cells from associatively trained \([F(1,5) < 1.0]\) and pooled control animals \([F(1,9) = 2.13, P > 0.19; \text{because of the relatively small n values the data from untrained and random-control animals were combined}]\) failed to reveal any significant differences in amplitude or voltage dependence between the two cell types. Moreover, the amplitude differences between conditioned and control cells were approximately equivalent for lateral and medial cells (Fig. 9, C and D).

To assess the possible significance of these training-associated differences in \( I_{\text{Delayed}} \) for the behavior of intact animals, correlations were computed between \( I_{\text{Delayed}} \) amplitudes at \(+10\) mV and behavioral suppression scores. In computing these correlations, the issue arises as to at what membrane potential(s) the ionic currents should be measured. Although the potential range reached during the steady-state light response of the synaptically isolated, nonspiking cell (about \(-35 \text{ to } -20\) mV in the present study) might seem like a natural choice, it is probably inappropriate, because it severely underestimates the time-averaged potential of synaptically intact A cells that are spiking during the steady-state
EXCITABILITY CHANGES IN HERMISSEND A TYPE A CELLS

FIG. 6. Characteristics of voltage-dependent, sustained outward K⁺ current (I_{Delayed}) in type A photoreceptors. A: representative current traces from a lateral type A photoreceptor from an untrained preparation. Currents were elicited by depolarizing command steps to potentials ranging from −25 to +15 mV, in 5-mV increments, from a V_h of −30 mV. Note rapid activation of the currents and lack of any appreciable inactivation. B: I_{Delayed} current-voltage plot for cell in A. Currents were measured 398 ms after onset of depolarization. For this cell, significant activation of I_{Delayed} was first apparent at −10 mV. C: rate of activation of I_{Delayed} is weakly voltage dependent. τ_{On} Declines slightly over the range of −20–0 mV, but is essentially independent of membrane potential at more positive voltages.
light response in behaving animals. As the following indicates, current values measured at membrane potentials ranging between 0 and +10 mV are physiologically more meaningful, and thus were used in computing the correlations.

The action potential frequencies of A cells during their steady-state light response (i.e., 25 s or so after light onset) have been found to range between 0.90 and 7.4 Hz (Farley and Alkon 1982; Farley and Jin 1997; Frysztak and Crow 1993), depending on training history, cell type, and light intensity. Thus the somatic membrane potential of synaptically intact A cells passively invaded by action potentials of 35–45 mV (Alkon and Fuortes 1972; Farley and Alkon 1982) at frequencies of 1–7 Hz fluctuates between a lower limit of about –30 mV (the value due to the generator potential alone, in the absence of impulse activity) and +5 to +15 mV (at the peak of the action potential). Because the action potential duration of A cells (recorded from the soma at room temperature) is ~4–5 ms (time to peak is ~2–3 ms), which is 2–3 times the time constant for activation of the composite outward current ($\tau_{on}$; Fig. 4), the outward current will be activated at a potential of ~10 mV during each impulse. The membrane time constant of the unclamped cell (~120 ms) is approximately equal to the average interspike interval for A cells responding to light, as well as to the deactivation time constant (~110 ms) for the composite outward current. Thus the sustained K$^+$ conductance elicited during each action potential (from about +10 mV) in A cells firing action potentials at ~8 Hz would be expected to decay little during the interspike interval.

Correlations between $I_{delayed}$ amplitudes at +10 mV and behavioral suppression scores revealed a strong, statistically significant negative correlation for associatively trained ani-
External tetraethylammonium (TEA) blocks $I_{\text{Delayed}}$. Current traces for $I_{\text{Delayed}}$ in the presence (B) and absence (A) of 50 mM TEA. $I_{\text{Delayed}}$ in this cell showed some inactivation at potentials more positive than $+5 \text{ mV}$. Currents elicited by depolarizing command steps from a $V_h$ of $-30 \text{ mV}$. C: current-voltage plot for $I_{\text{Delayed}}$ depicting inhibition of the current by TEA. Currents were measured 398 ms after depolarization. The inhibition observed in this experiment was particularly strong. D and E: TEA bath addition greatly enhanced steady-state light response of type A cells. Representative light responses of a medial A cell before (D) and after (E) bath addition of 50 mM TEA. TEA addition (E) increased the peak and steady-state component of the light response, presumably through reduction of $I_{\text{Delayed}}$ (B). Cell was current clamped at its original resting potential after TEA addition. Similar results observed in 4 other cells.
FIG. 9. Pairings of light and rotation increase $I_{\text{Delayed}}$. 

A: representative voltage-clamp traces of currents elicited by 400-ms depolarizations of medial type A photoreceptors from preparations exposed to indicated training conditions. Depolarizations were to membrane potentials spanning the range from $-25$ to $+10$ mV, in 5-mV increments, from a $V_h$ of $-60$ mV. Note larger sustained outward currents in cell from associatively trained (Paired) preparation. 

