The effects of serotonin (5-HT) and GABA on two Ca\textsuperscript{2+} currents, a transient low-voltage-activated current (tLVA) and a sustained high-voltage-activated current (sHVA) were examined in isolated photoreceptors of *Hermissenda*. The sHVA current was blocked by 5-HT and reduced by activation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate. The effects of 5-HT were transiently reversed by staurosporine and partially blocked by the PKC inhibitor peptide [PKC(19–36)]. GABA enhanced both the tLVA and sHVA currents at low concentrations (5 nM to 5 \mu M) and reduced the sHVA current at high concentrations (>10 \mu M). The GABA-mediated enhancement of the Ca\textsuperscript{2+} current at low concentrations was sensitive to block by picrotoxin. The protein kinase A (PKA) inhibitor peptide [PKI(6–22)amide] blocked enhancement of both Ca\textsuperscript{2+} currents produced by cAMP analogs and GABA, suggesting that the effects at low concentrations may be PKA mediated. Caged GTP-\gamma-S released by flash photolysis reduced the sHVA current, and pretreatment of the photoreceptors with pertussis toxin blocked the effects of higher concentrations of GABA, indicating that at higher concentrations, the effects may be G-protein mediated.

**Key words:** calcium current; \gamma-aminobutyric acid; neuromodulation; cellular plasticity; *Hermissenda*; serotonin

Neural networks can perform diverse functions by the combined actions of classical transmitters and neuromodulators (for review, see Harris-Warrick and Marder, 1991). Modulatory inputs may produce diverse activity patterns of neural networks by activating different second messengers that affect several membrane conductances. The photoreceptors of *Hermissenda crassicornis* are sites not only for the process of phototransduction but also for Ca\textsuperscript{2+}-dependent neuronal plasticity (Alkon and Rasmussen, 1987; Crow, 1988). Numerous studies have provided evidence that Ca\textsuperscript{2+}, neurotransmitters/neuromodulators and second messengers contribute to enhanced excitability of identified type B photoreceptors observed after classical conditioning (Alkon, 1984; Farley and Auerbach, 1986; Crow et al., 1991; Falk-Vairant and Crow, 1992; Matzel and Alkon, 1991; Matzel and Rogers, 1993). Voltage-clamp studies of the B photoreceptors in conditioned animals have revealed a reduction in the amplitude of two K\textsuperscript{+} currents, the transient (\textit{I}\textsubscript{A}) and Ca\textsuperscript{2+}-activated K\textsuperscript{+} (\textit{I}\textsubscript{K,CA}) currents (Alkon et al., 1985; Farley, 1988). Serotonin (5-HT) produces reductions of \textit{I}\textsubscript{A} and \textit{I}\textsubscript{K,CA} that are similar to changes found in conditioned animals (Farley and Wu, 1989; Acosta-Urquidi and Crow, 1993). The reduction of \textit{I}\textsubscript{A} and \textit{I}\textsubscript{K,CA} by 5-HT may be mediated by the activation of protein kinase C (PKC) (Farley and Auerbach, 1986). In addition, GABA paired with depolarization of the photoreceptors produces enhanced excitability of the B photoreceptors (Matzel and Alkon, 1991; Alkon et al., 1993). Although the mechanism for the contribution of GABA to enhanced excitability in type B photoreceptors is unknown, recent evidence suggests that GABA increases intracellular Ca\textsuperscript{2+} at the synaptic terminals of the photoreceptors (Alkon et al., 1993).

To further examine modulation of membrane currents in *Hermissenda*, we studied the effects of GABA and 5-HT on two recently characterized Ca\textsuperscript{2+} currents in the type A and type B photoreceptors, a transient low-voltage-activated (tLVA) current and a sustained high-voltage-activated (sHVA) current (Yamoah and Crow, 1994a), and provide evidence for the contribution of second messengers to the modulation. At low concentrations (≤5 \mu M), GABA increased the tLVA and sHVA Ca\textsuperscript{2+} currents, whereas at high concentrations (>10 \mu M), GABA blocked the sHVA current. The sHVA Ca\textsuperscript{2+} current was reduced by 5-HT. cAMP analogs enhanced both the tLVA and sHVA Ca\textsuperscript{2+} currents. The effects of GABA and cAMP were blocked with inhibitors of protein kinase A (PKA). High concentrations of GABA may produce effects that are mediated by a G-protein, as shown by the reduction in the sHVA current by release of caged GTP-\gamma-S and pretreatment with pertussis toxin (PTX). Activators of PKC reduced the sHVA Ca\textsuperscript{2+} current; however, the time course of the effect was delayed (>10 min) compared with the effects of 5-HT (<5 min) on Ca\textsuperscript{2+} currents. These results suggest that the effect of 5-HT on Ca\textsuperscript{2+} currents may involve PKC, a direct action on the channels, or an as yet unidentified second messenger. A preliminary report of these results has been presented (Yamoah and Crow, 1994b).

