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Post-light Potentiation at Type B to A Photoreceptor Connections in *Hermissenda*

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We investigated whether the long (~30-s) or short (~3-s) light stimuli that have been used during behavioral training would induce post-light potentiation (PLP) at the type B to A photoreceptor connections of the isolated nervous system of *Hermissenda.* We found that a single ~30-s light step induced PLP at these connections relative to both pre-light baseline and seawater control preparations, as did a series of nine short (~3-s) light steps. In addition, a 30-s step of depolarization-elicited type B cell activity induced potentiation comparable to that induced by a ~30-s light step, indicating that light-elicited type B cell activity contributes to the induction of PLP. By contrast, even though a series of short (3-s) light steps induced potentiation, short steps of depolarization-evoked type B cell activity did not. Hence, light-evoked processes other than type B cell depolarization or activity (e.g., perhaps intracellular Ca^{2+} release) also contribute to the induction of PLP. Further results suggest that these other light-evoked processes interact nonadditively with type B cell activity to generate PLP. Some but not all instances of synaptic potentiation were accompanied by various changes in parameters of type B cell action potentials and afterhyperpolarizing potentials, suggesting diverse underlying mechanisms, including increases in neurotransmitter release. Given that the type A cells have been proposed to polysynaptically excite the motor neurons that drive phototaxis, a light-evoked potentiation of synaptic strength at the inhibitory type B to A photoreceptor connections may play a mechanistic role in light-elicited nonassociative learning.

Key Words: post-tetanic potentiation; PTP; nonassociative learning; synaptic plasticity; invertebrate.

The marine mollusk *Hermissenda crassicornis* instinctively locomotes toward a light source. This positive phototactic response is suppressed after classical conditioning with

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a light conditioned stimulus (CS) and a rotation unconditioned stimulus (US) (Crow & Alkon, 1978). Although associative learning has been the most extensively analyzed in this system, nonassociative suppression of phototaxis has been observed in response to training with US-alone presentations (Alkon, 1974; Crow & Alkon, 1978), CS-alone presentations (Crow & Alkon, 1978; Grover, Farley, & Vold, 1987), random CS/US presentations (Crow, 1983; Farley & Alkon, 1980, 1982; Grover et al., 1987), or unpaired US/CS presentations (Crow & Alkon, 1978; Matzel, Lederhendler, & Alkon, 1990; Rogers, Talk, & Matzel, 1994).

Several cellular correlates of learned suppression of phototaxis have been identified. The paired eyes of *Hermissenda* each contain five photoreceptors, three type B and two type A cells. The type B photoreceptors inhibit the type A photoreceptors as well as each other (Alkon & Fuortes, 1972). It has been proposed that the type A cells have excitatory connections with interneurons that in turn excite the motor neurons that mediate phototaxis (Goh & Alkon, 1984). After behavioral conditioning, the type B photoreceptors exhibit enhanced excitability, as manifested by a greater number of spikes elicited by a constant light stimulus, enhanced depolarizing generator potentials, and increased input resistance (Crow & Alkon, 1980; Farley & Alkon, 1982; West, Barnes, & Alkon, 1982). Hence, when type B cells become more excitable, they presumably have a greater inhibitory effect on type A cells, leading to less excitation of the motor neurons and, consequently, reduced phototactic behavior (Goh & Alkon, 1984; Goh, Lederhendler, & Alkon, 1985; Hodgson & Crow, 1992).

Synaptic strength changes at the inhibitory type B to A cell connections are another means by which phototactic behavior can be altered. For instance, synaptic facilitation that occurs at the inhibitory type B to A cell connections after either behavioral training (Frysztak & Crow, 1994) or *in vitro* US-alone manipulations (Clark & Schuman, 1992; Schultz & Clark, 1997a; Schuman & Clark, 1994) may also contribute to learned suppression of phototaxis (Crow & Alkon, 1980; Farley & Alkon, 1982; Frysztak & Crow, 1994; West et al., 1982). Although type B to A cell synaptic transmission is depressed *during* the actual activation of the CS pathway (Schultz, Lee, & Clark, 1998), a light-evoked potentiation of synaptic strength at these connections expressed *after* the light presentations could play a mechanistic role in CS-elicited nonassociative learning. Hence, we investigated whether the long (~30-s) or short (~3-s) light stimuli that have been used during behavioral training would yield post-light potentiation (PLP) at the type B to A cell connections.

Light has several effects on the photoreceptors that could conceivably contribute to PLP induction. It yields depolarizing generator potentials that are accompanied by high-frequency spike discharge (Alkon & Fuortes, 1972; Alkon & Grossman, 1978; Dennis, 1967; Detwiler, 1976). It also triggers voltage-independent processes such as intracellular Ca^{2+} release (Connor & Alkon, 1984; Muzzio, Talk, & Matzel, 1998; Talk & Matzel, 1996), presumably through a phospholipase-C-mediated phototransduction cascade (Talk, Muzzio, & Matzel, 1997) in conjunction with ryanodine receptor activation (Blackwell & Alkon, 1999). The present series of experiments addresses the following related questions: (1) Does light induce PLP at the type B to A photoreceptor connections? (2) If so, what are the critical signals for the induction of PLP? In particular, do type B cell activity (i.e., depolarization and associated action potential discharge) as well as other light-evoked

processes (i.e., intracellular Ca^{2+} release or other second-messenger cascades) each contribute to the induction of PLP at these connections? (3) What are the mechanisms for maintenance and expression of PLP? In particular, does PLP arise in part from changes in action potential parameters in type B cells?

Some of these results have previously appeared in abstract form (Schultz & Clark, 1997b).

EXPERIMENT 1

Our first objective was to determine whether light is capable of promoting PLP at the type B to A cell connections. Given that behavioral experiments have utilized both long (~30-s) and short (~3-s to 5-s) light presentations, we used both types of stimuli in the present series of experiments (Experiments 1 through 3). Experiment 1 addresses the questions of (1) whether a single, ~30-s presentation of light can induce PLP at the type B to A cell connections and (2) whether depolarization-elicited type B cell activity produces a comparable level of potentiation.

Methods

Animals. *H. crassicornis* were obtained from Marinus (Long Beach, CA). Animals were housed individually in perforated 50-mL tubes in a tank of 12°C artificial seawater (ASW). They were maintained on a 12-h dark / 12-h light cycle and fed small pieces of mussel three times weekly. Experiments were conducted during the light phase using animals that ranged in length from 2 to 3 cm. Animals were maintained in the laboratory for 1 to 7 days prior to experiments.

Dissection. After anesthesia by injection of a nominal volume of isotonic $MgCl_2$ into the posterior–ventral surface of the animal, the circumesophageal nervous system (including eyes, statocysts, and the optic, cerebropleural, and pedal ganglia) was removed and pinned to the Sylgard (Dow-Corning, Midland, MI) surface of a 0.6-mL-volume recording chamber. This preparation was incubated for 9–10 min at room temperature with a nonspecific protease (XXVII, 1.5 mg/mL ASW; Sigma, St. Louis, MO) to facilitate subsequent impalement with microelectrodes. Preparations were bathed in 0.5–0.6 mL ASW with a pH of 7.6 and the following composition (in mM): NaCl, 430; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50; Tris·HCl (pH 7.6), 10.

Electrophysiology. Pairs of synaptically connected type B and A photoreceptors were recorded with conventional intracellular recording techniques as previously described (Schultz & Clark, 1997a). Briefly, single action potentials were evoked in the dark-adapted (5–10 min) type B photoreceptor at 30-s intervals via intracellular current injection, and the amplitudes of the resulting inhibitory postsynaptic potentials (IPSPs), the primary dependent variable, were recorded in the type A cell. In addition, as indexes of type B cell action potential parameters, the type B cell action potential duration (from peak to baseline), the peak amplitude of the afterhyperpolarizing potential (AHP), and the area of the initial portion of the AHP (from baseline crossing to 100 ms after action potential peak) were also measured. Action potential peak amplitude was not formally analyzed because, in many preparations, the measurement of the amplitude (but not time) of the

action potential peak was obscured by the capacitive transient at the offset of the depolarizing stimulus used to evoke the action potential. Finally, as a rough index of cellular excitability, the input resistance of both type A and type B photoreceptors was measured using 0.2-nA hyperpolarizing steps. To avoid possible confounds introduced by spontaneous firing, cellular membrane potentials were held constant throughout the experiment at −60 to −65 mV (a value that is below action potential threshold) via a nominal hyperpolarizing holding current. On the basis of microelectrode position, we believe that all possible B photoreceptor subtypes (medial, intermediate, and lateral) and A photoreceptor subtypes (medial and lateral) were used during the various experimental conditions. All experiments were conducted at room temperature.

Treatment protocol. Measurements of the IPSP, action potential, and input resistance were obtained at a 30-s interstimulus interval (ISI) for five trials before (pretests) and after (posttests) one of the following three experimental manipulations: ~30 s of light ($n = 6$), 30 s of depolarization-induced (+1 to +2 nA intracellular current injection) type B cell activity ($n = 7$), or a 30-s control period of seawater exchange ($n = 4$). The actual duration of the manually delivered light stimuli was 29.5 ± 0.3 s, whereas the electronically gated depolarization stimuli were precisely 30 s. We used a red light (Dolan-Jenner filter FR1-60; Lawrence, MA) stimulus generated by a 150-W quartz–halogen lamp (Dolan-Jenner Model 180) coupled to a 5/8″ diameter fiber optic light guide (Dolan-Jenner) which yielded a light intensity of ~3 mW/cm^2 (as measured by a Liconix 45-PM power meter; Sunnyvale, CA) at the preparation site. One minute elapsed between the termination of the stimulus and the first posttest trial.