B–D: summary current-voltage plots for $I_{\text{Delayed}}$. Each data point represents the average current amplitude for cells from the indicated training condition. In B, the data for all cells have been pooled together. In C and D, the data for medial (C) and lateral (D) cells are presented separately. $I_{\text{Delayed}}$ was greater in type A cells from paired animals at all potentials more positive than $-10$ mV ($n = 6, 7,$ and 4 for untrained, paired, and random conditions, combining medial and lateral cells). The training-produced differences were similar for medial ($C: n = 4, 4,$ and 2) and lateral ($D: n = 2, 3,$ and 2) cells. Error bars: mean $\pm$ SE.

$V_h = -30$ mV) and behavioral suppression scores were as follows: $r = 0.39$ for untrained animals ($P > 0.29$), $r = -0.58$ for associatively trained animals ($P < 0.04$), and $r = -0.49$ for random controls ($P > 0.50$). The average behavioral suppression scores were $0.47 \pm 0.01$ for untrained animals, $0.29 \pm 0.04$ for associatively trained animals, and $0.44 \pm 0.04$ for random controls. An ANOVA of these data indicated a significant training effect [$F(2,32) = 9.25, P < 0.001$], with the associatively trained animals exhibiting significantly more suppression than untrained ($t_{28} = 3.90, P < 0.01$), and random ($t_{21} = 2.14, P < 0.05$) controls. The two latter groups failed to differ significantly ($t_{15} = 0.81$).

When the contribution of $I_A$ and any other 4-AP–sensitive currents to $I_{\text{Delayed}}$ were further minimized by switching to 4-AP ASW, the training-associated differences in steady-state currents elicited from $-30$ mV were once again appar-
FIG. 10. Pairing-specific enhancement of $I_{\text{delayed}}$ when $I_A$ has been reduced. A: representative voltage-clamp traces of currents elicited by 400-ms depolarizations of cells from preparations exposed to indicated training conditions. Depolarizations were to membrane potentials spanning the range from $-25$ to $+10$ mV, in 5-mV increments, from a $V_h$ of $-30$ mV, which inactivated $I_A$. Note larger sustained outward currents in cell from paired preparation. B–D: summary current-voltage plots for $I_{\text{delayed}}$. Each data point represents the average current amplitude for cells from the indicated training condition. In B, the data for all cells have been pooled together. In C and D, the data for medial (C) and lateral (D) cells are presented separately. $I_{\text{delayed}}$ was greater in type A cells from paired animals at all potentials more positive than $-15$ mV ($n = 12, 17, \text{ and } 5$ for untrained, paired, and random conditions, combining medial and lateral cells). The training-produced differences were similar for medial (C: $n = 9, 10, \text{ and } 3$) and lateral (D: $n = 3, 7, \text{ and } 2$) cells. Error bars: mean ± SE.

ANOVA indicated that the currents were significantly greater in cells from associatively trained versus control animals [$F(2,10) = 8.89, P < 0.006$]. As before, the results from the two control conditions did not differ significantly from one another. Separate analyses of lateral versus medial A cells indicated no significant differences in amplitude between the two cell types within any of the behavioral conditions. The training-associated differences were similar for the two cell types.

Correlations between $I_{\text{delayed}}$ magnitudes in 4-AP at $+10$
mV and behavioral suppression scores were as follows: \( r = -0.23 \) for untrained animals \((P > 0.66)\), \( r = -0.89 \) for associatively trained animals \((P < 0.05)\), and \( r = -0.37 \) for random controls \((P > 0.62)\). The average behavioral suppression scores were \(0.47 \pm 0.02\) for untrained animals, \(0.24 \pm 0.03\) for associatively trained animals, and \(0.43 \pm 0.03\) for random controls. An ANOVA of these data indicated a significant training effect \([F(2,12) = 22.16, P < 0.001]\), with the associatively trained animals exhibiting significantly more suppression than untrained \((t_0 = 6.58, P < 0.01)\) and random \((t_0 = 4.20, P < 0.01)\) controls. The two latter groups failed to differ significantly \((t_0 = 1.29, \text{not significant})\).

\( I_{k,ca} \). In addition to residual \( I_h \), \( I_{delayed} \) consists of at least two separable components: 1) a rapidly activating but sustained voltage-dependent \(K^+\) current \((I_{k,v})\) and 2) a slow calcium-dependent \(K^+\) current \((I_{k,ca})\) (Farley et al. 1990). \( I_{k,ca} \) can be isolated from \( I_h \) and \( I_{k,v} \) by blocking the former current with 5 mM 4-AP and the latter with 50 mM bath-applied TEA. Under these conditions \((4-AP + TEA ASW)\), in the presence of normal concentrations of extracellular calcium \((10 \text{ mM})\), \( I_{k,ca} \) increases slowly during prolonged depolarizations and shows little or no inactivation (Fig. 11). Single-exponential functions were found to provide better fits of the rising portions of \( I_{k,ca} \) traces than power functions \((n = 1-4)\). And \( \tau_{ON} \) was found to be only weakly voltage dependent, decreasing with membrane depolarization over the range from \(-20\) to \(-5\) mV. At more positive potentials, \( \tau_{ON} \) declined further only slightly (Fig. 11, B and C).