**MATERIALS AND METHODS**

**Cell preparation.** Adult *Hermissenda* were obtained from Sea Life Supply (Sand City, CA). The photoreceptors were isolated using the protocol outlined in Yamoah and Crow (1994a). Briefly, the nervous systems were dissected from the animal and incubated for 10 min at 4°C in 1.0 mg/ml protease type VIII (Sigma, St. Louis, MO) and 7 mg/ml dispase grade II (Boehringer Mannheim, Mannheim, Germany) in artificial seawater (ASW) composed of (in mM): 420 NaCl, 10 KCl, 10 CaCl\textsubscript{2}, 22.9 MgCl\textsubscript{2}, 25.5 MgSO\textsubscript{4}, 15 Hepes (free acid) at pH of 7.8 (NaOH). The initial enzyme treatment allows slow diffusion into the interstitial space of the nervous system. The nervous systems were transferred to room temper-
nature (21°C) for 10 min and then incubated at 4°C for 20–30 min in fresh ASW. We cut the posterior tip of the photoreceptor to allow digestion of the connective tissue and capsule around the photoreceptors, but also loosened the capsule surrounding them. The eyes were pinched off from the nervous system, desheathed in 35 mm sterile culture dishes, and placed under an inverted microscope. Photoreceptors were identified as type A or type B on the basis of their position relative to the lens and the optic nerve. Isolated eyes without a lens and the stump of the optic nerve were therefore discarded. Desheathed photoreceptors were isolated from the eyes using mechanical agitation and a fire-polished pipette. The average yield for this procedure was two photoreceptors per eye. Isolated cells were incubated in ASW with 10 mM glucose and 50 mg/ml gentamicin sulfate (Sigma) at 4°C before electrophysiological experiments were performed.

Unless indicated, all chemicals were obtained from Sigma. A stock solution of 5 mM GABA was made with bath solution and stored at −20°C. Aliquots of the stock solution were added to bath solutions to achieve a desired concentration (5 nm–100 μM) and perfused into the experimental chamber (0.8–1.0 ml) at a rate of 1.0–1.5 ml/min. A solution of 10 mM of 5-hydroxytryptamine creatinine sulfate complex (5-HT) was also stored at −20°C; aliquots were added to the bath solution to achieve a final concentration of 5–20 μM in the peptide. All experiments with peptide inhibitors were performed within 48 h of reconstitution of the stock solution. PTX and guanosine 5′-O-(2-thiodiphosphate) trilithium salt (GDP-β-S) and guanosine 5′-O-(3-thiotriphosphate) tetralithium salt, (GTPT-γ-S) were obtained from Sigma and Calbiochem. “Caged” GTP-γ-S was obtained from Molecular Probes (Eugene, OR).

**Recording solutions.** Bath solutions were made from (in mM): 400 tetraethylammonium (TEA) acetate, 10 CaCl₂, 50 MgSO₄, 5 4-aminopyridine (4-AP), 15 HEPES (free acid), pH 7.7, with TEA-hydroxide and CsOH (TEA/OH). The pipette solution was made of (in mM): 20 NaCl, 2 MgCl₂, 10 EGTA, 20 TEACl, 300 CsCl, 300 N-methyl-D-glucamine (NMG), 10 glutathione (reduced), 5 MgATP, 1 Na₂GTP, 40–50 HEPES, pH 7.4 (TEA/0H). Nystatin (0.1 μM) was prepared in 100% methanol. Osmolarity of all solutions ranged from 0.96 to 1.00 Osm. The pipette solution was stored in 15 ml aliquots at −20°C, and fresh pipette solutions were used daily.