Statistical analysis. Mean IPSP amplitude and input resistance values were computed for each preparation across the five trials preceding the experimental manipulation (pretest score) and the five trials after it (posttest score). Data are reported throughout as means ± SEM. IPSP difference scores (i.e., posttest score − pretest score) and the changes in input resistance of the type B and A photoreceptors were analyzed using one-way ANOVAs, followed by Duncan pairwise comparisons to assess between-group differences. Two-tailed, paired Student's *t* tests were used for within-group comparisons. Action potential duration, AHP peak, and AHP area were analyzed similarly. One-way ANOVAs were also used to determine whether the type B cell spike frequency, number of spikes, and peak depolarization elicited by depolarizing current injection were comparable to those elicited by light. Any major outliers (defined as those values that fall more than 3 interquartile ranges above the 3rd quartile or below the 1st quartile) were excluded from each experimental condition prior to the above analyses.

RESULTS AND DISCUSSION

Long Light Steps Induce PLP at Type B to A Cell Connections

Consistent with our primary hypothesis, IPSPs evoked in type A photoreceptors by type B photoreceptors were potentiated after a single, ~30-s presentation of light (0.79 ± 0.27 mV increase from a baseline of 2.96 ± 0.50 mV; $t(5) = 2.90$, $p < .03$). IPSPs were also potentiated after type B cell depolarization (0.55 ± 0.22 mV increase from a baseline of 1.69 ± 0.48 mV; $t(6) = 2.47$, $p < .05$), but not after control ASW exchange

(0.40 ± 0.26 mV decrease from a baseline of 3.15 ± 0.43 mV; $t(3) = 1.57$, $p < .21$) (Fig. 1). Both light and depolarization potentiated IPSPs relative to ASW control preparations (one-way ANOVA ($F(2, 15) = 5.43$, $p < .02$), followed by Duncan comparisons, p's $< .05$), but their effects did not differ from each other ($p > .05$). We do not know how long this synaptic potentiation persisted in this experiment (or other experiments below). However, given that short-term potentiation resulted after a single training trial, it is plausible that reasonably persistent changes could occur with the multiple trials (e.g., 150 trials; Crow & Alkon; 1978, Crow, 1983) that are used in behavioral training of the intact animal and result in nonassociative learning.

In sum, a 30-s period of type B cell depolarization and spike discharge, elicited by either light or depolarizing current injection, was sufficient to induce potentiation at type B to A photoreceptor connections. Given that the magnitude of the potentiation induced by depolarization-evoked type B cell activity was comparable to that yielded by light, these data imply that light-evoked processes other than type B cell activity, such as intracellular Ca^{2+} release, are not *necessary* for the induction of PLP at type B to A cell connections with these stimulus parameters (but see other experiments below for different results with short light steps). Nonetheless, because these experiments did not examine whether other light-evoked processes are *sufficient* to induce PLP, they do not rule out the possibility that these other light-evoked processes may also contribute to PLP induction, as indicated in further experiments below.

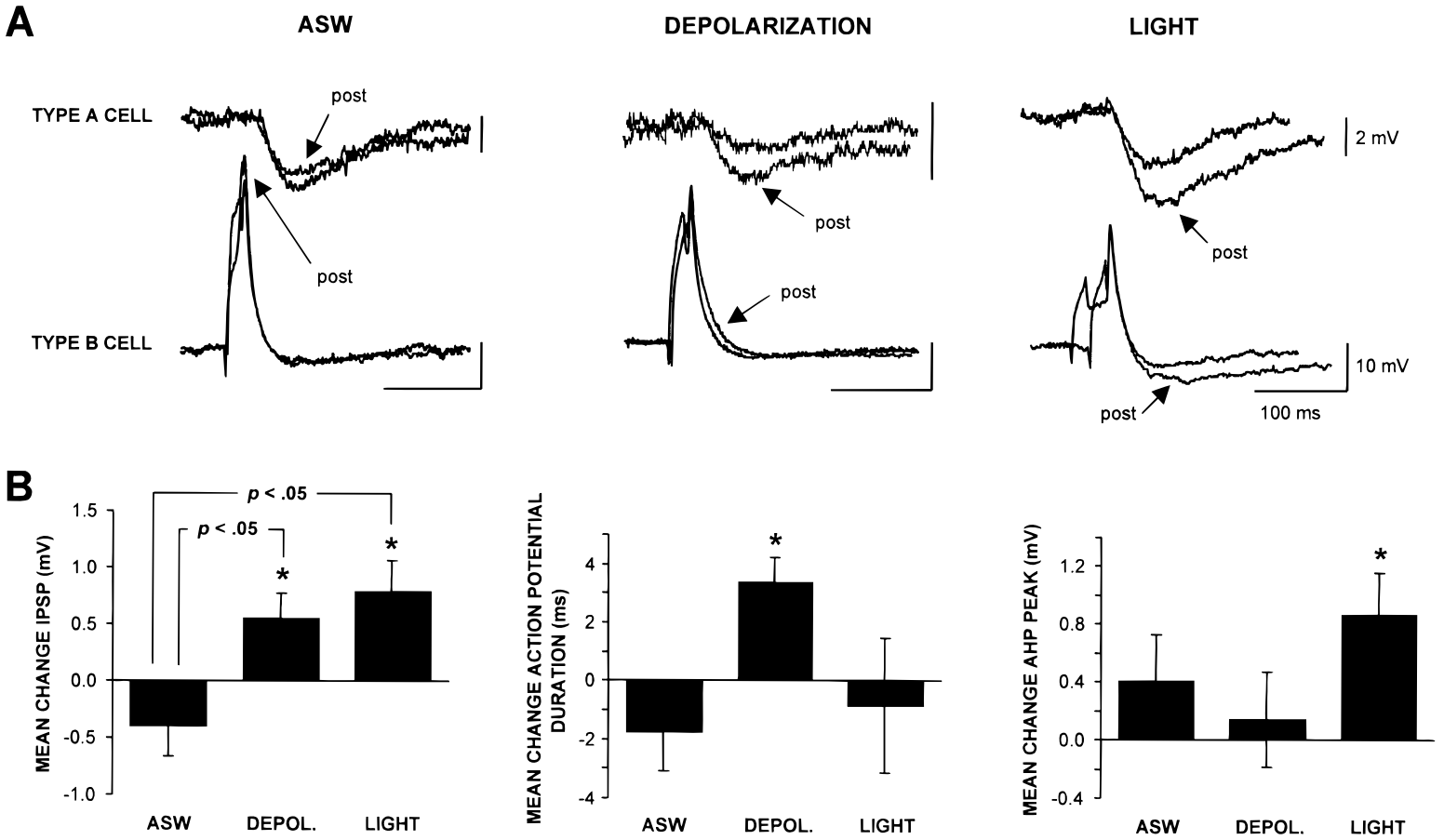
PLP Does Not Arise Simply from Increases in Type A Cell Input Resistance

There were no significant between-group differences for either type B cell input resistance ($F(2, 11) = 0.26$, $p < .78$) or type A cell input resistance ($F(2, 13) = 1.35$, $p < .29$). Likewise, there were no within-group differences in input resistance for either cell type (p's $> .05$), although a light-evoked increase in type A cell input resistance approached significance (4.58 ± 1.94 MΩ increase from a baseline of 31.22 ± 3.97 MΩ, $t(5) = 2.37$, $p < .06$). Hence, it is unlikely that increases in IPSP amplitude are a secondary consequence of increased resistance of the postsynaptic type A cell target.

Changes in Type B Cell Action Potential Parameters

In principle, changes in ion channel function (e.g., reduction in K^+ currents) that contribute to training-related modifications of type B cell excitability (Alkon, 1984; Alkon, Lederhendler, & Shoukimas, 1982; Alkon, Sakakibara, Forman, Harrigan, Lederhendler, & Farley, 1985) could also modify action potential parameters and thereby alter neurotransmitter release from type B cells (Fost & Clark, 1996a; Leszczuk, Gandhi, Muzzio, & Matzel, 1997). Alternatively, changes in synaptic strength could arise from other mechanisms, including changes in transmitter release from type B cells that are independent of action potential parameters, or postsynaptic changes in type A cell receptors.