The amplitude of \( I_{k,ca} \) varied in the expected manner with changes in both extracellular and intracellular \(Ca^{2+}\). Increasing extracellular calcium from 10 to 30 mM \((\text{high-calcium ASW})\) consistently increased the current by 100–200% \((\text{not shown})\). In contrast, reducing extracellular calcium from 10 mM to \(1 \mu M\) by extensive wash with an ASW containing \(<1 \mu M \text{Ca}^{2+}\) and 30 mM EGTA \((4-AP + \text{TEA + low-calcium/EGTA ASW})\) typically reduced the TEA- and 4-AP-resistant current by only 30–55% \((n = 5 \text{ cells})\). Occasionally \((n = 2 \text{ cells})\) suppression was \(\geq 80\%\). In ASW containing 10 mM \text{Ca}^{2+} \((4-AP + \text{TEA ASW})\), the TEA- and 4-AP-resistant current could also be blocked \((75–90\%)\) by switching to a solution \((\text{Ca}^{2+}/\text{ASW})\) containing 2 mM \text{Co}^{2+}, an inorganic blocker of voltage-dependent \(Ca^{2+}\) channels \((\text{not shown})\). With 10 mM calcium present in the bath, iontophoresis of EGTA into type A cells also invariably reduced the TEA- and 4-AP-resistant current \((\text{Fig. 12})\) by an average of \(\geq 50\%\) \((\text{see also Fig. 14 in Farley et al. 1990})\). Collectively, the results of these manipulations of both extracellular and intracellular calcium are consistent with the slow, TEA-resistant current requiring increases in intracellular calcium for activation. The moderate \((30–55\%)\) but incomplete suppression of the currents by reduction of extracellular calcium to \(1 \mu M\) may arise from some combination of a small dissociation constant of \(I_{k,ca}\) channels for calcium and/or residual calcium influx. The generally greater block of \(I_{k,ca}\) by extracellular \(\text{Co}^{2+}\) is consistent with this. Alternatively, exposing the nervous system to low external calcium may stimulate release of intracellular calcium and/or affect calcium pumps, with the result that basal intracellular calcium levels increase above normal physiological levels.

When \( I_{k,ca} \) was elicited from a \(V_h \) of \(-60\) mV in 4-AP + TEA ASW, and the effects of the learning experience were assessed for the pooled data from medial and lateral A cells, we found no significant differences due to conditioning \((\text{Fig. 13})\). ANOVA indicated a nonsignificant effect of training \([F(2,10) < 1.0, \text{not significant}]\) and a nonsignificant interaction of test potential and training \([F(16,80) < 1.0]\). Thus it appears unlikely that the training-associated increase in \( I_{delayed} \) was due to enhancement of \(I_{k,ca}\). Separate analyses of the results for the two cell types failed to reveal significant training-associated differences in \(I_{k,ca}\) for either lateral \([F(1,8) = 1.88, P > 0.22]\) or medial \([F(1,8) < 1.0, \text{not significant}]\) A cells \((\text{Fig. 13, C and D})\), although there was a suggestive trend for currents of lateral A cells from trained animals to be larger than those of controls \((\text{Fig. 13D})\).

None of the correlations between \(I_{k,ca}\) magnitudes at +10 mV and behavioral suppression scores were significant: \(r = 0.22\) for untrained animals \((P > 0.68)\), \(r = 0.51\) for associatively trained animals \((P > 0.49)\), and \(r = 0.01\) for random controls \((P > 0.98)\). The average behavioral suppression scores were \(0.46 \pm 0.02\) for untrained animals, \(0.25 \pm 0.03\) for associatively trained animals, and \(0.45 \pm 0.03\) for random controls. An ANOVA of these data indicated a significant training effect \([F(2,16) = 15.44, P < 0.001]\), with the associatively trained animals exhibiting significantly more suppression than untrained \((t_{11} = 4.62, P < 0.001)\) and random controls \((t_{11} = 3.17, P < 0.01)\). The two latter groups failed to differ significantly \((t_8 < 1.0, \text{not significant})\).

\( I_{k,v} \). A third component of \(I_{delayed}\) elicited by depolarizations to potentials more positive than \(-15\) mV, distinct from \(I_h\) and \(I_{k,ca}\), was also isolated. After reduction of \(I_h\) by 4-AP and \(I_{k,ca}\) by lowering extracellular calcium to \(<1 \mu M (4-AP + \text{low-calcium/EGTA ASW})\) and iontophoretic injection of EGTA, a voltage-dependent outward current was apparent \((\text{Fig. 14B})\), with values of \(\tau_{ON}\) ranging from 1 to 3 ms over the range of \(-10\) to \(+5\) mV. The current inactivated only slightly over this same potential range. Changing the external solution to one that also contained 10 mM TEA \((4-AP + \text{TEA + low-calcium/EGTA ASW})\) reduced this current by \(\geq 90\%\) \((\text{Fig. 14C})\). Increasing the external \(K^+\) concentration from 10 mM \((4-AP + \text{low-calcium/EGTA ASW})\) to 100 mM \((4-AP + \text{low-calcium/EGTA + high-K$^+$ ASW})\) decreased this current in the manner expected for \(K^+\)-selective channels \((\text{not shown})\). Thus we have termed this current \(I_{k,v}\) to emphasize that it is a voltage-dependent \(K^+\) current that apparently is not dependent on increases in intracellular calcium for its activation.