**Voltage clamp.** Ca²⁺ currents were recorded with a standard patch-clamp protocol using the whole-cell configuration (Hamill et al., 1981) using the Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA). Patch pipettes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) with a Flaming Brown microprobe puller, model P800/PC (Sutter Instruments, San Rafael, CA). The tips of the pipettes were fire polished and coated with SYLGARD #184 (Dow Corning, Midland, MI). Pipettes had a final resistance of 0.7–1.6 MΩ. Nystatin patches were established by filling the tips of the pipettes with the nystatin solution and backfilling with the pipette solution. Seals were established by the application of negative pressure at the end of the tubing connected to the pipette holder. Seal resistance ranged from 0.8 to 1.2 GΩ. Series resistance was 3.3 ± 1.2 MΩ (n = 29) and 7.8 ± 2.0 MΩ (n = 6) for nystatin patches. Series resistance was minimized with a compensatory circuit. Current records were filtered at 5 kHz and digitized at a frequency of 10 kHz with a Digidata interface (Axon Instruments). Ca²⁺ currents were evoked with command pulses from a personal computer (Gateway, Sioux City, SD) at a sample rate of 250 μsec/pulse using PCLAMP software version 5.7.1 and an A/D converter (Digidata, Axon Instruments). Passive leakage current was subtracted on-line and the current was corrected for the hyperpolarization of the hyperpolarized pulse (bath potential) to the test potential. Photolysis was achieved by a Chadwick Helmuth Strobex Model 278 xenon arc flash lamp (230 J maximum output). The light was focused onto an ellipsoidal mirror and the spectrum reflected by a dichroic mirror (Acton Research, Acton, MA) onto the aperture of a liquid light guide (Oriel, Stratford, CT). Aperture output of the liquid light guide was focused onto the cell using a plano-convex condenser lens and the microscope objective (Nikon Fluor 20×, M.A. 0.4). Wavelength selection was enhanced between the condenser and the preparation by using a UV light off a second dichroic mirror centered at −350 nm. Electromagnetic impulse-dependent current artifact lasted a few milliseconds.

**Data analysis.** Analysis of the current traces was made with CLAMPFIT software (Axon Instruments). The criteria used for acceptance of current records were similar to that outlined in Yamoah and Crow (1994a). (1) Series resistance (except for nystatin-containing electrodres) and seal resistance were <4.5 MΩ and >0.6 GΩ, respectively. (2) Active current traces did not exhibit “pumps.” (3) The current–voltage relation had a negative peak as the step potentials approached the apparent reversal potential of Ca²⁺. Statistics were computed with SigmaPlot software (Jandel Scientific, San Rafael, CA). Descriptive data are presented as means ± SD. Statistical differences within groups were determined using t tests for correlated means, and between-group differences consisted of t tests for independent groups. Two-tailed tests were used in the statistical analysis unless otherwise indicated.

**RESULTS**

**Effect of 5-HT on I_{Ca}.** Calcium currents and their modulation by 5-HT and GABA were studied in both type A and type B photoreceptors by blocking the outward currents I_{A}, I_{K,CA}, and I_{K,CA} with 4-AP, TEA, and Cs⁺. Inward Na⁺ current was blocked by choline substitution of Na⁺, and the inward rectifier current was blocked by Cs⁺. Two components of Ca²⁺ currents, a tLVA and an sHVA, have been identified previously in the photoreceptors (Yamoah and Crow, 1994a,b). Type A and type B photoreceptors were identified on the basis of their anatomical positions in the eyes. Isolated cells near the lens were classified as type A, and cells in the posterior part of the eye near the stunt of the optic nerve were classified as type B. Further classification of A and B photoreceptors as medial or lateral was not possible with these isolation procedures.

An example of the tLVA and sHVA Ca²⁺ currents recorded from type A and type B photoreceptors as medial or lateral is shown in Figure 1A. The whole-cell currents were elicited from a holding potential of −80 mV. With EGTA, ATP, and GTP in the patch pipette, the Ca²⁺ currents remained stable for at least 60 min after establishing the whole-cell configuration. As shown in Figure 1B, the application of 25 μM 5-HT blocked the sHVA current and had little, if any, effect on the tLVA current (see Fig. 1C). The magnitude of the reduction in the sHVA current in both type A and type B cells by 5-HT was similar (type A control: X = −1.1 ± 0.4 nA; n = 16; type B: X = −1.2 ± 0.4 nA; n = 15; t_{29} = 0.66, NS). The sHVA Ca²⁺ current recorded from both type A and type B photoreceptors was reduced by the application of 5-HT. An example of the tLVA and sHVA Ca²⁺ currents recorded from a type A cell is shown in Figure 1A. The whole-cell currents were elicited from a holding potential of −80 mV. With EGTA, ATP, and GTP in the patch pipette, the Ca²⁺ currents remained stable for at least 60 min after establishing the whole-cell configuration. As shown in Figure 1B, the application of 25 μM 5-HT blocked the sHVA current and had little, if any, effect on the tLVA current (see Fig. 1C). The magnitude of the reduction in the sHVA current in both type A and type B cells by 5-HT was similar (type A control: X = −1.1 ± 0.4 nA; 5-HT: X = −0.59 ± 0.3 nA; n = 15; t_{29} = 8.8; p < 0.0001) (type B control: X = −1.2 ± 0.4 nA; 5-HT: X = −0.65 ± 0.3 nA; n = 15; t_{29} = 10.9; p < 0.0001). The time course of the effect of 5-HT on the sHVA current (<5 min) was faster than the time course of current rundown (>60 min). The current–voltage relationship shown in Figure 1C revealed two apparent peaks (−5 and 30 mV) measured under control conditions (open circles) before the application of 5-HT. However, in the presence of 25 μM 5-HT, only one peak was expressed at −5–10 mV (filled circles, Fig. 1C). At holding potentials greater than or equal to −30 mV, at which the sHVA Ca²⁺ current was predominantly activated, 5-HT reduced the magnitude of the current without altering the inactivation kinetics. The effects of 5-HT were reversible within 5–7 min after washout (see Fig. 2C). Figure 1D illustrates the dose–response relationship for the effects of 5-HT on the sHVA Ca²⁺ current.
Ca\textsuperscript{2+} current. The half-blocking concentration of 5-HT on the shVACa\textsuperscript{2+} current was 2.4 μM. The concentration of 5-HT applied to isolated photoreceptors were lower than that reported previously for isolated nervous systems (Farley and Wu, 1989). Presumably higher concentrations are required for intact isolated nervous systems to overcome diffusional barriers and possible 5-HT uptake by both neurons and glial cells.