In the present experiment, type B cells that had been exposed to light showed, in addition to an increase in IPSP amplitude, a significant increase in the amplitude of the AHP peak (0.86 ± 0.30 mV increase, from a pretraining value of 4.37 ± 0.51 mV, $t(4) = 2.89$, $p < .05$) (Fig. 1), although they showed no significant changes in action potential duration (0.84 ± 2.28 ms decrease, $t(4) = 0.37$, $p < .73$) or AHP area (53.5 ±


A
ASW
DEPOLARIZATION
LIGHT
TYPE A CELL
TYPE B CELL
post
2 mV
10 mV
100 ms
B
p < .05
p < .05
MEAN CHANGE IPSP (mV)
MEAN CHANGE ACTION POTENTIAL DURATION (ms)
MEAN CHANGE AHP PEAK (mV)
ASW
DEPOL.
LIGHT

25.3 mV·ms increase, $t(4) = 2.11$, $p < .11$). Increases in AHP peak may reflect an increase in K^+ currents, possibly arising from increased intracellular Ca^{2+}; such increases in intracellular Ca^{2+} could enhance neurotransmitter release as well as increase the Ca^{2+}-dependent K^+ current. Thus, although increases in AHP amplitude would not themselves increase transmitter release, they may serve as an indirect readout of Ca^{2+}-related processes that do enhance transmitter release from type B cells and consequently the amplitude of the IPSP evoked in type A cells. Learning-related modifications of AHPs also occur in hippocampal pyramidal cells (Disterhoft, Coulter, & Alkon, 1986) as well as in *Aplysia* siphon sensory cells (Clark, Hawkins, & Kandel, 1994). By contrast to light-alone training, depolarization training resulted in a significant increase in action potential duration (3.34 $\pm$ 0.86 ms increase from a pretraining value of 22.2 $\pm$ 3.3 ms, $t(4) = 3.88$, $p < .02$), although there was no significant change in AHP area ($p < .59$) or AHP peak ($p < .68$). Broadening of the action potential could in principle enhance transmitter release from type B cells and contribute to the increases in IPSP amplitude recorded in type A cells. ASW control preparations showed no significant change in any action potential parameter (all p's $> .26$). That changes occurred in type B cell action potential parameters after light or depolarization is not necessarily in conflict with the absence of significant changes in input resistance, because the voltage-dependent currents activated during the action potential would contribute relatively little to the membrane resistance at the hyperpolarized membrane potentials at which input resistance measurements were obtained.

Despite the above-mentioned within-group differences, there were no significant between-group differences in type B cell action potential parameters. Thus, one cannot necessarily conclude that between-group differences in IPSP potentiation arise from training-dependent differences in action potential currents.

Light-Elicited Spiking and Depolarization of Photoreceptors

Our ~30-s red light stimulus evoked a mean type B cell firing frequency of 10.73 $\pm$ 0.92 Hz and type A cell firing frequency of 7.07 $\pm$ 0.75 Hz. Using the definitions put

FIG. 1. Long light steps induce potentiation of inhibitory postsynaptic potentials (IPSPs) (Experiment 1). (A) Representative results. Action potentials were evoked in type B photoreceptors by depolarizing current injection, and the resulting IPSPs were recorded in type A photoreceptors before (unlabeled traces) and 1 min after (*post*) one of three treatment conditions: control seawater exchange (*ASW*), depolarization-induced type B cell activity (*depolarization*), or a single ~30-s presentation of 3 mW/cm^2 red light (*light*). Note the increases in IPSP amplitudes after light or depolarization manipulations and accompanying changes in action potential parameters. In this and subsequent figures, stimulus artifacts that precede the evoked type B cell action potentials have been electronically cropped, and traces are temporally aligned relative to action potential peaks. (B) Group data for mean change in IPSP amplitude (left), type B cell action potential duration (middle), and type B cell afterhyperpolarizing potential (*AHP*) peak amplitude (right). (Left) IPSP amplitudes were significantly potentiated after either ~30-s light ($n = 6$; $p < .03$) or depolarization-induced type B cell activity ($n = 7$; $p < .05$), but not after control ASW exchange ($n = 4$; $p < .21$). (Middle, right) Similarly, type B cell action potential parameters were altered after type B cell depolarization (*depol.*) or long light steps, but not after ASW exchange. The depolarization step increased the action potential duration ($n = 5$, $p < .05$), whereas the 30-s light step increased the AHP peak ($n = 5$, $p < .05$). Possibly, the increased action potential duration after depolarization may contribute to enhanced transmitter release and the observed synaptic potentiation. The enhanced AHP after light may reflect increased Ca^{2+}-dependent K^+ currents and increased intracellular Ca^{2+} concentrations, which may also contribute to PLP. Here and in subsequent figures, data are depicted as means $\pm$ SEM, significant within-group differences at $p < .05$, .01, or .001 are denoted by one, two, or three asterisks, respectively, and significant between-group differences are indicated by brackets.

forth by Lederhendler and Alkon (1987), we also determined the peak firing frequency (averaged across the first 5 s) and steady-state firing frequency (averaged across the last 10 s). As previously described (Crow, 1985; Farley, 1987a; Farley & Alkon, 1982; Lederhendler & Alkon, 1987), type B cell spike frequency declined across the light step, from a peak of 15.87 ± 1.45 Hz to a steady-state of 8.15 ± 0.99, with the level of light-elicited type B cell depolarization (peak: 27.57 ± 5.31 mV; steady-state: 18.94 ± 2.55 mV) showing a similar decline. Type A cell firing frequencies (peak: 14.44 ± 3.48 Hz; steady-state: 3.49 ± 0.66 Hz) abruptly dropped after 2 s and then gradually declined across the remainder of the step, with the level of light-elicited type A cell depolarization (peak: 25.76 ± 3.45 mV; steady-state: 18.19 ± 1.40 mV) showing a similar trend. As described later in the General Discussion, this adaptation may reflect an increase in intracellular Ca^{2+} that could contribute to PLP induction.

Given that the photoreceptors are relatively insensitive to red light (Dennis, 1967), it is quite likely that the photoreceptors sensed the intensity of our red light stimulus as being considerably lower than the nominal intensity of 3 mW/cm^2. In fact, the photoreceptor responses evoked in this experiment are comparable to responses evoked by lower intensity white light stimuli used in Experiments 2 and 3 below (600 μW/cm^2), as well as in other studies (e.g., Crow, 1985; Farley & Alkon, 1982; Frysztak & Crow, 1993; Lederhendler & Alkon, 1987).

30-s Light or Depolarizing Current Steps Yield Comparable Levels of Type B Cell Depolarization and Spike Discharge

In order to address the question of whether light-induced type B cell activity contributes to PLP induction, we attempted to equate the depolarization and action potential discharge elicited by depolarizing current injection (~2 nA) with the levels generated by our light stimulus. One-way ANOVAs revealed that steps of light or depolarizing current injection yielded comparable mean peak depolarization (27.57 ± 5.31 mV vs 16.47 ± 7.17, $F(1, 8) = 1.09$, $p < .33$), comparable type B cell spike frequency (10.73 ± 0.92 Hz vs 12.31 ± 0.79 Hz, $F(1, 9) = 1.62$, $p < .23$), and comparable type B cell spike number (333.5 ± 23.8 spikes vs 328.4 ± 54.1 spikes, $F(1, 9) = 0.01$, $p < .91$). We did not explicitly attempt to match for spike pattern.

EXPERIMENT 2

Experiment 1 demonstrated that type B cell activity, elicited by a 30-s step of light or depolarizing current injection, induces potentiation at the type B to A photoreceptor connections. Given that both long (e.g., Crow & Alkon, 1978) and short (e.g., Matzel et al., 1990; Rogers et al., 1994) light presentations have been used during behavioral training, we were interested in determining whether a series of short light steps would also induce PLP at the type B to A cell connections. Furthermore, we wished to explicitly address the question of whether light-evoked processes other than type B cell activity/depolarization also contribute to PLP induction. Hence, this experiment addresses the related questions of (1) whether nine 3-s light steps are capable of inducing PLP and (2) whether type B cell activity and other light-evoked processes each contribute to the PLP induced by 3-s stimuli.

Methods

Animals. *Hermissenda* (2–4 cm long) were obtained from Marinus. Animals were housed individually in perforated 50-mL tubes in a tank of 12°C artificial seawater for 1 to 14 days prior to experiments. They were maintained on 11 to 13 h of light per day and fed small pieces of mussel three times weekly. Experiments were conducted during the light phase.

Electrophysiology. Pairs of synaptically connected type B and A photoreceptors were recorded from the eyes of the isolated circumesophageal nervous system as detailed for Experiment 1.

Treatment protocol. IPSP and input resistance measurements were obtained at a 30-s ISI for five trials before (pretests) and after (posttests) nine ~3-s presentations of one of the following: (1) white light (600 μW/cm^2) delivered through a 3/8″ diameter fiber-optic light guide (Dolan-Jenner) as described for Experiment 1 ($n = 9$), (2) depolarization-induced type B cell activity (+2 to +2.5 nA intracellular current injection) ($n = 8$), (3) light combined with hyperpolarization-induced blockade of type B cell activity (−2 to −2.5 nA intracellular current injection for the entire 4.5-min treatment period) ($n = 7$), or (4) control ASW exchange for an equivalent period of time ($n = 6$). Figure 2 illustrates sample responses of type B and A photoreceptors to each of these stimuli. Two minutes elapsed between the termination of the final stimulus and the start of the first posttest trial.

Statistical analysis. Data were analyzed as described for Experiment 1.

RESULTS AND DISCUSSION

Short Light Steps Also Induce Potentiation at Type B to A Cell Connections

The primary results of this experiment are that IPSPs evoked in type A photoreceptors by type B photoreceptors were potentiated after nine, ~3-s white light (600 μW/cm^2) steps, but were reduced after the other manipulations (Fig. 3). The increase in IPSP amplitudes induced by light (0.60 ± 0.24 mV increase from a baseline of 1.61 ± 0.34 mV) was statistically significant relative to prestimulation baseline ($t(8) = 2.51$, $p < .04$). Likewise, the decreases in IPSP amplitudes observed after control ASW exchange (0.27 ± 0.05 mV decrease from a baseline of 1.99 ± 0.38 mV; $t(5) = 5.61$, $p < .003$), depolarization-induced type B cell activity (0.67 ± 0.11 mV decrease from a baseline of 1.97 ± 0.22 mV; $t(7) = 6.06$, $p < .0005$), or light combined with hyperpolarization blockade of type B cell activity (1.22 ± 0.38 mV decrease from a baseline of 2.36 ± 0.40 mV; $t(6) = 3.21$, $p < .02$) were also statistically significant.