\( I_{k,v} \) could also be isolated without EGTA injection, by holding the cell at \(-30\) mV \((\text{to inactivate} I_h \text{ and} I_{k,ca})\) and reducing extracellular calcium to \(<1 \mu M\) \((\text{low-calcium/EGTA ASW})\). Comparison of current traces in standard ASW versus low-calcium/EGTA ASW indicated that reduction of external calcium to \(\equiv 1 \mu M\) had little or no effect on the amplitude of this current for up to \(8–9\) min after the solution change, in contrast to the results for \(I_{k,ca}\) \((\text{see above})\), which clearly decreased over this same time period.
FIG. 11. Characteristics of slow, TEA-sensitive calcium-activated $K^+$ current ($I_{K-Ca}$) in type A photoreceptors. A: representative current traces from a lateral type A photoreceptor from an untrained preparation. Currents were elicited by depolarizing command steps to potentials ranging from $-25$ to $+20$ mV, in 5-mV increments, from a $V_h$ of $-60$ mV. Note slow activation of the currents and lack of any appreciable inactivation. B: $I_{K-Ca}$ current-voltage plot for cell in A. Currents were measured 398 ms after onset of depolarization. For this cell, significant activation of $I_{K-Ca}$ was first apparent at $-20$ mV. C: rate of activation of $I_{K-Ca}$ is moderately voltage dependent. $\tau_{on}$ Declines by $\sim 50$ ms over the range of $-20$ to $-5$ mV, but is essentially independent of membrane potential at more positive voltages.

In low-calcium/EGTA ASW, $I_{K-Ca}$ first activated at about $-20$ mV (Fig. 15, A and B), showed a moderate rate of activation that was moderately dependent on membrane potential (Fig. 15C), and showed slow-to-nonexistent inactivation over the range of $-20$ to $+5$ mV. At potentials more positive than $+5$ mV, moderate rates of inactivation were observed (Fig. 15, A and D). Changing the solution to one that also contained 10 mM TEA (low-calcium/EGTA + TEA ASW)
FIG. 12. Reduction of $I_{K\text{-Ca}}$ by injection of ethyleneglycolbis(aminooethyl)ether-tetra-acetate (EGTA) into a type A photoreceptor. A: currents elicited by depolarizing command steps spanning the range from −15 to +20 mV (in 5-mV increments), in 10 mM calcium, TEA, and 4-aminopyridine (4-AP) ASW, before EGTA injection. B: reduction of currents measured ∼5 min after EGTA injection at same membrane potentials. $I_{K\text{-Ca}}$ was suppressed by ≥90%. C: summary current-voltage plot depicting average reduction of $I_{K\text{-Ca}}$ by injection of EGTA into cells (n = 4).
FIG. 13. $I_{K\text{-Ca}}$ is not selectively affected by associative training. A: representative voltage-clamp traces of currents elicited by long-duration (400-ms) depolarizations of type A photoreceptors from preparations exposed to indicated training conditions. Currents elicited by depolarizing command steps spanning the range from $-25$ to $+15$ mV (in 5-mV increments). $V_h$ was $-60$ mV. There were no conspicuous differences in amplitudes or kinetics as a function of training. B–D: summary current-voltage plots for $I_{K\text{-Ca}}$. Each data point represents the average current for cells from the indicated training condition. In B, the data for all cells have been pooled together. In C and D: the data for medial (C) and lateral (D) cells are presented separately. $I_{K\text{-Ca}}$ did not differ significantly as a function of training condition ($n = 7, 10, \text{ and } 4$ for untrained, paired, and random conditions, combined data for medial and lateral A cells). There were no significant training-produced differences for medial (C: $n = 4, 6, \text{ and } 2$) or lateral (D: $n = 3, 4, \text{ and } 2$) A cells. Error bars: mean ± SE.

Reduced this current by $\sim 75–90\%$ (Fig. 15, E and F). Beginning some 10 min after the solution change from standard ASW to low-calcium/EGTA ASW, an increase in resting membrane conductance and an increase in the currents elicited by depolarization was often ($\sim 50–67\%$ of the time) observed (not shown). Thus, although activation of $I_{K\text{-Ca}}$ does not apparently depend on increases in intracellular calcium, it does appear to be modulated by external calcium.