**Effects of PKC activators and inhibitors on Ca\textsuperscript{2+} current**

Previous research has shown that the effects of 5-HT on diverse K\textsuperscript{+} currents may be mediated by PKC (Farley and Auerbach, 1986; Crow et al., 1991). A potential role of PKC in mediating the effects of 5-HT on the shVACa\textsuperscript{2+} current was examined by measuring currents after the application of the PKC activator phorbol 12-myristate 13-acetate (PMA). The dose–response curve is shown in Figure 2A. The phorbol ester PMA is an effective activator of PKC in *Hermissenda* photoreceptors (Crow et al., 1991). Whereas 5-HT completely blocked the shVACa\textsuperscript{2+} current, PMA partially reduced the current as shown in Figure 2B1. The statistical analysis of the difference scores for the 5-HT and PMA group data showed that 5-HT resulted in a significantly larger reduction in the shVACa\textsuperscript{2+} current compared with PMA (5-HT: D = 0.78 ± 0.05 nA; n = 9; PMA: D = 0.40 ± 0.08 nA; n = 9, t\textsubscript{16} = 4.11, p < 0.005). The inactive analog of phorbol ester, 4α-phorbol, did not affect either component of the Ca\textsuperscript{2+} current as shown in Figure 2B2. It is possible that PMA not only reduced shVACa\textsuperscript{2+} but also removed the inactivation of the transient component of the Ca\textsuperscript{2+} current. However, this is unlikely, because PMA...
did not affect currents from cells that predominantly expressed the tLVA current (data not shown). Thus, the effects of PMA cannot be attributed to its secondary effect on either the changes in the magnitude or the kinetics of the tLVA current.

The phorbol ester produced different kinetic effects on the sHVA current compared with 5-HT. However, this finding may be the result of the effect of phorbol esters on other cellular processes in addition to activation of PKC. A specific inhibitor of PKC (PKCI), [PKC(19–36)], reduced but did not completely block the effects of 5-HT on the sHVA current (Fig. 2B3). In addition, staurosporine, a broad-spectrum kinase inhibitor (Tamaoki et al., 1986; Yanagihara et al., 1991) transiently (3–5 min) reversed the effects of 5-HT (note that 5-HT was applied 3 min before staurosporine was perfused into the bath) (see Fig. 2B4). However, 10 min after application of both 5-HT and staurosporine, the Ca\(^{2+}\) current was reduced. It is conceivable that activation of PKC by 5-HT might increase over time and that prolonged 5-HT exposure may overcome the inhibitory effects of staurosporine.

We observed that the reduction in the Ca\(^{2+}\) current produced by 20 nM PMA was delayed relative to the effects of 20 \(\mu\)M 5-HT, as shown in Figure 2C. The effects of 5-HT on sHVA currents were observed within 3 to 5 min of application; however, the effects of PMA were not observed until 10–15 min after bath application. A summary of the group data and statistical results for the effects of 5-HT, active and inactive phorbol esters, and PKC inhibitors is presented in Table 1.
Effect of GABA on Ca\(^{2+}\) current

We investigated the effects of GABA on the two voltage-activated Ca\(^{2+}\) currents by using known analogs and inhibitors of GABA receptors. The effect of GABA on the sHVA current was studied from a holding potential greater than \(-35\) mV at which the tLVA current is predominantly inactivated. At low concentrations (5–1000 nM), GABA enhanced the sHVA current in both type A and B photoreceptors (type A control: \(\bar{X} = 1.18 \pm 0.32\) nA, 15 nM; GABA: \(\bar{X} = 1.43 \pm 0.38\) nA; \(n = 6, t_5 = 6.08, p < 0.005\)) (type B control: \(\bar{X} = 1.13 \pm 0.47\) nA, 15 nM; GABA: \(\bar{X} = 1.43 \pm 0.45\) nA; \(n = 7, t_6 = 7.66, p < 0.005\)).