The various manipulations produced multiple between-group differences, as revealed by a one-way ANOVA ($F(3, 26) = 11.34$, $p < .0001$), followed by Duncan comparisons (Fig. 3B). The light-induced synaptic potentiation was statistically significant relative to the changes in each of the other three experimental groups (relative to ASW control preparations: $p < .05$; relative to type B cell depolarization: $p < .01$; relative to light combined with hyperpolarization-induced blockade of type B cell activity: $p < .01$). The light + hyperpolarization manipulation yielded a significant decrease in IPSP amplitudes relative to ASW control preparations ($p < .05$), which is intriguing given that control

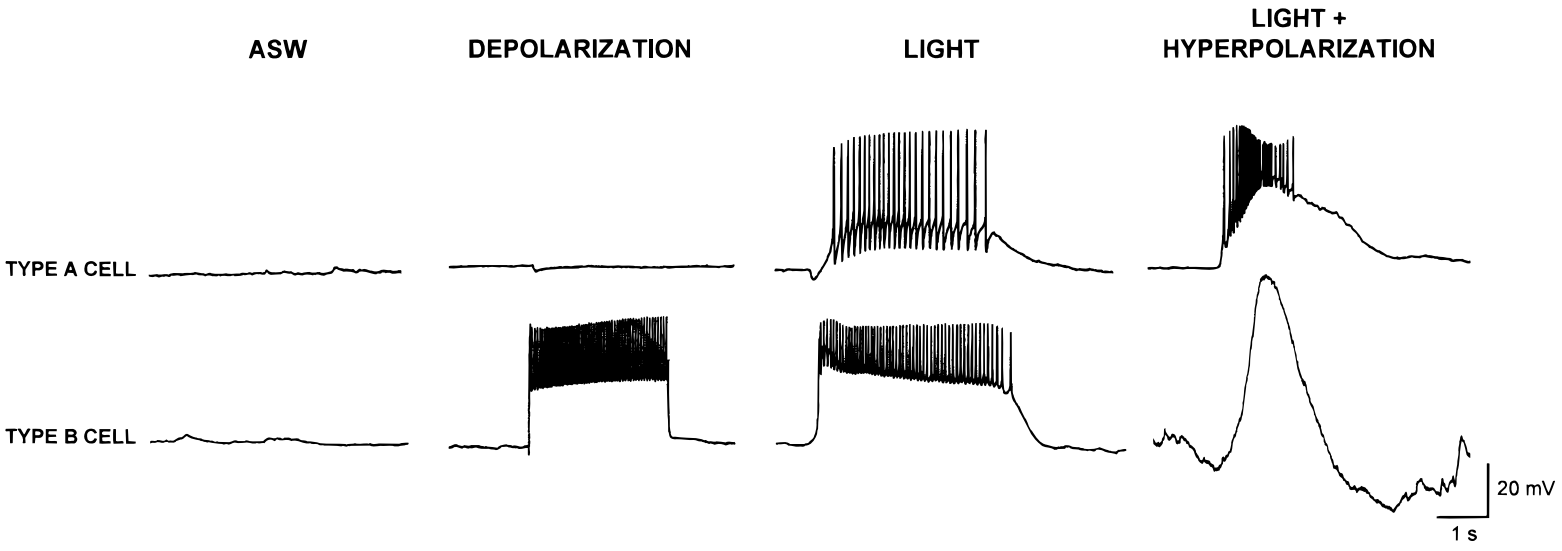
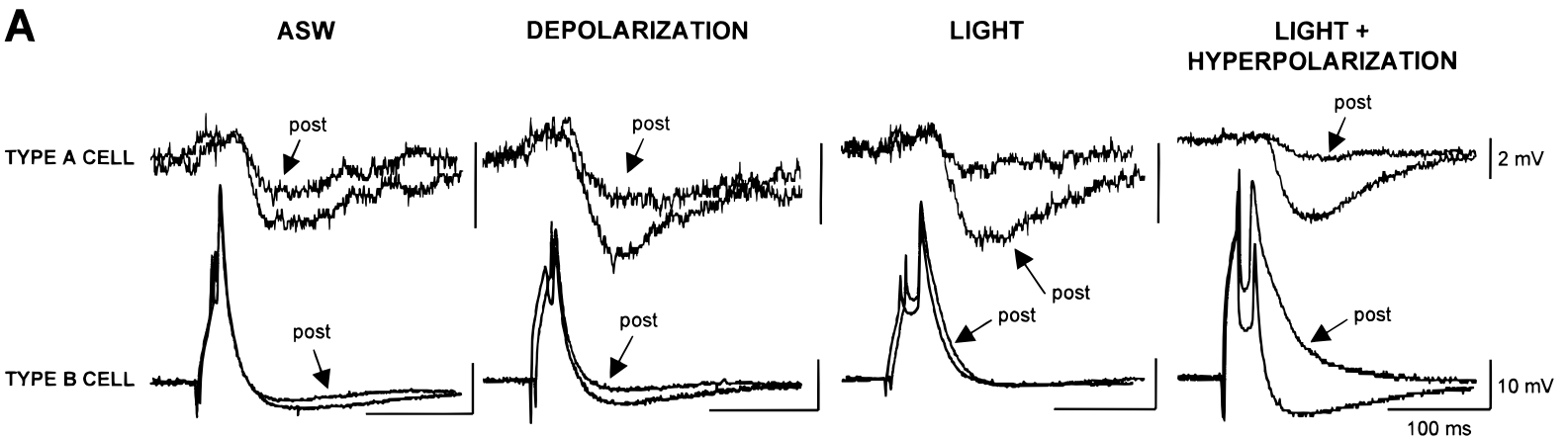


FIG. 2. Type B and A photoreceptor responses to various stimuli (Experiment 2). Preparations received either control seawater exchange (*ASW*); nine approximately 3-s presentations of depolarization-induced type B cell activity (*depolarization*); presentations of 600 μW/cm^2 white light (*light*); or presentations of light in conjunction with hyperpolarization, which blocked light-evoked type B cell activity (*light + hyperpolarization*). Stimuli were delivered at a 30-s ISI. The duration of manually delivered light stimuli was not always precisely 3 s.


A
ASW
DEPOLARIZATION
LIGHT
LIGHT + HYPERPOLARIZATION
TYPE A CELL
TYPE B CELL
post
2 mV
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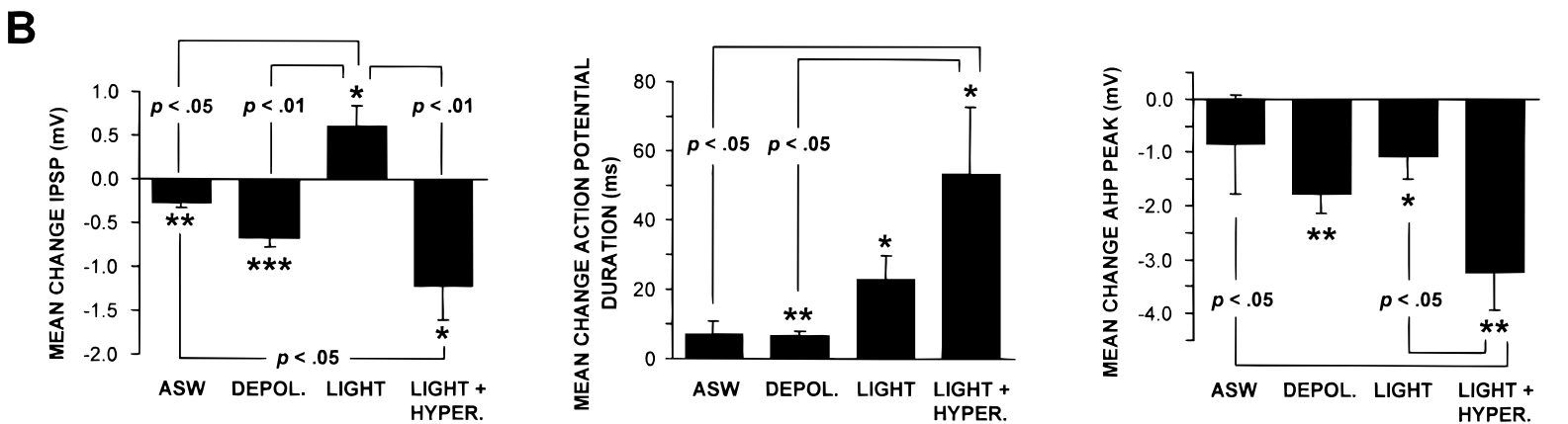

B
MEAN CHANGE IPSP (mV)
MEAN CHANGE ACTION POTENTIAL DURATION (ms)
MEAN CHANGE AHP PEAK (mV)
p < .05
p < .01
ASW
DEPOL.
LIGHT
LIGHT + HYPER.

IPSP amplitudes were depressed relative to their own prestimulation baseline. By contrast, the decrease observed after depolarization-elicited type B cell activity was not significantly different from that observed in control preparations ($p > .05$), suggesting that the modest decreases in these two groups probably resulted from homosynaptic depression arising from the testing procedure (Schultz et al., 1998).

