Putative Synaptic Mechanisms of Inhibition in *Limulus* Lateral Eye

**ALAN R. ADOLPH**

From the Neuroscience Laboratory, Eye Research Institute of Retina Foundation, Boston, Massachusetts 02114

dedicated to the memory of Detlev W. Bronk

**ABSTRACT** Serotonin (5-HT) perfusion of a thin section of *Limulus* lateral eye hyperpolarizes retinular and eccentric cell membrane potential, and blocks spike action potentials fired by the eccentric cell. The indoleamine does not directly affect retinular cell receptor potential or eccentric cell generator potential in response to light stimuli. LSD perfusion blocks both this inhibitory action of 5-HT and light-evoked, synaptically mediated, lateral inhibition. Iontophoretic application of 5-HT to the synaptic neuropil produces shorter latency and duration and larger amplitude of inhibition than does the perfusion technique. This inhibition is dose dependent; the accompanying inhibitory postsynaptic potential (IPSP) appears to have an equilibrium potential more hyperpolarized than normal resting potential levels of ca. -50 mV. IPSP amplitude is sensitive to extracellular potassium ion concentration: it increases with decreased $[K^+]_o$ and decreases with increased $[K^+]_o$. LSD blocks the inhibition produced by iontophoretic application of 5-HT. Interaction between light-evoked, natural synaptic transmitter-mediated IPSP's and 5-HT IPSP's suggests a common postsynaptic receptor or transmitter-receptor-permeability change mechanism.

The spike response to a light stimulus of one ommatidium is reduced or suppressed by simultaneous illumination of neighboring ommatidia in the lateral compound eyes of the horseshoe crab, *Limulus polyphemus*. The physiology of this lateral inhibition has been extensively studied by Hartline and coworkers, 1974 (Wolbarsht and Yeandle [1967] have reviewed some of this work and the findings of other workers in this field), and the results of such studies provide strong physiological evidence for a chemical synaptic basis (Adolph and Tuan, 1972).

The pharmacology and neurochemistry of the possible chemical synaptic basis of lateral inhibition nonetheless, has received relatively little study (Adolph, 1966; Behrens and Wulff, 1970; Adolph and Tuan, 1972). There have been somewhat more extensive studies of its histology (Hartline et al., 1961; Gur et al., 1972; Adolph and Tuan, 1972; Fahrenbach, 1969), although ultrastructural histochemical localization and formaldehyde fluorescence techniques have had more limited application (Adolph and Tuan, 1972; Gur et al., 1972; Adolph and Ehinger, 1975).
In this paper I present the results of further pharmacological studies of lateral inhibition which include the effects on intracellular measurements of membrane potentials of perfused and iontophoretically applied serotonin (5-hydroxytryptamine; 5-HT), modifications of the ionic environment, and the serotonergic synapse blocker, lysergic acid diethylamide (LSD).

METHODS

Animals

Adult *Limulus polyphemus*, 10–12 inches or larger across the carapace, were obtained from the Marine Biological Laboratory Supply Department, Woods Hole, Mass. or the Gulf Specimen Company, Panacea, Fla. The animals were maintained in a 150-gallon aquarium at 17°C and pH ca. 8. The aquarium light environment was operated on a 12:12, light:dark schedule with illumination during the light phase by two 40-W "daylight" fluorescents about 2 feet above the glass tank top. The animals were given a weekly feeding of minced clams.

Optical

A large (ca. 1.5-mm diameter) and a small (ca. 70-μm diameter) light spot were transmitted directly to the corneal surface by two individual fiber optic bundles (American Optical Corp., Bedford, Mass.). The corneal ends of the bundles were positioned by individual micromanipulator mechanisms which had X, Y, Z, and angular movements (Narashige, Labtron Scientific Corp., Farmingdale, N. Y.). The light source for the larger bundle was a 50-W tungsten-halogen lamp (Klinger Scientific Apparatus Corp., Jamaica, N. Y.) whose filament image was focused on the fiber bundle end through a heat-absorbing filter, any desired neutral density filters, and an electromagnetic shutter (Uniblitz, Velmex, Inc., Holcomb, N. Y.). An 18-W tungsten lamp, the light of which was beamed through a similar filter and shutter chain, illuminated the aperture of a microscope substage condenser which focused the filament image on the smaller fiber bundle. Either bundle alone, or both combined, could be used as the stimulus source in experiments on single receptors, a group of receptors, or the interaction between a group and a single receptor. Both lamps were powered by regulated current supplies and operated at their nominally rated color temperatures, without modification of their visible spectra by any filtering.