The data were obtained from the peak current elicited at 20 mV. An example from a type B cell of enhancement of sHVA by 10 nM GABA is shown in Figure 3. The peak current elicited at 20 mV. An example from a type B cell of enhancement of sHVA by 10 nM GABA is shown in Figure 3.

The peak current elicited at 20 mV. An example from a type B cell of enhancement of sHVA by 10 nM GABA is shown in Figure 3.

\[ V_h = -80 \text{ mV}, \text{ and the step voltage was } +20 \text{ mV}. \]

Data are expressed as the mean peak current \( \pm SD \) (number of cells). Computed \( t \) statistics are for correlated means. NS, Not significant; Staur \(*\) 3–5 min after staurosporine application; Staur *, 7 or more min after staurosporine application.

### Table 1. Effects of 5-HT, active and inactive phorbol esters, and PKC inhibitors on calcium currents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control nA</th>
<th>Experimental nA</th>
<th>df</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>(-1.29 \pm 0.14) (9)</td>
<td>5-HT</td>
<td>(-0.50 \pm 0.03) (9)</td>
<td>8</td>
<td>15.1</td>
</tr>
<tr>
<td>—</td>
<td>(-1.02 \pm 0.22) (9)</td>
<td>PMA</td>
<td>(-0.61 \pm 0.14) (9)</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>—</td>
<td>(-1.10 \pm 0.07) (6)</td>
<td>4-α-Phorbol</td>
<td>(-1.09 \pm 0.07) (6)</td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td>PKCI</td>
<td>(-0.99 \pm 0.22) (7)</td>
<td>PKCI + 5-HT</td>
<td>(-0.82 \pm 0.21) (7)</td>
<td>6</td>
<td>2.8</td>
</tr>
<tr>
<td>5-HT</td>
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<td>5-HT + Stauro</td>
<td>(-0.90 \pm 0.21) (7)</td>
<td>6</td>
<td>14.6</td>
</tr>
<tr>
<td>5-HT</td>
<td>(-0.20 \pm 0.09) (7)</td>
<td>5-HT + Stauro</td>
<td>(-0.24 \pm 0.12) (7)</td>
<td>6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\( V_h = -80 \text{ mV}, \text{ and the step voltage was } +20 \text{ mV}. \) Data are expressed as the mean peak current \( \pm SD \) (number of cells). Computed \( t \) statistics are for correlated means. NS, Not significant; Staur * 3–5 min after staurosporine application; Staur *, 7 or more min after staurosporine application.
rent. The effects of PMA on the current waveform and time course exhibited different kinetics from the effects of GABA. To further investigate a possible relationship between GABA and PKC, we examined the effects of GABA on the Ca\(^{2+}\) currents in the presence of the PKC inhibitor PKCI (PKC(19–36)). In the presence of PKCI (5 μM) in the patch pipette solution, micromolar concentrations of bath-applied GABA (25 μM) reduced the sHVA Ca\(^{2+}\) current. Thus, PKC does not appear to contribute to the modulation of Ca\(^{2+}\) currents by GABA. The results of the statistical tests of data generated from various treatments are summarized in Table 2.

**G-protein-mediated reduction of Ca\(^{2+}\) current**

Previous research has implicated G-proteins as a target for Ca\(^{2+}\)-mediated plasticity in the photoreceptors (Matzel and Alkon, 1991). We examined this further by studying the effects of G-proteins on Ca\(^{2+}\) currents to determine which second messengers are responsible for the GABA-induced effects. As shown in the current–voltage plot in Figure 6A, the nonhydrolyzable form of GTP (GTP-γ-S) reduced the sHVA Ca\(^{2+}\) current compared with normal controls or controls that were dialyzed with GDP-β-S. The analysis of the group data revealed a statistically significant reduction in Ca\(^{2+}\) currents after the application of GTP-γ-S (control: \(X = -2.5 \pm 0.24\) nA; GTP-γ-S: \(X = -1.5 \pm 0.11\) nA; \(n = 3\), \(t_2 = 3.15\), \(p < 0.05\), one-tailed test). In contrast, GDP-β-S did not produce a significant reduction in the Ca\(^{2+}\) current (control: \(X = -2.7 \pm 0.18\) nA; GDP-β-S: \(X = -2.23 \pm 0.33\) nA; \(n = 2\), \(t_1 = 2.9\), NS, one-tailed test). We further confirmed the results by using a photolabile form of GTP-γ-S. Release of caged GTP-γ-S caused a decline in the magnitude of the COF current as shown in Figure 6B1. UV light alone did not affect the currents as
shown in Figure 6B2. In cells treated with the G-protein inhibitor PTX, the differential effects of GABA on Ca$^{2+}$ currents were abolished. After treatment with PTX, high concentrations of GABA enhanced the Ca$^{2+}$ current as shown in Figure 6C. The analysis of group data revealed that GABA (30 μM) enhanced Ca$^{2+}$ currents if cells were pretreated with PTX (PTX control: \( X = -8 \pm 0.08 \) nA, 30 μM; GABA: \( X = -4.44 \pm 0.09 \) nA; \( t_2 = -43.9, p < 0.005 \)). These results suggest that GABA may be acting through at least two second messengers, G-proteins and PKA.