These results corroborate some of the findings of Experiment 1, but differ from others. Once again, light induced synaptic potentiation, relative to both pre-light baseline and to ASW control preparations, at the type B to A cell connections. Hence, both long (~30-s) and short (~3-s) light presentations are capable of inducing PLP. However, the PLP generated by these two stimuli showed some mechanistic differences. In Experiment 1, not only was depolarization-induced type B cell activity sufficient to induce potentiation, but this potentiation was of a comparable magnitude to that induced by light, suggesting that the type B cell activity elicited by a 30-s light stimulus is sufficient to induce the full PLP effect. By contrast, in Experiment 2, 3-s steps of depolarization-induced type B cell activity did not produce any synaptic potentiation, even though 3-s light steps did. It therefore seems that processes other than type B cell activity are necessary in order for 3-s light stimuli to induce PLP. Although possible reasons for this dissociation will be discussed later, at this point we can reasonably conclude that, under certain circumstances, both type B cell activity and other light-evoked processes contribute to PLP induction.

Is Type B Cell Activity Necessary for Induction of PLP at Type B to A Cell Connections?

No PLP occurred when hyperpolarizing current injection was used to prevent light-evoked type B cell activity. At first, this result may seem to imply that type B cell activity during the light presentations is necessary in order to produce PLP at these connections. However, if the only effect of hyperpolarization were a cancellation of light-evoked type B cell activity, then this manipulation simply would have reduced synaptic strength to ASW control levels. By contrast, there was instead a decrease in synaptic strength at the type B to A cell connections relative to ASW control preparations, as well as relative to the prestimulation baseline. Given that a substantial amount of hyperpolarization was needed in order to prevent the type B cell from spiking during the light steps (−2 to −2.5 nA, yielding membrane potentials of −100 mV or lower), it is conceivable that this

FIG. 3. Short light steps also induce potentiation at type B to A cell connections (Experiment 2). (A) Representative results. Action potentials were evoked in type B photoreceptors by depolarizing current injection, and the resulting IPSPs were recorded in type A photoreceptors before (unlabeled traces) and 2 min after (*post*) one of four treatment conditions: *ASW, depolarization, light,* or *light + hyperpolarization.* Light presentations produced an increase in the amplitude of the IPSP evoked in the type A cell, accompanied by changes in type B cell action potential kinetics. (B) Group data for IPSP amplitude (left), type B cell action potential duration (middle), and AHP peak amplitude (right). (Left) IPSP amplitudes were significantly potentiated after light presentations (*light,* $n = 9$; $p < .04$), but were reduced after depolarization-induced type B cell activity (*depol.,* $n = 8$, $p < .0005$), light presented in conjunction with hyperpolarization-induced blockade of type B cell activity (*light + hyper.,* $n = 7$; $p < .02$), or control *ASW* exchange ($n = 6$; $p < .003$). (Middle, right) Short (3-s) light stimuli and other stimuli also modified action potential parameters of type B cells. Light alone, type B cell depolarization, and light plus hyperpolarization each produced significant increases in type B cell action potential duration and decreases in AHP peak (all n's = 6 to 8, all p's < .05 or .01), whereas ASW treatments produced no significant changes in action potential parameters. The relatively large changes in action potential parameters after light plus hyperpolarization may reflect nonspecific damage induced by the large current injections.

intense hyperpolarization damaged or altered the type B cell in such a way as to reduce subsequent synaptic transmission. This interpretation is further supported by analyses of action potential parameters (below).

The likelihood that our hyperpolarization manipulation had debilitating effects other than blocking light-induced type B cell activity prevents us from concluding at this point that type B cell activity contributes to PLP induced by short light steps (but see Experiment 3 below, which in fact confirms this interpretation). In principle, other light-evoked processes may have been sufficient to induce PLP in the present experiment, but any potentiation was masked by the co-occurrence of the hyperpolarization-induced damage.

PLP Does Not Arise Simply from Increases in Type A Cell Input Resistance

There were no significant between-group differences for either type B cell ($F(3, 25) = 1.05$, $p < .39$) or type A cell input resistance ($F(3, 24) = 1.28$, $p < .30$). However, we observed significant within-preparation increases in type A cell input resistance in both the light group (9.09 ± 2.06 MΩ increase from a baseline of 34.77 ± 3.13 MΩ; $t(6) = 4.42$, $p < .004$) and the light + hyperpolarization group (12.38 ± 4.12 MΩ increase from a baseline of 43.43 ± 5.17 MΩ; $t(6) = 3.01$, $p < .02$). Given that there was an increase in type A cell input resistance coupled with a *decrease* in synaptic strength when light was presented in conjunction with type B cell hyperpolarization, it is unlikely that the increase in synaptic strength observed after normal light presentations was merely a reflection of the corresponding increase in type A cell input resistance.

Changes in Type B Cell Action Potential Parameters

Several changes occurred in type B cell action potential parameters that may have contributed to changes in IPSPs at type B cell to type A cell connections (Fig. 3). The 3-s light presentations produced an increase in the duration of the action potential (22.6 ± 6.9 ms increase from a baseline of 38.1 ± 3.0 ms, $t(6) = 3.29$, $p < .02$), and a decrease in the AHP peak (1.08 ± 0.40 mV decrease from a baseline of 3.40 ± 0.61 mV, $t(6) = 2.67$, $p < .04$) and AHP area (91.8 ± 25.0 mV·ms decrease from a baseline of 163.2 ± 38.1 mV·ms, $t(6) = 3.67$, $p < .02$). This set of changes could arise from an underlying decrease in K^+ currents, and, in principle, the broadened action potential could have contributed to enhanced neurotransmitter release and to the observed IPSP potentiation that resulted from light presentations.

Light plus hyperpolarization training also dramatically increased action potential duration (53.1 ± 19.4 ms increase from a baseline of 40.1 ± 12.2 ms, $t(5) = 2.74$, $p < .05$) and significantly decreased AHP peak (3.23 ± 0.73 mV decrease from a baseline of 4.79 ± 0.83 mV, $t(5) = 4.44$, $p < .007$) and AHP area (232.6 ± 70.3 mV·ms decrease from a baseline of 262.1 ± 76.9 mV·ms, $t(5) = 3.31$, $p < .03$), even though this manipulation decreased IPSPs below ASW control levels. We suspect that these large increases in action potential duration were not physiological and may instead reflect membrane damage resulting from the large, long-lasting (4.5 min) hyperpolarization command. Our method of measuring AP duration, from action potential peak to return to baseline, may also have maximized these changes, which appeared informally to be most pronounced in the latter phases of the action potential (as indicated also in the modifications of the AHP).

Depolarization training also produced a significant increase in action potential duration (6.45 ± 1.28 ms increase from a baseline of 26.4 ± 2.7 ms, $t(6) = 5.05$, $p < .003$) and significant decreases in AHP peak (1.79 ± 0.36 mV decrease from a baseline of 3.98 ± 0.42 mV, $t(7) = 4.96$, $p < .002$) and AHP area (111.2 ± 25.3 mV·ms decrease from a baseline of 216.8 ± 32.9 mV·ms, $t(7) = 4.40$, $p < .004$), despite showing no IPSP potentiation. ASW control preparations showed no significant changes in action potential parameters (all p's $>.14$).

The increases in action potential duration resulting from light plus hyperpolarization were greater than the changes that occurred in other groups ($F(3, 20) = 4.16$, $p < .02$; Duncan's: $p < .05$ compared with ASW or depolarization; $p < .10$ compared with light). Similarly, the decreases in AHP peak resulting from light plus hyperpolarization were greater than the changes that occurred in other groups ($F(3, 22) = 3.13$, $p < .05$; Duncan's: $p < .05$ compared with ASW or light; $p < .10$ compared with depolarization). There were no other significant between-group differences.

One plausible, albeit speculative, interpretation of the above findings is that the spike broadening that occurred after light presentations was physiological and contributed to enhanced transmitter release from type B cells and IPSP potentiation, whereas the large spike broadening that occurred after light plus hyperpolarization was not physiological and reflected cell damage at the point of intracellular current injection, as proposed above. In contrast to the physiological changes produced by light presentations, this damage-related broadening induced by the strong hyperpolarization may be expected to be relatively confined to the electrode site and not occur at the synaptic terminal to the same degree. Hence, such damage-related broadening would not strongly influence transmitter release.

Finally, it is worth noting that the multiple 3-s light presentations of the present experiment produced spike broadening, whereas the single 30-s light presentation of Experiment 1 did not, suggesting possible mechanistic differences between the PLP produced by these two types of light presentations. Consistent with this possibility is that in Experiment 1, the facilitatory effects of a 30-s light presentation were mimicked by a 30-s depolarization of the type B cell, whereas in Experiment 2, the facilitatory effects of multiple 3-s light presentations were not mimicked by multiple 3-s depolarizations. However, given the various parametric differences between the light stimuli used in the two experiments, it is not possible to identify with certainty which factor or factors (e.g., stimulus length, stimulus wavelength, number of trials) underlie the different results obtained in the two experiments.

Firing Frequencies of Type B and A Photoreceptors Decline across Multiple Light Presentations

The multiple light steps used in this experiment enabled us to ascertain the effects of repeated light presentations on the firing frequencies of type B and A photoreceptors. Photoreceptor firing frequencies declined with each additional light presentation ($F(8, 128) = 11.83$, $p < .0001$), with type B cells consistently firing at a higher rate than did the type A cells ($F(1, 16) = 28.55$, $p < .0001$). The type B cell firing frequency evoked during the first 3-s light step (22.81 ± 2.76 Hz) was comparable to that elicited during the initial 3 s of the 30-s light step used in Experiment 1 (17.06 ± 1.73 Hz) ($F(1, 13) = 2.43$, $p < .14$), suggesting that these stimuli were physiologically comparable, despite

their nominal differences. Likewise, the type A cell firing frequency evoked during the first 3-s step (14.47 ± 3.72 Hz) was consistent with that elicited during the initial 3 s of a 30-s light step (16.93 ± 4.73 Hz) ($F(1, 12) = 0.16$, $p < .69$). As would be expected, the mean firing frequencies of the type B (14.97 ± 0.95 Hz) and type A (9.07 ± 0.90 Hz) cells evoked by the 3-s stimulus were both higher than the mean frequencies evoked by the 30-s light stimulus (Experiment 1), given the decline in firing frequency that occurred from peak to steady-state firing rate during the 30-s stimulus.