When the amplitude of $I_{K\text{-Ca}}$ was measured in low-calcium/EGTA ASW for cells from preparations exposed to the different behavioral training conditions, $I_{K\text{-Ca}}$ was $\sim 60–150\%$ greater in cells from associatively trained animals (Fig. 16, A and B) [$F(2,9) = 5.98, P < 0.025$]. A significant difference in current amplitudes was apparent at the most negative membrane potential tested ($-15$ mV) ($t_9 = 2.28, P < 0.05; \text{ paired vs. random}$). There were no significant differences in current amplitudes between untrained and random-control cells. Most of the enhancement of $I_{K\text{-Ca}}$ by light-rotation pair-
FIG. 14. Isolation of delayed-rectifier-type, voltage-dependent K$^+$ current ($I_{K,v}$) in type A photoreceptor. A: currents elicited by depolarizing command steps spanning the range from −25 to +15 mV (in 5-mV increments) in normal ASW from −60 mV. Both transient and sustained components are apparent. B: rapidly activating, voltage-dependent, sustained outward currents ($I_{K,v}$) measured 5 min after addition of 4-AP (5 mM) to the bath, reduction of extracellular calcium to <1 mM, and injection of EGTA into the cell. C: $I_{K,v}$ is blocked by addition of 10 mM TEA to the bath.

ings can be explained by an apparent ~15- to 20-mV shift in the voltage dependence of the current toward more negative potentials (Fig. 16B). Extrapolation from the current-voltage relationships for cells from paired animals suggests an amplitude of ~0.5–1.0 nA for $I_{K,v}$ at −25 mV. There were no significant differences in $\tau_{ON}$ (Fig. 16E) or $\tau_{OFF}$ (Fig. 16F) due to training. Addition of TEA (low-calcium/EGTA + TEA ASW) suppressed the currents, regardless of conditioning history, and abolished any differences due to training (not shown).

Analysis of the results from lateral ($n = 3, 3$) and medial ($n = 3, 4$) type A cells from associatively trained [$F(1,4) = 0.92, P > 0.37$] or pooled control animals [$F(1,4) = 0.13, P > 0.72$] failed to reveal any significant differences in amplitude or voltage dependence between the two cell types (not shown). The training-associated differences in $I_{K,v}$ amplitude were approximately equivalent for the two cell types.

Correlations between $I_{K,v}$ magnitudes at +10 mV and behavioral suppression scores were as follows: $r = 0.30$ for untrained animals ($P > 0.26$), $r = −0.93$ for associatively trained animals ($P < 0.03$), and $r = 0.59$ for random controls ($P > 0.28$). Correlations computed at 0 mV were as follows: $r = 0.65$ for untrained animals ($P > 0.26$), $r = −0.93$ for associatively trained animals ($P < 0.03$), and $r = 0.59$ for random controls ($P > 0.28$). The average behavioral suppression scores were 0.50 ± 0.04 for untrained animals, 0.24 ± 0.02 for associatively trained animals, and 0.47 ± 0.02 for random controls. An ANOVA of these data indicated a significant training effect [$F(2,9) = 28.3, P < 0.001$], with the associatively trained animals exhibiting significantly more suppression than untrained ($t_5 = 6.35, P < 0.01$) and random ($t_5 = 8.14, P < 0.01$) controls. The two latter groups failed to differ significantly ($t_5 < 1.0$, not significant).

DISCUSSION

Changes in light responses and resting membrane excitability

Type A photoreceptors from conditioned animals exhibited smaller steady-state light responses, which were correlated with the degree of phototactic suppression, and smaller
FIG. 15. Characteristics of $I_{K,v}$ in type A photoreceptor. A: currents elicited by depolarizing command steps spanning the range from $-25$ to $+20$ mV (in 5-mV increments) from a lateral type A photoreceptor of an untrained preparation, from a $V_h$ of $-30$ mV, in $\sim 1$ mM calcium ASW. B: current-voltage plot of traces in A. C: rate of activation of $I_{K,v}$ is moderately voltage dependent. $\tau_{on}$ declined by $\sim 1$ ms per 5 mV of depolarization for the cell in A. D: rate of inactivation of $I_{K,v}$ was also voltage dependent. At potentials $>5$ mV, the currents showed small relaxations during sustained depolarizations. Note log scale for ordinate. E and F: $I_{K,v}$ is decreased by 10 mM external TEA. Current traces elicited by depolarizations spanning the potential range from $-25$ to 10 mV, in 5-mV increments, before (E) and after (F) addition of TEA to the bath.

resting input resistances than those of control preparations. These differences were apparent for $\leq 2$ days after training, the longest retention interval examined. Because these excitability differences were recorded in synaptically isolated cells, they are indicative of training-associated changes in one or more ionic conductance systems within the somatic membrane of type A photoreceptors. For animals that received the same conditioning treatment, there were no conspicuous differences in light responses, resting membrane potential, or input resistance between lateral and medial type A cells.