Electrophysiological

A thin (ca. 1 or 2 mm) slice of lateral eye, including the bulk of optic nerve fibers in a bundle 1–2 cm long, was mounted with its corneal surface accessible to optical stimulation, in a 10-ml perfusion chamber. The chamber was operated in a constant volume mode and was gravity-fed from appropriate solution reservoirs. Five volumes (50 ml) of solutions were perfused through the chamber during solution changes and this volume gave over 95% of final solution concentration in slightly over 1 min, as measured by dye concentration photometry. Flow rate was approximately 1 ml/s. For extracellular recording the vascular sheath was removed from the optic nerve and bundles with several nerve fibers were teased from the optic nerve. Extracellular responses were recorded from fibers within a bundle by means of a drawn-glass capillary suction electrode and conventional recording, display, and analysis techniques (e.g. Adolph, 1973). Intracellular recording electrodes were drawn of theta-section capillary tubing (R. Barlow, personal communication) which could be filled by rapid diffusion techniques and gave 60–100-MΩ...
electrodes when filled with 4 M potassium acetate. The tips of these electrodes were significantly smaller than 1-μm diameter, probably several tenths of a micron. Electrodes of similar configuration to the intracellular recording electrodes were used for iontophoretic application of serotonin and hydrogen ions arising in HCl and creatinine sulfate control solutions. Two iontophoretic electrodes, one carrying serotonin creatinine sulfate solution (pH ca. 3.5) and the other carrying either HCl or creatinine sulfate solutions at the same pH (ca. 3.5) as the usual serotonin ionophore, were mounted in a double microelectrode holder for the hydrogen ion control experiments. Although these small iontophoretic electrodes probably had transport numbers ca. 0.1 and could not pass currents greater than 100-200 nA, they were quite satisfactory in practice and allowed highly localized applications of 5-HT. More conventionally sized iontophoretic electrodes (ca. 1–3 μm) required excessively high braking currents, with associated long and erratic ejection latencies, to counteract the potent effect of even spontaneous 5-HT onto the highly sensitive synaptic regions in the proximal neuropil.

Pharmacological

The bathing medium in the perfusion chamber was an artificial seawater (ASW), prepared either from a commercially available salt mix (Instant Ocean) or from component reagents when ionic substitutions were required (Brown and Mote, 1974). The ASW was also used as the carrier medium for perfusion solutions of the 5-HT creatinine sulfate (Sigma Chemical Co., St. Louis, Mo.) and LSD-tartrate (Sandoz). Table I shows the statistics of experiments performed.

| Bath environment          | Extracellular recording (light-evoked lateral inhibition) | Intracellular recording |
|---------------------------|------------------------------------------------------------|-------------------------|
| 5-HT                      | (25:Adolph and Tuan, 1972)                                 | 3 retinular cells; 5 eccentric cells |
| 5-HT/0-[Ca++]             | 7 (1 with LSD)                                             |                         |
| 5-HT/Bufotenine           | 7                                                          |                         |
| LSD                       | 7                                                          | 4 eccentric cells with 5-HT |
|                           |                                                            | 1 retinular cell with 5-HT |
| Brom-LSD                  | 2                                                          |                         |
| 5-HT iontophoresis        | 9 eccentric cells with LSD, 0-[K⁺], 0-[CL⁻], and TEA environments. |                         |
|                           | [H⁺] iontophoresis controls                                |                         |
| Totals                    | 24                                                         | 22                      |

RESULTS

Serotonin (5-HT) is a potent inhibitor of spike firing in the lateral eye. This inhibition is apparently mediated by a mechanism which ultimately hyperpolarizes the membrane potential without affecting the eccentric cell generator potential or retinular cell receptor potential response to light stimuli. Fig. 1 shows an intracellular recording from an eccentric cell and the changes produced by
perfusion with 10 μM 5-HT. There is a complete blockade of spikes accompanied by a substantial hyperpolarization of resting membrane potential (ca. 10 mV), but no reduction in amplitude or modification of time-course of the generator potential. In fact, there is a slight increase in generator potential amplitude in response to the increased driving potential due to the hyperpolarized resting membrane potential. There is a long recovery period during which resting potential and spike rate return to control levels.