Comparable Levels of Type B Cell Spike Discharge, but Not Depolarization, Are Yielded by 3-s Light or Depolarizing Current Steps

Once again, we confirmed that our light (14.60 ± 0.91 Hz) and depolarization (16.35 ± 1.26 Hz) stimuli yielded comparable type B cell firing frequencies ($F(1, 15) = 1.30$, $p < .27$). Likewise, the absolute number of spikes elicited by a light (53.46 ± 2.79 spikes) or depolarization stimulus (50.96 ± 4.04 spikes) did not differ ($F(1, 15) = 0.27$, $p < .61$), which suggests that these stimuli yielded a comparable level of type B cell spike activity despite the relatively longer duration of our hand-delivered light stimuli (3.7 ± 0.2 s, as opposed to the 3.0-s duration of electronically gated depolarization stimuli). However, light elicited significantly more type B cell depolarization (37.76 ± 2.99 mV) than did intracellular current injection (26.43 ± 1.76 mV) ($F(1, 15) = 9.98$, $p < .007$). Given the multiple conductances underlying the light-evoked generator potential (Alkon & Grossman, 1978; Detwiler, 1976), it is not surprising that we were not always able to match both spike activity and depolarization with a simple depolarizing current step. Nonetheless, the comparable levels of type B cell spike discharge evoked by our light and depolarization stimuli enable us to compare the effects of these stimuli on synaptic strength.

EXPERIMENT 3

In Experiment 2, 3-s steps of type B cell activity did not produce any potentiation, even though 3-s steps of light did. These results indicate that, under these stimulus conditions (by contrast to those of Experiment 1), type B cell activity by itself was not sufficient to induce potentiation. Nonetheless, a remaining question is whether type B cell activity contributes at least in part to the induction of PLP with short light steps, in which case light in conjunction with type B cell activity would produce greater PLP than light alone, without type B cell activity. Although the results of Experiment 2 are consistent with this possibility, they do not provide a conclusive answer to this question, because the apparent blockade of PLP with light plus hyperpolarization could have resulted from nonspecific cell damage, rather than from the blockade of action potential activity per se.

Hence, our final experiment was designed to address this question explicitly. Preparations received either overlapping or alternating presentations of light and type B cell hyperpolarization. In overlapping presentations, the hyperpolarization blocked light-evoked action potential activity, whereas in alternating presentations it did not. Given that these conditions were equated for any nonspecific hyperpolarization-induced effects as well as for any light-elicited effects other than type B cell activity, any between-group differences would imply that type B cell activity does indeed contribute to the PLP generated by short light steps.

Methods

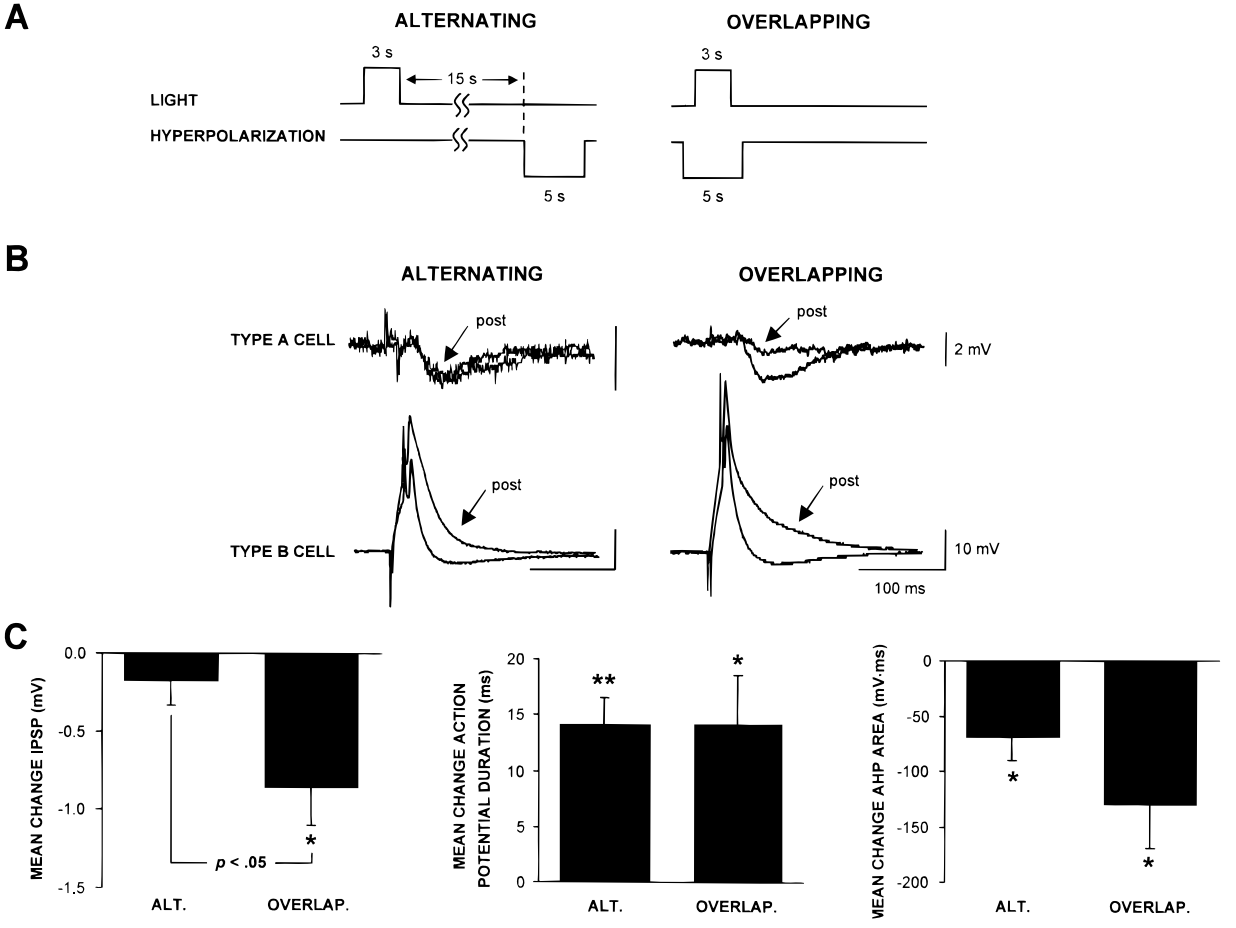
Animals. *Hermissenda* were maintained as described for Experiment 2.

Electrophysiology. Pairs of synaptically connected type B and A photoreceptors were recorded from the eyes of the isolated circumesophageal nervous system as detailed for Experiment 1.

Treatment protocol. IPSP and input resistance measurements were obtained at a 30-s ISI for five trials before (pretests) and after (posttests) either overlapping or alternating presentations of light plus type B cell hyperpolarization, as depicted in Fig. 4. The overlapping condition ($n = 5$) consisted of nine overlapping presentations of 3-s white light (600 μW/cm^2, gated electronically) and 5-s type B cell hyperpolarization (-2 nA intracellular current injection), delivered at a 30-s ISI. The onset of a hyperpolarizing current command to the type B cell preceded the light stimulus by 1 s, and the offset occurred 1 s after termination of the light stimulus. This condition was intended to block type B cell action potentials during the light presentations. The alternating condition ($n = 5$) consisted of nine alternating presentations of these same light and hyperpolarization stimuli. In this case, light stimuli were gated electronically at a 30-s ISI, with a hyperpolarizing current step delivered 15 s after the termination of each light stimulus. This condition did not block action potential activity during the light presentations and was intended as a control for nonspecific effects of hyperpolarization. For both groups, a period of 2 min elapsed between the termination of the last stimulus and the onset of the first posttest.

Statistical analysis. Data were analyzed as described for Experiments 1 and 2.

FIG. 4. Type B cell activity contributes to PLP evoked by short light steps (Experiment 3). (A) Combinations of light plus type B cell hyperpolarization used during Experiment 3. (Left) Preparations in the *alternating* condition received alternating presentations of 3-s light and 5-s type B cell hyperpolarization. Nine light stimuli were delivered at a 30-s ISI, with a hyperpolarization step given 15 s after the offset of each light stimulus. (Right) Preparations in the *overlapping* condition received nine overlapping presentations of 3-s light and 5-s type B cell hyperpolarization delivered at a 30-s ISI. The onset of hyperpolarization preceded that of light by 1 s, and its offset followed that of light by 1 s. Hence, in the overlapping but not the alternating condition, hyperpolarization blocked the type B cell activity normally evoked by light. (B) Representative results. Action potentials were evoked in type B photoreceptors by depolarizing current injection, and the resulting IPSPs were recorded in type A photoreceptors before (unlabeled traces) and 2 min after (*post*) either alternating or overlapping light plus hyperpolarization stimuli. Note the large decrease in IPSP amplitude in the overlapping condition, in which type B cell activity was blocked. (C) Group data for IPSP amplitude (left), type B cell action potential duration (middle), and AHP area (right). (Left) Mean IPSP amplitude was significantly reduced after overlapping presentations of light and hyperpolarization ($n = 5$, $p < .02$), but not after alternating presentations ($n = 5$, $p < .29$). Overlapping stimuli yielded a significantly greater reduction of synaptic strength than did alternating stimuli ($p < .03$), suggesting that the type B cell activity present in the alternating but not overlapping condition contributed to synaptic potentiation. (Middle, right) Alternating presentations (*alt.*) and overlapping presentations (*overlap.*) of light plus hyperpolarization both modified action potential parameters of type B cells, increasing the action potential duration and decreasing the AHP (all n's $= 4$ or 5, all p's $< .05$ or .01). Given the different effects of alternating and overlapping presentations on synaptic strength, the changes in action potential parameters common to the two procedures may reflect nonspecific damage resulting from the large hyperpolarizing command.