The conditioning-produced differences in light responses of type A cells were markedly attenuated when cells were exposed to a low-calcium ASW. Reducing extracellular calcium enhanced the light response of type A photoreceptors, regardless of conditioning history. This is unlikely to have occurred because of saturation of the cells’ light responses, because all cells responded to a brighter light with a larger
Fig. 16. Pairings of light and rotation enhance $I_{K,v}$. A: currents elicited by depolarizing command steps spanning the range from $-15$ to $+20$ mV (in 5-mV increments) from medial type A photoreceptors of preparations exposed to indicated training conditions, 24 h after training. Note larger currents from associatively trained (Paired) animal. Currents elicited by depolarizations to indicated potentials, from a $V_h$ of $-30$ mV, in low-calcium ASW. B–D: summary current-voltage plots for $I_{K,v}$ measured under present conditions. In B, each data point represents the average current from 4 (Untrained), 6 (Paired), and 3 (Random) cells for the respective training condition. $I_{K,v}$ was greater in cells from paired animals at all potentials more positive than $-15$ mV. In C and D, the results for medial and lateral cells are presented separately. D and E: summary $\tau_{on}$ (E) and $\tau_{off}$ (F) data for cells whose data appear in B. There were no significant differences as a function of training in the rates of either activation or inactivation. Error bars: mean ± SE.
receptor potential. A consideration of mechanisms that might account for the calcium dependence of expression of the training-produced changes in the type A photoreceptors’ light response is deferred until the voltage-clamp results have been discussed.

Changes in sustained, outward K+ current

A major finding of the present study was that pairings of light and rotation produced a two- to threefold enhancement of sustained outward K+ current (\(I_{\text{Delayed}}\)). Enhancement of \(I_{\text{Delayed}}\) would be expected to contribute to the training-produced reductions in the light response of sympathetically isolated type A cells by increasing the ratio of outward to inward current during the cell’s plateau response to light. Although \(I_{\text{Delayed}}\) is small over the potential range typically reached during an A cell’s steady-state light response (range of \(-30\) to \(-15\) mV), our results indicate that even small changes in the absolute size of \(I_{\text{Delayed}}\) can produce substantial changes in its relative contribution to total membrane current. Our observation that the A cells’ light responses were enhanced by TEA inhibition of \(I_{\text{Delayed}}\) demonstrated that \(I_{\text{Delayed}}\) is an important determinant of the light response amplitude. Voltage-clamp analysis indicated that the major ionic currents of type A cells that are activated (at steady state) during a light step are 1) light-activated sodium current (\(I_{\text{Na-light}}\), typically \(-1.0\) to \(-2.0\) nA for untrained cells at \(-30\) to \(-15\) mV) and 2) \(I_{\text{Delayed}}\) (\(-0.5\) to \(-1.5\) nA in untrained cells at \(-30\) to \(-15\) mV). Thus \(I_{\text{Delayed}}\) constitutes \(20\%\) to \(60\%\) of the net current, and increases in \(I_{\text{Delayed}}\) would be expected to decrease the light response of type A cells, consistent with the effects of associative training observed here.

\(I_{\text{Delayed}}\) is a composite current that consists of residual \(I_{\text{A}}\), voltage-dependent K+ current (\(I_{\text{K-v}}\)), and a slow, TEA-resistant, calcium-activated K+ current (\(I_{\text{K-Ca}}\)). An inward voltage-dependent calcium current, masked by the outward K+ currents and not yet carefully characterized, is also activated during elicitation of \(I_{\text{Delayed}}\). Which of the above components of \(I_{\text{Delayed}}\) are changed by associative training?

Three observations establish that a change in \(I_{\text{K-v}}\) is the most likely possibility.

1) The pairing-specific enhancement of \(I_{\text{Delayed}}\) was observed when \(I_{\text{A}}\) was minimized, either by holding the cell at \(-30\) mV or by pharmacological block with 4-AP. These observations, coupled with the failure to observe any training-associated changes in \(I_{\text{A}}\) amplitude or kinetics, argue against the difference in \(I_{\text{Delayed}}\) being due to differences in residual \(I_{\text{A}}\).

2) The enhancement of \(I_{\text{Delayed}}\) by conditioning was also apparent when \(I_{\text{Delayed}}\) was measured in low-calcium/EGTA ASW, from a \(V_h\) of \(-30\) mV. These conditions would be expected to reduce the contributions of \(I_{\text{A}}\), \(I_{\text{K-Ca}}\), and any calcium (or other calcium-activated) currents to the net outward current. Isolation and measurement of \(I_{\text{K-Ca}}\) failed to reveal clear, training-associated differences, although we cannot at present rule out the possibility that \(I_{\text{K-Ca}}\) may have been slightly increased in lateral A cells from paired animals. However, contrary to our original speculation (Farley et al. 1990), changes in \(I_{\text{K-Ca}}\) do not appear to be of primary importance for the expression of conditioning-related excitability changes in type A photoreceptors. The training-produced enhancement of \(I_{\text{Delayed}}\) does not appear to be primarily due to an increase in \(I_{\text{K-Ca}}\).

3) Isolation and measurement of \(I_{\text{K-v}}\) indicated that it was selectively enhanced in both lateral and medial A cells from conditioned animals. In addition, blocking \(I_{\text{K-v}}\) with TEA greatly suppressed \(I_{\text{Delayed}}\) and abolished training-associated differences in the remaining current.

Although \(I_{\text{K-v}}\) was selectively enhanced by light-rotation pairings and no doubt contributed to the training-associated increases in the composite current (\(I_{\text{Delayed}}\)), it may not have been the only current changed by training. It is possible that an additional, not yet isolated or characterized component of outward current in A cells is increased by conditioning (or an inward current is decreased). This current may also be reduced by the manipulations used to isolate \(I_{\text{K-v}}\) (4-AP and/or \(V_h\) = \(-30\) mV). Alternatively, these manipulations might modify \(I_{\text{K-v}}\) channels (e.g., induce steady-state inactivation, shift the voltage dependence) and either mask or interfere with full expression of training-produced differences.