A continuous recording of retinular cell membrane potential during which light stimuli are periodically applied, is shown in Fig. 2 A. A control flush of ASW is given between passes 6 and 7, and a test flush of 10 μM 5-HT is perfused between 11 and 12. The remaining parts of Fig. 2 show the retinular cell responses on expanded time base and illustrate the finding that only the resting membrane potential is markedly affected by application of 5-HT. The amplitude and time-course of the receptor potential are essentially unchanged.

Blockade of the inhibitory action of directly perfused 5-HT by a preceding flush with 5 μM LSD, is illustrated in Fig. 3. The LSD had been applied about 70 min before the eccentric cell response in Fig. 3 A. Two minutes later, a 10 μM 5-HT flush gave only moderate inhibition (Fig. 3 B). Thirty-eight minutes later, after full recovery of the light-evoked response, a second 10 μM 5-HT flush gave a somewhat greater inhibition, showing progressive recovery from the usual prolonged effects of LSD.

In addition to the ability of LSD to block the direct inhibitory action of 5-HT, it also blocks light-evoked, synaptically mediated, lateral inhibition. The light-evoked inhibitory action, of a group of ommatidia, on the response of a neighboring test ommatidium, in a thin slice of lateral eye is illustrated in Fig. 4.
Figure 2. Retinular cell response to light during 5-HT perfusion. Upper trace in records A–F, shutter gate signal. Lower trace, intracellular recording of membrane potential. Reversed in G. (A) Long-duration record of responses to control ASW perfusion followed by test 5-HT perfusion. Calibration, 1 min and 10 mV. (B) Before ASW. (C) After ASW. (D) 12 min after C. (E) 3 min later, after 10 μM 5-HT. (F) 100 min after E (75 min after ASW flush at cycle 37 of experimental sequence, cf. part A). Calibration of graph lines in B–F, 1 s and 10 mV. (G) Responses at D and E superimposed with same zero reference (not shown).

Figure 3. Blockade of 5-HT inhibition of eccentric cell response by LSD. Upper trace, shutter gate signal. Lower trace, intracellular recording of membrane potential. (A) 70 min after perfusion with 5 μM LSD. (B) 2 min later after 10 μM 5-HT. (C) 2 min after B. (D) 2 min after C. (E) 50 min after D. (F) 2 min after E. (G) 2 min later, after 10 μM 5-HT. (H) 2 min after G. (I) 2 min after H. (J) 25 min after I. Calibration of graph lines, 1 s and 10 mV.
FIGURE 4. LSD blockade of light-evoked lateral inhibition. Firing rates of extracellularly recorded spike action potentials. Open circles, control spike response of optically isolated ommatidium to light stimulus in the absence of lateral inhibition. Filled circles, inhibitory decrement/control, i.e. inhibitory constant. Inhibitory decrement is the difference between control spike response and the response during activation of lateral inhibition by concurrent illumination of ommatidia neighboring the test unit. Any direct pharmacological actions affect the neighbors as well as the test unit; thus dividing the decrement by control response compensates for direct effects. For example, direct reduction of an inhibitory unit's firing rate would result in less inhibition on the test unit even though the inhibitory stimulus light intensity was unchanged. The ratio of inhibitory decrement to control response is also equivalent to the lateral inhibitory coefficient in the Hartline-Ratliff equations (Hartline, 1974). For further details of method and data presentation, refer to Adolph (1973).

The points represent the normalized firing rates of extracellularly recorded spike potentials, and normalized inhibitory coefficients (inhibitory decrement/control response). The results of the experiment illustrated by Fig. 4 show that, in addition to a direct inhibitory action on spike firing, LSD causes a marked, maintained diminution of inhibitory coefficient which does not recover with a time-course coincident with control, uninhibited response.