A
ALTERNATING
OVERLAPPING
3 s
15 s
LIGHT
HYPERPOLARIZATION
5 s
3 s
5 s
B
ALTERNATING
OVERLAPPING
TYPE A CELL
post
post
2 mV
TYPE B CELL
post
post
10 mV
100 ms
C
MEAN CHANGE IPSP (mV)
0.0
-0.5
-1.0
-1.5
p < .05
*
ALT.
OVERLAP.
MEAN CHANGE ACTION POTENTIAL DURATION (ms)
20
15
10
5
0
**
*
ALT.
OVERLAP.
MEAN CHANGE AHP AREA (mV·ms)
0
-50
-100
-150
-200
*
*
ALT.
OVERLAP.

RESULTS AND DISCUSSION

Type B Cell Activity Contributes to PLP

As illustrated in Fig. 4, IPSP amplitudes were significantly reduced after overlapping presentations of light and type B cell hyperpolarization, which blocked light-evoked type B cell spike activity (0.86 ± 0.24 mV decrease from a baseline of 1.97 ± 0.35 mV; $t(4) = 3.62$, $p < .02$). By contrast, IPSP amplitudes were not significantly changed after alternating stimuli, which allowed normal light-evoked type B cell activity (0.18 ± 0.15 mV decrease from a baseline of 1.59 ± 0.43 mV; $t(4) = 1.22$, $p < .29$). Most importantly, overlapping light and hyperpolarization presentations yielded a greater decrease in synaptic strength than did alternating presentations ($F(1, 8) = 5.87$, $p < .04$).

The most straightforward interpretation of these results is that type B cell activity, which was present in the alternating but not in the overlapping condition, contributes to PLP induced by short light steps, and this activity-dependent PLP is superimposed upon the debilitating effects of the hyperpolarizing stimulus. If activity had not potentiated the IPSP, then the two manipulations would have instead had comparable effects on synaptic strength.

High Levels of Type B Cell Hyperpolarization Have an Adverse Effect on Subsequent Synaptic Transmission

The large decrease in IPSP amplitude observed in the overlapping condition is consistent with our earlier conclusion (Experiment 2) that the extreme level of hyperpolarization required to block light-elicited type B cell activity has a debilitating effect on subsequent synaptic transmission. As noted above, if we assume that the hyperpolarization-related decreases in IPSP amplitude were comparable in the overlapping and alternating groups, then it seems likely that light-evoked type B cell activity, which was present only in the alternating group, contributed to the between-groups difference (i.e., the smaller reduction in IPSP amplitudes in the alternating group). By extension, the lack of an absolute increase in IPSP amplitudes in the alternating group implies that nonspecific adverse effects of type B cell hyperpolarization counteracted the potentiating effects of light.

Changes in Input Resistance Cannot Account for Differences in IPSP Amplitudes

There were no significant light-related changes in type A cell input resistance after either overlapping presentations (6.62 ± 2.82 MΩ increase from a baseline of 41.74 ± 3.92 MΩ; $t(4) = 2.35$, $p < .08$) or alternating presentations (12.59 ± 6.26 MΩ increase from a baseline of 38.86 ± 1.46 MΩ; $t(4) = 2.01$, $p < .11$) of light and type B cell hyperpolarization. Furthermore, there was no between-group difference in changes in type A cell input resistance ($F(1, 8) = 0.6$, $p < .41$).

Type B cell input resistance was increased after either overlapping (24.76 ± 7.63 MΩ increase from a baseline of 39.60 ± 3.30 MΩ; $t(4) = 3.25$, $p < .03$) or alternating (28.52 ± 4.55 MΩ increase from a baseline of 41.76 ± 7.26 MΩ; $t(4) = 6.26$, $p < .003$) presentations of light and type B cell hyperpolarization. However, there was no significant between-group difference ($F(1, 10) = 1.39$, $p < .27$).

Changes in Type B Cell Action Potential Parameters

The overlapping condition resulted in a significant increase in action potential duration (14.1 ± 4.4 ms increase from a baseline of 29.3 ± 4.1 ms, $t(3) = 3.20$, $p < .05$), along with a significant decrease in AHP area (130.2 ± 38.6 mV·ms decrease from a baseline of 241.5 ± 38.7 mV·ms, $t(4) = 3.37$, $p < .03$) and a marginally significant decrease in AHP peak (1.67 ± 0.64 mV decrease from a baseline of 4.19 ± 0.49 mV, $t(4) = 2.61$, $p < .06$) (Fig. 4.). Similarly, the alternating condition resulted in a significant increase in action potential duration (14.1 ± 2.4 ms increase from a baseline of 41.4 ± 9.4 ms, $t(3) = 5.99$, $p < .01$), along with a significant decrease in AHP area (69.0 ± 21.4 mV·ms decrease from a baseline of 164.5 ± 58.6 mV·ms, $t(4) = 3.21$, $p < .04$), although there was no significant change in AHP peak ($t(4) = 0.91$, $p < .42$). There were no significant between group differences for any of these three measures (action potential duration: $F(1, 6) = 0.000$, $p < .996$; AHP area: $F(1, 8) = 1.92$, $p < .21$; AHP peak: $F(1, 8) = 2.39$, $p < .17$).

These effects on action potential parameters—the broadening of the action potential and reduction of AHP—are largely consistent with the effects of hyperpolarizing manipulations on action potential parameters that were found Experiment 2. They further suggest that the nonspecific effects of hyperpolarization were comparable between the alternating and overlapping conditions in the present experiment, implying that the relatively larger IPSP amplitude in the alternating condition is in fact attributable to the facilitatory interactions of spike activity with other light-evoked processes.

GENERAL DISCUSSION

Induction of PLP

This series of experiments has demonstrated that both long (30-s) and short (3-s) light steps are capable of inducing potentiation at the type B to A cell connections, a phenomenon that we have termed "post-light potentiation." Moreover, both type B cell activity and other light-evoked processes contribute, under some circumstances, to PLP induction.

Type B Cell Activity Is Sometimes, but Not Always, Sufficient to Induce Potentiation

What role does light-evoked type B cell activity play in PLP induction? A 30-s step of depolarization-elicited type B cell activity yielded potentiation relative to both prestimulation baseline and control preparations, and this potentiation was comparable in magnitude to that induced by a ~30-s light step. Thus, light-elicited type B cell activity can contribute importantly to the induction of PLP by long light steps. By contrast, short (3-s) steps of depolarization-evoked type B cell activity did not yield any potentiation. This latter result indicates that brief bursts of type B activity per se are not sufficient to induce PLP and thereby implies that other light-evoked processes must be involved in the induction of PLP by short light steps. However, even though brief type B cell activity by itself is not sufficient to produce potentiation, it nonetheless plays a contributory role in PLP induction by 3-s light stimuli, given that light plus activity yields a larger IPSP than does light alone, without activity (Experiment 3).

Light-Evoked Processes Other Than Type B Activity Can Also Contribute to PLP

We were also interested in determining the role of light-evoked processes other than type B cell activity in PLP induction. As described above, these other processes are not necessary for the induction of PLP by 30-s light stimuli (Experiment 1), given that 30 s of activity produces facilitation comparable to that produced by light. By contrast, these other processes are necessary for the induction of PLP by 3-s light stimuli (Experiment 2), because 3 s of type B cell activity, without light, does not produce facilitation. However, given that light presentations associated with spike activity (Experiment 3, alternating condition) resulted in larger IPSPs than light presentations where spike activity was blocked (Experiment 3, overlapping condition), these other light-evoked processes by themselves are not sufficient to induce the full PLP effect yielded by 3-s light stimuli.

Type B Cell Activity and Other Light-Evoked Processes Can Interact Synergistically to Induce PLP

Finally, 3 s of type B cell activity contributed to PLP induction when it occurred during light presentations (Experiment 3, alternating condition), but it was not sufficient to induce potentiation when light was absent (Experiment 2, depolarization condition). Type B cell activity also enhanced the potentiating effects of light (Experiment 3, alternating vs overlapping conditions). Taken together, these findings suggest that type B cell activity and other light-evoked processes can interact synergistically to induce PLP.

Why Is Type B Cell Activity Not Always Sufficient to Induce Potentiation?

Although depolarization-induced type B cell activity was sufficient to induce potentiation in Experiment 1, this was not the case in Experiment 2. The simplest explanation for this difference is that activity-dependent potentiation at the type B to A cell connections has a parametric sensitivity and that 30-s spike trains (as used in Experiment 1) yield post-tetanic potentiation (PTP), whereas 3-s trains (as used in Experiment 2) do not. PTP is commonly attributed to the build-up of presynaptic $[Ca^{2+}]_i$ that occurs during a period of high-frequency spike activity (Kretz, Shapiro, & Kandel, 1982; Mulkey & Zucker, 1992). Although a depolarization-triggered influx through voltage-gated channels would provide one source of Ca^{2+}, the short (3-s) depolarization stimuli used in Experiment 2 may not have yielded a large enough increase in $[Ca^{2+}]_i$ to induce PTP. By contrast, the relatively longer (30-s) depolarization stimulus used in Experiment 1 may have increased $[Ca^{2+}]_i$ to a level sufficient to induce PTP.