### DISCUSSION

These results show that both 5-HT and GABA modulate Ca$^{2+}$ currents in the photoreceptors of *Hermissenda*. 5-HT blocks the small conductance Ca$^{2+}$ current; GABA increases both the tLVA and sHVA currents at low concentrations, and blocks only the sHVA current at higher concentrations. Studies of the photoreceptors in *Hermissenda* have suggested that the mechanism of plasticity is Ca$^{2+}$-dependent (Falk-Vairant and Crow, 1992; Matzel and Rogers, 1993). However, the mechanism of the induction and maintenance of Ca$^{2+}$-dependent neuronal plasticity is not understood. Previous studies have implicated GABA in the mediation of decreases in intracellular Ca$^{2+}$ at the synaptic terminals of the photoreceptors (Alkon et al., 1992). In addition, 5-HT produces enhanced excitability of type B photoreceptors and synaptic facilitation of inhibitory synaptic connections between type B and type A photoreceptors (Farley and Wu, 1989; Crow and Forrester, 1991; Schuman and Clark, 1994). Recently, it was reported that GABA, which under normal conditions is inhibitory, may transform a synapse into an excitatory one by an unknown mechanism (Alkon et al., 1992).

### 5-HT modulation of Ca$^{2+}$ current

Modulation of Ca$^{2+}$ currents by 5-HT has been demonstrated in several neural systems. 5-HT reduces a high-voltage-activated Ca$^{2+}$ current in dorsal raphe neurons (Penington and Kelly, 1990; Penington et al., 1992), embryonic chick sensory neurons (Dunlap and Fischbach, 1981), and spinal cord neurons (Sah, 1990). As a result of Ca$^{2+}$ current inhibition, neuronal excitability is reduced (Penington et al., 1992) and action potential durations are shortened (Dunlap and Fischbach, 1981; Penington et al., 1992). In contrast, a low-voltage-activated Ca$^{2+}$ current in spinal motor neurons is enhanced by 5-HT (Berger and Takahashi, 1990). Calcium currents in *Helix aspera* neurons (Paupardin-Tritsch et al., 1986) and *Aplysia* sensory neurons are enhanced by 5-HT through activation of PKC (Edmonds et al., 1990; Braha et al., 1993). However, in neuron R15 of *Aplysia*, potentiation of Ca$^{2+}$ currents by 5-HT is mediated by activation of PKA (Levitan and Levitan, 1988). Previous work in *Hermissenda* suggested that 5-HT increased Ca$^{2+}$ currents in type B photoreceptors (Farley and Wu, 1989) and pedal neurons (Jacket and Acosta-Urquidi, 1985). The initial studies of Ba$^{2+}$ currents in the type B photoreceptors did not identify the two components of Ca$^{2+}$ currents that were recently characterized by Yamashita and Crow (1994a). The failure to isolate these currents in earlier studies may be attributable to differences in the experimental procedures and potential current contamination in the voltage-clamp data. Previous studies have reported that in type B photoreceptors, 5-HT reduced \( I_a \) and \( I_{K,Ca} \) (Farley and Wu, 1989), slowed the rate of inactivation of \( I_{K,V} \), and in some cases reduced \( I_{K,V} \) (Acosta-Urquidi and Crow, 1993). Moreover, recent evidence suggests that the reduction of \( I_{K,Ca} \) by 5-HT is a consequence of modulation of \( I_{Ca} \) by 5-HT (Yamashita and Crow, 1995). In addition, 5-HT enhances an inward rectifier current \( (I_{IR}) \) (Acosta-Urquidi and Crow, 1993), which is conducted by Na$^{+}$ and K$^{+}$ (Matzel et al., 1995). Voltage-clamp protocols used in earlier studies to measure Ba$^{2+}$ currents in the photoreceptors may not have eliminated other potential ionic current contamination (Farley and Wu, 1989). Because some of these currents are not exclusively carried by K$^{+}$, shifting \( E_K \) to 0 mV does not necessarily indicate that a null potential for these conductances will be attained. If inward Ca$^{2+}$ currents are recorded in the presence of any 5-HT-sensitive inward or outward currents, the reduction of outward K$^{+}$ currents or enhancement of the inward rectifier current by 5-HT may be manifested in an apparent increase in a putative Ca$^{2+}$ current.