Perfusing the eye, even with relatively low micromolar concentrations of 5-HT, results in very long-lasting effects, desensitization to subsequent doses, and delayed recoveries. In addition, the site of action of the putative transmitter cannot be localized. Iontophoretic application of the chemical circumvents these difficulties and also allows repetitive testing of response to drug application under a variety of experimental conditions.
The results of a series of 5-HT doses iontophoretically applied to synaptic loci in the neuropil proximal to an ommatidium are shown in Fig. 5. The intracellular recording is from an eccentric cell in the ommatidium firing maintained, spontaneous spikes at a regular rate of ca. 6-7/s. The responses show that the inhibitory response to iontophoretically applied 5-HT is dose dependent. The 5-HT has an extremely potent inhibitory action when applied locally in the neuropil proximal to the ommatidium, i.e., the known locus of presumably inhibitory synaptic contacts (Gur et al., 1972). The inhibition, which outlasts the short (1 s) iontophoretic pulse, is evidenced by a transient suppression or reduction in spike frequency accompanied by a membrane hyperpolarization. The blockade of spike and hyperpolarization during 5-HT application, reveals underlying quantum bumps in the dark-adapted ommatidium being tested (Fig. 5 A).

The finely tipped iontophoretic electrodes probably have transport numbers no greater than 0.1 (Bradley and Candy, 1970). Thus a 1-s, 80-nA pulse of 5-HT ejects about $80 \times 10^{-18}$ mol.

Extrinsically hyperpolarizing the resting membrane potential by passing current through the recording electrode reduces the amplitude of the response to a

---

**Figure 5.** Eccentric cell response to iontophoretic pulse of 5-HT. Top trace, eject current indicated by voltage across 1-MΩ series resistor in ion electrode circuit. Bottom trace, intracellular recording of membrane potential. (A) 80-nA eject current, 1-s duration. (B) 60 nA. (C) 40 nA. (D) 20 nA. Calibration of bottom traces, 1 s and 10 mV.
5-HT pulse (Fig. 6). This suggests that if the membrane response to 5-HT is a conductance increase, it is to an ionic species the equilibrium potential of which is more hyperpolarized than the ~50-mV or so resting potential. We know from the work of Purple (1964) that antidromically evoked, synaptically mediated, lateral inhibition produces a conductance increase of this type. An ionic species having the appropriate strong hyperpolarizing driving potential is potassium.

The extracellular potassium ion concentration, \([K^+]_0\), was varied from zero to three times normal (ca. 30 mM) and the hyperpolarizing response to a 5-HT pulse determined, in the experiment illustrated in Fig. 7. The resting membrane potential was reset by intracellular current injection of the appropriate direction to control for modifications of the resting potential caused by extracellular potassium ion changes. Reducing \([K^+]_0\) resulted in a larger 5-HT-evoked hyperpolarization and greater suppression of eccentric cell spike firing. Such a \([K^+]_0\) reduction should cause an increased potassium equilibrium potential and this factor may be the basis of the effect on 5-HT response. Conversely, increasing \([K^+]_0\) decreases the potassium equilibrium potential and is seen to decrease the membrane hyperpolarization and spike suppression evoked by the iontophoretic application of 5-HT.

**Figure 6.** Effect of extrinsic membrane hyperpolarization on the IPSP response to a 5-HT pulse. Top trace, eject current monitor. Bottom trace, intracellular recording of eccentric cell membrane potential. (A) Control, no current, potential at resting level. (B–F) Responses at increasing levels of membrane hyperpolarization. Nonmembrane resistance components were not completely balanced out by bridge so that absolute level of membrane potential in the records is not significant with respect to determining IPSP equilibrium potential. The hyperpolarization of the membrane potential makes the ongoing quantum bump activity quite apparent. Calibration, 1 s and 10 mV.
LSD is effective in blocking the inhibitory action of iontophoretically applied 5-HT. This is illustrated in Fig. 8, where the control inhibition produced by a pulse of 5-HT is transiently reduced by a low, 0.5 \( \mu \text{M} \), LSD dose and blocked for a much more prolonged period by a stronger, 5 \( \mu \text{M} \) dose of LSD. The lower dosage is also less than 1/10 the dose level found necessary to block the action of perfused 5-HT, and LSD perfusion may thus be more effective in blocking the highly localized application of 5-HT by the iontophoretic technique.