There are several other differences between Experiments 1 and 2 that could conceivably have contributed to the different results, although we consider it likely that these other factors were not of primary importance.

First, although the light and depolarizing current steps used in Experiment 1 yielded comparable levels of both spike discharge and depolarization in type B cells, the stimuli utilized in Experiment 2 did not. Although the 3-s light and depolarization did generate comparable levels of type B cell spike discharge, the 3-s light presentations yielded considerably more depolarization than did the depolarizing current steps. Thus, it is conceivable that both the high-frequency type B cell spike discharge and the depolarization that are elicited by light can contribute to the induction of PLP.

Second, in principle, potentiation at type B to A cell connections could be enhanced by simultaneous presynaptic and postsynaptic activity (Hebb, 1949). This condition was satisfied by our light manipulations, given that both type A and type B photoreceptors spike during a light step. Hence, in principle such a mechanism may have contributed to the potentiation induced by light, at least in the case of 3-s light steps (Experiment 2). By contrast, during the comparable short depolarization manipulations (Experiment 2), only the type B cell was active. Hence, the lack of potentiation after nine 3-s steps of depolarization-induced type B cell activity may have been due to a lack of conjunctive type A cell activity. The fact that type B cell to type A cell synapses are nominally inhibitory (but see Fost & Clark, 1996b, and Yamoah, Matzel, & Crow, 1998) may seem to make a Hebbian-type mechanism unusual, given that type B cell activity would hyperpolarize rather than "take . . . part in firing" (Hebb, 1949) type A cells. However, it does not necessarily rule out Hebbian-type contributions. Indeed, recent data suggest that activity-dependent associative mechanisms can occur at inhibitory synapses (Bell, Caputi, Grant, & Serrier, 1993; Bastian, 1998; Ganguly, Bi, Schinder, Berninger, & Poo, 1999). A different situation exists for the long 30-s light stimuli used in Experiment 1, in which 30-s depolarizing stimuli restricted to type B cells induced potentiation comparable to that induced by 30-s light steps. Thus, irrespective of whether conjunctive pre- and postsynaptic activity contributes to PLP with short 3-s light steps (Experiment 2), the fact that 30 s of type B cell activity is by itself sufficient to produce PLP rules out the possibility that a pseudo-Hebbian or anti-Hebbian mechanism plays an essential role in the PLP generated by 30-s light steps.

Finally, in principle, it is also possible that PLP at type B to A cell connections has a heterosynaptic origin. Light activates cells other than the presynaptic type B cell utilized in a given experiment—other photoreceptors and other neurons are also activated, directly or indirectly. Hence, it is conceivable that the activity of one of these other cells plays a critical role in the production of PLP at a given type B to A cell synapse. This cell may not have been strongly activated by 3-s depolarizing current steps delivered to the type B cell in Experiment 2, which could explain why this manipulation failed to yield potentiation. Although we cannot rule out this possibility, the fact that a 30-s step of depolarization-induced type B cell activity *was* sufficient to induce potentiation in Experiment 1 tends to argue against a heterosynaptic PLP mechanism.

Intracellular Ca^{2+} Release May Contribute to both Light Adaptation and PLP Induction

Given that depolarization and activation of type B cells are not always sufficient to produce potentiation comparable to that evoked by light itself (Experiment 2), some other light-evoked process must also contribute to PLP. What then is the nature of this process?

One possibility—light-evoked intracellular Ca^{2+} release—is suggested by the fact that type B and A photoreceptors experienced light adaptation during a single, sustained 30-s light step (Experiment 1) as well as during a series of short 3-s light steps (Experiment 2), consistent with earlier work (Detwiler, 1976; Farley, Richards, & Grover, 1990; Frysztak & Crow, 1993; Muzzio et al., 1998; Rogers et al., 1994). Light triggers a release of Ca^{2+} from intracellular stores that is inversely related to the amplitude of depolarizing generator potentials during sustained or repetitive light steps (Muzzio et al., 1998). This

relationship suggests that rising levels of intracellular Ca^{2+} contribute to light adaptation, perhaps by suppressing a light-activated sodium current (Farley, 1987b; Talk & Matzel, 1996). That light adaptation occurred during our experiments provides indirect evidence for intracellular Ca^{2+} release, a process that may enhance transmitter release and contribute to the induction of PLP. However, it is not known how light-evoked Ca^{2+} release, which initiates near the photoreceptor soma, would influence transmitter release at synaptic terminals. One speculative possibility is that waves of Ca^{2+}-induced Ca^{2+} release (Blackwell & Alkon, 1999; Tsien & Tsien, 1990) may propagate to synaptic regions.

Changes in Type B Cell Action Potential Parameters

As discussed above, PLP induction involves both type B cell activity and other light-evoked processes. A separate but related issue concerns the mechanisms underlying PLP maintenance and expression.

Increases in synaptic strength can arise from various presynaptic or postsynaptic mechanisms, which are not mutually exclusive. Two established presynaptic mechanisms for increasing transmitter release are (1) changes in action potential parameters, resulting from underlying changes in ionic conductances, and (2) other processes, such as spike release coupling, or changes in buffering of Ca^{2+}, which is critical for release. In *Aplysia* mechanosensory cells, nonassociative or associative training produces decreases in K^+ currents that prolong the action potential and reduce the AHP, thereby enhancing Ca^{2+} influx and transmitter release (Baxter & Byrne, 1989, 1990; Clark et al., 1994; Klein, Camardo, & Kandel, 1982; Klein & Kandel, 1978, 1980; Siegelbaum, Camardo, & Kandel, 1982; Hochner, Klein, Schacher, & Kandel, 1986a). Other mechanisms, including steps independent of action potential parameters (e.g., spike-release coupling), also contribute (Braha, Dale, Hochner, Klein, Abrams, & Kandel, 1990; Dale, Schacher, & Kandel, 1988; Ghirardi, Braha, Hochner, Montarolo, Kandel, & Dale, 1992; Hochner, Klein, Schacher, & Kandel, 1986b; Klein, 1994). In *Hermissenda,* computational simulations have suggested that training-induced changes in ionic currents of type B cells would also result in modifications of action potential parameters that could enhance transmitter release (Fost & Clark, 1996a). In support of this possibility, recent preliminary electrophysiological evidence (Leszczuk et al., 1997) suggests that *in vitro* associative training, but not unpaired training, alters action potential parameters of *Hermissenda* type B cells, leading to an increase in action potential height and duration and a decrease in the AHP. Such action potential changes would be expected to enhance synaptic transmission, further suggesting that changes in type B cell action potential parameters may contribute to synaptic facilitation in this system as well. However, it has not yet been empirically demonstrated that *in vitro* associative conditioning results in increases in synaptic strength at B to A cell connections.

We therefore investigated whether changes occurred in type B cell action potential parameters that may contribute to the synaptic potentiation we observed. In brief, we found that certain action potential parameters were modified after some manipulations that potentiated synaptic strength, but not others. These findings provide the first empirical evidence that training-related manipulations that produce synaptic potentiation also modify action potential parameters in type B cells. These results further indicate that both ionic and nonionic mechanisms contribute to the observed potentiation and that different manipulations produce nominally comparable potentiation through different mechanisms. More

specifically, for example, the single 30-s light presentation (Experiment 1) and the multiple 3-s light presentations (Experiment 2) both produced PLP, but they produced different modifications of action potential parameters. The 3-s light presentations broadened the action potential and reduced the AHP, perhaps reflecting a decrease in K^+ currents; by contrast, the 30-s light presentation did not change action potential duration but did increase the AHP, perhaps reflecting an increase in intracellular Ca^{2+} and an increase in a Ca^{2+}-activated K^+ current. Such differences in modifications of action potential parameters suggest that multiple mechanisms contribute differentially to potentiation resulting from different types of light stimuli or from light and type B cell activity.

PLP May Contribute to Light-Elicited Nonassociative Suppression of Phototaxis

Nonassociative suppression of phototaxis occurs after several forms of training involving light stimuli (Crow, 1983; Crow & Alkon, 1978; Farley & Alkon, 1982; Grover et al., 1987; Matzel et al., 1990; Rogers et al., 1994). Given that the type A cells have been proposed to drive phototaxis, a PLP-related increase in synaptic strength at the inhibitory type B to A cell connections could conceivably contribute to short-term, light-elicited nonassociative learning. This PLP would be expected to translate into reduced type A cell spike activity during subsequent light steps (as in Experiment 2) and, hence, reduced phototaxis. Type B cell input resistance is not consistently increased by light-alone training (Experiments 1–3; Richards, Farley, & Alkon, 1984; Rogers et al., 1994), and both type B and type A cells fire less frequently in response to each additional light presentation (Experiment 2). Hence, it is unlikely that a light-triggered increase in the type B cell firing rate plays a major role in light-induced nonassociative learning, although light adaptation in type A cells could in principle contribute. By contrast, a PLP-related increase in type B to A cell synaptic strength could conceivably contribute to a reduction of type A cell activity and, hence, to a reduction of phototaxis, separate from any intrinsic changes in type B cell firing frequency.

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