Calcium dependence of training-associated differences in light response

The mechanisms responsible for the calcium dependence of expression of the training-produced changes in the type A cells’ light response have not yet been conclusively determined. Several possibilities merit careful consideration, and they may be of different degrees of importance for lateral versus medial type A cells. First, one or more ionic currents changed by training may be activated by calcium. Reduction of such a current by lowering extracellular Ca2+ concentration ([Ca2+]o) would then minimize one of the primary effects of training, and thus reduce differences in light responses. Such a process might make a minor contribution to the reduction in training-associated differences in light responses of lateral A cells in low-calcium ASW, because small training-associated differences in \(I_{\text{K-Ca}}\) were observed in these cells. This explanation is considerably less likely for the similar effects observed in medial A cells. Here, we failed to observe any training-associated changes in \(I_{\text{K-Ca}}\), yet reduction of [Ca2+]o still enhanced the light response of these cells, regardless of conditioning history, and greatly reduced (although it did not completely abolish) the training-associated differences apparent in normal ASW.

However, we cannot exclude the possibility that activation of \(I_{\text{K-Ca}}\) by light (which is mediated in part by light-induced release of intracellular calcium) might differ in some critical way from activation by influx of calcium through voltage-gated calcium channels, and that our means of activating \(I_{\text{K-Ca}}\) here (by membrane depolarization) fails to duplicate some essential gating requirement. Perhaps, for example, light activation of \(I_{\text{K-Ca}}\) involves calcium pools that are released by the cooperative action of inositol 1,4,5-triphosphate (IP3) and calcium entering through plasmamembrane channels.

In a similar vein, we also cannot exclude the possibility that other faster, TEA-sensitive, calcium-dependent K+ channels might also be present in both lateral and medial A cells (e.g., ICF channels in Drosophila flight muscle and neurons) (Elkins et al. 1986; Salkoff 1983), and these may
be modulated by conditioning. Activation of such channels would be expected to overlap with that of $I_{K,v}$ channels (Singh and Wu 1989) and thus might contribute to $I_{delayed}$. However, such channels would be expected to be inhibited (by TEA) during isolation of slow $I_{K,Ca}$ in A cells, and thus would not contribute much to the macroscopic currents we measured.

An additional complication is that $I_{Kv}$ may also be dependent on $[Ca^{2+}]_o$. Ten minutes or more after reduction of $[Ca^{2+}]_o$ to low levels, we often observed that type A cells became quite “leaky.” The holding current and resting membrane conductance increased. These observations are reminiscent of those reported by Armstrong and Lopez-Barneo (1987) for voltage-dependent K’ channels of squid neurons. Those researchers suggested that extracellular calcium is necessary for these channels to close and to retain their selectivity for K’ (see also Kostyuk and Martynyuk 1988). If $I_{Kv}$ channels in *Hermissenda* A cells are subject to similar regulation, reduction of $[Ca^{2+}]_o$ may transform $I_{Kv}$ from an outward to an inward current, enhance the light response, and thereby reduce training-produced differences in amplitude.

Finally, reduction of $[Ca^{2+}]_o$ may increase the light-induced Na’ current in *Hermissenda* photoreceptors. In many invertebrate photoreceptors, light-induced increases in intracellular calcium plays an important role in mediating light adaptation (Bacigalupo et al. 1990; Brown and Lisman 1975). If calcium played the same role in A cells, then an additional consequence of reducing extracellular calcium (all else being equal) would be a slowing and reduction in the inactivation of $I_{Na-light}$, resulting in a larger ratio of inward to outward current, and a possible masking of differences in light responses due to training-associated differences in the outward currents.

The above factors, alone or in combination, may explain an otherwise puzzling dissociation. Bathing the A cells in low-Ca’ solution greatly reduced training-associated differences in light responses, yet failed to abolish the training-associated differences in $I_{Kv}$. This seems to suggest that the differences in $I_{Kv}$ cannot explain the differences in the light responses, because the latter are demonstrably dependent on external calcium whereas the former are not. Two considerations merit emphasis, however. For several reasons discussed above, prolonged exposure to low external calcium may transform the photoresponses in such a way that differences in $I_{Kv}$ now constitute a relatively minor component of total membrane current during the photoresponse. Also, the differences in $I_{Kv}$ may be necessary for expression of differences in A cell light responses, but not sufficient. A precedent for this possibility has previously been observed for the type B photoreceptors of *Hermissenda*. For type B cells, associative training increases the photoresponse, and this is accompanied by reduction of $I_{A}$ (Alkon et al. 1982, 1985) and $I_{K,Ca}$ (Farley 1988), with the maximal A conductance being reduced by ~30% and the calcium-activated K’ conductance being reduced by ~50–60%. Although $I_{A}$ is clearly reduced by conditioning, eliminating its contribution to total membrane current (by 4-AP or depolarization) exaggerated rather than diminished the training-associated difference in light responses (Farley 1988). This observation has subsequently been explained by the recognition that complete elimination of $I_{A}$ results in greater than normal light-induced depolarization of B cells, at which level conditioning-produced differences in $I_{K,Ca}$ are greatest. A Hodgkin-Huxley type model of B cell membrane excitability that incorporated $I_{A}$, $I_{K,Ca}$, and five other distinct ionic currents that have been characterized in these cells (Koide and Farley 1990) indicated that although reductions in $I_{A}$ alone had a negligible effect on the steady-state light response of B cells (although reductions in $I_{K,Ca}$ alone produced ~50% of the training-associated difference), combined reductions of $I_{A}$ and $I_{K,Ca}$ resulted in enhanced simulated light responses that were ~75% of those produced by conditioning. It is possible that $I_{Kv}$, in type A cells may play a role analogous to $I_{A}$ in type B cells: its reduction may be necessary to allow full expression of training-produced differences in another conductance system. Alternatively, the training-associated reductions in $I_{Kv}$ in A cells may be entirely incidental, with the differences in the light response being accounted for by another, as yet uncharacterized, current.