### Table 2. Effects of GABA, baclofen, picrotoxin, cAMP analog, IBMX, PKAI, and PKCI on calcium currents

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>nA</td>
</tr>
<tr>
<td>—</td>
<td>—0.95 ± 0.29 (7)</td>
</tr>
<tr>
<td>—</td>
<td>—0.17 ± 0.06 (7)</td>
</tr>
<tr>
<td>—</td>
<td>—0.94 ± 0.30 (7)</td>
</tr>
<tr>
<td>—</td>
<td>—1.01 ± 0.11 (9)</td>
</tr>
<tr>
<td>GABA (50 nM)</td>
<td>—0.78 ± 0.21 (6)</td>
</tr>
<tr>
<td>—</td>
<td>—1.28 ± 0.16 (7)</td>
</tr>
<tr>
<td>—</td>
<td>—0.90 ± 0.18 (5)</td>
</tr>
<tr>
<td>PKAI (5 μM)</td>
<td>—1.00 ± 0.21 (5)</td>
</tr>
<tr>
<td>PKCI (5 μM)</td>
<td>—1.08 ± 0.26 (6)</td>
</tr>
</tbody>
</table>

Asterisk indicates that the \( V_C \) was ~30 mV and step potential was +20 mV. \( V_C \) for other experiments was ~80 mV, and the step voltage was ~20 mV. The transient component of the calcium current \( (I) \) was elicited from a holding potential of ~80 mV to step potentials of ~10 mV. Data are expressed as the mean peak current ± SD (number of cells). Computed \( t \) statistics are for tests of correlated means. Picro, Picrotoxin; dibu-cAMP, cAMP-γγ-dibutyryl.
The reduction of $I_{\text{Ca},\text{Ca}}$ and $I_{\text{A}}$ by 5-HT has been attributed to activation of PKC, because application of phorbol esters and microinjection of PKC into the B photoreceptors produced similar effects (Farley and Auerbach, 1986). The following evidence implicates PKC as a possible candidate for 5-HT-mediated reduction of the sHVA Ca$^{2+}$ current. PMA, but not 4α-phorbol, reduced the Ca$^{2+}$ current, and staurosporine transiently (3–5 min) reversed the effects of 5-HT. PKCI reduced the effect of 5-HT on the sHVA Ca$^{2+}$ current. However, the time course of PMA reduction of the Ca$^{2+}$ current in the photoreceptors was delayed relative to the effects of 5-HT. Similar delayed effects of phorbol esters have been demonstrated for changes of action potential amplitudes in Aplysia bag cells (Conn et al., 1989). In Aplysia sensory neurons, it has been reported that the time course of the effect of 5-HT lags behind the time course of the effects of phorbol ester (Braha et al., 1993). In this study, we found that effects of 5-HT on Ca$^{2+}$ current was expressed earlier than the effect of PMA on the Ca$^{2+}$ current. However, the sequences of activation of PKC by phorbol ester and transmitters are quite different, because phorbol esters must permeate through the cell membrane to activate PKC and, thus, the time courses of activation of their effects are not expected to be identical. Taken together, these results suggest that the reduction of the Ca$^{2+}$ currents in the photoreceptors by 5-HT may be PKC mediated. In addition to PKC modulation, a possible role of CAM kinase II in the modulation of the Ca$^{2+}$ currents has been suggested and requires further investigation (Matzel and Alkon, 1991).

**GABA modulation of Ca$^{2+}$ currents**

GABA has two effects on the amplitude of Ca$^{2+}$ currents in the photoreceptors that are similar to effects reported for DRG neurons (Scott et al., 1990, 1991). At nanomolar and micromolar
concentrations, GABA increases and decreases the Ca\(^{2+}\) currents, respectively. GABA increases intracellular Ca\(^{2+}\) at the axonal terminal of the photoreceptors as measured with Ca\(^{2+}\) indicator dyes (Alkon et al., 1993). Recently, it was proposed that the GABA-induced rise in intracellular Ca\(^{2+}\) may be the trigger for the induction of cellular plasticity (Alkon et al., 1993). In an independent study, Matzel and Rogers (1993) observed that Ca\(^{2+}\) is required for the induction of plasticity in the photoreceptors. Although the intracellular Ca\(^{2+}\) release hypothesis is intriguing, we propose that GABA-induced increases in Ca\(^{2+}\) influx through voltage-activated Ca\(^{2+}\) channels may augment the intracellular release process, such as a Ca\(^{2+}\)-induced Ca\(^{2+}\) release. The contribution of these two processes acting together may exceed a threshold for the induction of Ca\(^{2+}\)-dependent changes in the photoreceptors. It is conceivable that beyond the threshold and the capacity of intracellular Ca\(^{2+}\) buffers, reduction of the Ca\(^{2+}\) currents by GABA may serve an important physiological function by preventing possible cell death as a result of intracellular Ca\(^{2+}\) overload.