Functional consequences of changes in type A cell light responses

At present, it is difficult to say precisely what are the consequences of the diminished light responses of type A photoreceptors for the behavior of intact animals. Activity in the lateral A cell has not yet been explicitly linked to phototaxis through identified neural circuitry. In contrast, excitability changes in the medial A cell have been linked to changes in phototactic behavior. The evidence includes demonstrations of polysynaptic excitation of motoneuron number one (MN1) motoneurons by medial A cells (Goh and Alkon 1984) and significant correlations between the medial A cell’s photoresponse amplitude and phototactic suppression (Farley et al. 1990; present report). These results, combined with findings that light-elicted action potential frequencies in both types of A cells vary in the expected way with generator potential amplitude (Farley and Alkon 1982; Frysztak and Crow 1993) and a report that synaptic inhibition of the medial A cell by the medial B cell is selectively enhanced by conditioning (Frysztak and Crow 1994), lead to the expectation that medial A cells from conditioned (vs. control) animals would fire fewer action potentials in response to light (thereby producing less activation of MN1 cells and reduced phototaxis).

Contrary to expectation, Frysztak and Crow (1993) reported no statistically significant training-related differences in medial A cell light-elicted spike frequencies during the initial portion (5–30 s) of a 5-min light step. However, inspection of those data indicates that Frysztak and Crow (1993) (their Table 1) consistently observed smaller spike frequencies (by factors of 23–52%, depending on light intensity) for conditioned versus pseudorandom medial A cells during the last 30 s of a 5-min light step. Thus whether medial type A cells exhibit increased or decreased spike frequencies may depend on their state of light adaptation.

As Frysztak and Crow (1993) also noted, the difference in outcome between their study and our earlier one (Farley and Alkon 1982) may also reflect the importance of increased tonic inhibitory synaptic input on type A cells from caudal hair cells that occurs when preparations are oriented vertically. The vertical orientation appears to magnify ex-
pression of conditioning-related behavioral and neural changes (Farley and Alkon 1982).

Alternatively, it may prove to be the case that conditioning does not produce reliable changes in medial A cell spike frequencies, despite reducing the generator potential. But the changes in generator potential may nonetheless be physiologically relevant. Perhaps, for example, release of excitatory neurotransmitter (or a modulator) at medial A cell–interneuron synapses is not strictly dependent on the occurrence of action potentials, but instead on the degree of presynaptic terminal depolarization, as is the case for glutamate release at vertebrate photoreceptor-bipolar/horizontal cell synapses (Werblin and Dowling 1969). Reductions in A cell light responses might in this way effect reductions in visual activation of locomotor circuitry.

**Dissociation of changes in action potential frequency and receptor potential amplitude**

The cooccurrence of an increase in conditioned-stimulus-elicited spike frequency and a decrease in the light-evoked generator potential of the lateral A cell raises interesting questions concerning possible differences in somatic versus axonal conductance systems within type A cells. This observation also underscores the importance of careful separation of somatic and axonal conductances in future studies and examination of how each is affected by conditioning. Perhaps the training-associated spike frequency increase in the lateral A cell reflects modulation in the gating of axonal Na⁺ channels and/or the presence of a class of axonal K⁺ channels that is affected differently by training than the somatic K⁺ channels.

**Distributed plasticity and learning in Hermisenda**

The fact that type A and B photoresponses change with associative learning in *Hermisenda*, in opposite but apparently complementary fashion, parallels findings from studies of sensitization in *Aplysia* (Frost et al. 1988; Trudeau and Castellucci 1993) and habituation in the crayfish (Krasne and Teshiba 1995). It seems clear that learning-correlated changes in synaptic efficacy and/or neural excitability can occur at multiple sites within neural circuits that mediate behavior. More generally, our results provide some support for theories that emphasize the distributed nature of engrams. An important question to address in future research with *Hermisenda* is whether these multiple changes occur independently and in parallel or serially.

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Address reprint requests to J. Farley.

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