What GABA receptors are responsible for the modulation of Ca\(^{2+}\) current?

The finding that baclofen reduced the Ca\(^{2+}\) currents suggests that a GABA\(\beta\) receptor is involved in the modulation of Ca\(^{2+}\) channels. Muzzio et al. (1994) have reported similar reduction of Ca\(^{2+}\) currents by baclofen in the photoreceptors. This is similar to the GABA\(\beta\) receptor-mediated inhibition of Ca\(^{2+}\) currents in hippocampal neurons (Pfrieger et al., 1994), cerebellar Purkinje neurons (Mintz and Bean, 1993), and sensory neurons (Scott et al., 1991). However, blockage of current enhancement by picrotoxin, a known GABA\(\alpha\) receptor antagonist (Puia et al., 1990), suggests that there may be different subtypes of GABA receptors that are sensitive to picrotoxin, but not similar to the known GABA\(\alpha\) anion receptor. The GABA receptor mediating Ca\(^{2+}\) current enhancement may be a subtype of a GABA receptor with some homologies to the GABA\(\alpha\) receptor, because it is known that different subunits of GABA receptors can form receptors with different pharmacologies (Verdoorn et al., 1990; Shimada et al., 1992). The possibility that a novel GABA receptor (GABA\(\beta\)_c), which has been characterized in bipolar neurons in salamander retina (Lukasiewicz and Werblin, 1994), is also present in Hermissenda photoreceptors requires further investigation. Alternatively, Alkon et al. (1992) have suggested the presence of two subtypes of GABA\(\beta\) receptors that have differential effects on membrane conductances in the photoreceptors. In either case, the GABA enhancement of the Ca\(^{2+}\) currents appears to be mediated through activation of PKA, because cAMP and

Figure 5. Effects of a cAMP analog, IBMX, and PKAI on Ca\(^{2+}\) currents. Current traces were generated before and after the bath application of cAMP analogs (2.5 mM); both the dibutylryl and the chlorophenylthio analogs of cAMP had similar effects. The difference current traces after the treatment are represented by the dotted lines. The cAMP analogs increased both tLVA (A1) and tHVA (A2) Ca\(^{2+}\) currents. Similar results were obtained after the application of IBMX (200 mM), as shown in B1 and B2. After dialysis of cells with PKAI (5 mM), GABA (50 nM) reduced the magnitude of the Ca\(^{2+}\) currents as shown in C1 and C2. Traces in A and B were generated from type A cells, and traces in C were from a type B cell. D, Group data showing the effects of both cAMP analogs (n = 7) and IBMX (n = 5) on the tHVA Ca\(^{2+}\) current and the effect of GABA on the tHVA Ca\(^{2+}\) current in the presence of PKAI (n = 6).
IBMX essentially mimic the GABA effects, and PKAI blocked the GABA-induced enhancement of the Ca\(^{2+}\) currents.

**G-protein and GABA reduction of Ca\(^{2+}\) current**

GABA-mediated reduction of the SHVA Ca\(^{2+}\) current may result from activation of a G-protein. The evidence for this is as follows: GTP-\(\gamma\)-S, but not GDP-\(\beta\)-S, reduced the SHVA Ca\(^{2+}\) current. Micromolar concentrations of GABA and baclofen also reduced the current. PKCI had no effect on the actions of GABA. An inhibitor of some G-proteins (PTX) blocked the reduction in the SHVA current typically produced by GABA at micromolar concentrations. In PTX-treated cells, GABA enhanced the SHVA Ca\(^{2+}\) current both at nanomolar and micromolar concentrations. Thus, the dual effects of GABA on the Ca\(^{2+}\) current is mediated through activation of PKA and a G-protein.

Several examples of G-protein modulation of Ca\(^{2+}\) currents have been reported from different systems, e.g., G-protein augmentation of agonist and antagonist actions of the dihydropyridine-sensitive high-voltage-activated Ca\(^{2+}\) currents in sensory and sympathetic neurons (Dolphin and Scott, 1989). In sympathetic neurons, it has been suggested that G-proteins regulate Ca\(^{2+}\) channels tonically (Scott and Dolphin, 1990). Interestingly, in DRG neurons, GABA\(_B\) receptors have been found to be coupled to Ca\(^{2+}\) channels via a G-protein (Scott et al., 1990). Moreover, GABA has a dual effect on a low-voltage-activated Ca\(^{2+}\) channel in DRG neurons. In contrast, the dual effects of GABA seen in *Hermisenda* photoreceptors were observed on the SHVA Ca\(^{2+}\) current, but not the TLVA Ca\(^{2+}\) current.
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