The iontophoretically applied serotonin is ejected in a cationic moiety, serotonin creatinine sulfate solution at pH 3.0–3.5. Thus, a significant hydrogen ion concentration exists in the solution and these ions are ejected along with the serotonin. Brown et al. (1970) have shown that hydrogen ions can cause significant permeability changes, to both chloride and potassium ions, in the membrane of \textit{Aplysia} neurons. In order to test for any such effects in \textit{Limulus} eye several control experiments were done using HCl and creatinine sulfate as \( [H^+] \) sources. In these experiments, double iontophoretic electrodes were used, one electrode containing the serotonin complex and the other electrode, either HCl or creatinine sulfate (CRSO\(_4\)), at comparable pH levels (3.0–3.5). Fig. 9 illustrates
Figure 8. Blocking action of LSD on the IPSP response to a 5-HT pulse. Top and bottom traces, as in Fig. 7. (A) Control, before perfusion with 0.5 μM LSD. (B) 7½ min after LSD. (C) 10 min after B. (D) 5 min after 5.0 μM LSD. (E) 5 min after D. (F) 2 min after ASW flush. (G) Double the control 5-HT pulse duration, i.e. 2 × 5-HT. (H) Triple the 5-HT pulse duration, i.e. 3 × 5-HT. This higher dose of 5-HT was necessary to overcome the prolonged blocking action of the higher LSD dose (i.e. 5 μM). Calibration, 1 s and 10 mV.

The results of such control experiments. In Fig. 9 A, hydrogen ions (H+) from a HCl electrode were ejected by a quantity of charge equal to the ejecting charge in a succeeding serotonin (5-HT) pulse. The hydrogen ions were ineffective but there was an immediate and pronounced inhibition in response to the serotonin. Fig. 9 B and C, show a similar result when the control substance was CRSO₄. These results indicate that neither CRSO₄ itself (the same substance with which the test serotonin was complexed) nor any hydrogen ions accompanying it during the ejection process had inhibitory activity comparable to serotonin.

Finally, the interaction between the natural, light-evoked IPSP and the IPSP evoked by 5-HT locally applied to the neuropil by microiontophoresis, is illustrated in Fig. 10. Light alone produced a significant hyperpolarizing IPSP, recorded intracellularly in the eccentric cell, and comparable to the last re-
sponses in traces A and B of Fig. 10. However, the same inhibiting illumination applied during 5-HT application does not produce any IPSP and the response recovers progressively only after cessation of the 5-HT ejection and in parallel with the return of the membrane potential from the 5-HT-evoked hyperpolarization to its resting level. The interaction during 5-HT and subsequent recovery are also functions of the rate and quantity of the indoleamine ejected, as illustrated in Fig. 10 A and B. Fig. 10 C illustrates the fact that the order in which the inhibiting factors are presented is not significant. Thus a short 5-HT pulse ejected during a long light-evoked IPSP (second and fourth iontophoretic responses in the figure) results in a much attenuated 5-HT IPSP compared to controls evoked in the dark. One possible conclusion that may be drawn from these results is that both the natural inhibitory transmitter released in response to light-induced activity in neighboring ommatidia and 5-HT directly applied to the synaptic neuropil act on a common postsynaptic receptor in what may be some form of competition for the receptor sites or the membrane permeability changes in response to transmitter-receptor interactions.

**Figure 9.** Ineffectiveness of hydrogen ion and ionized creatinine sulfate pulses compared to 5-HT pulses in inhibiting eccentric cell firing. Top and bottom traces, as in Fig. 7. (A) [H+] from HCl solution and 40-nA pulse of 5-HT. (B) 5-HT from 5-HT creatinine sulfate complex and pure creatinine sulfate solution. (C) Increasing durations of creatinine sulfate pulses followed by a 5-HT pulse. Calibration, 5 s and 10 mV in A, 5 s and 4 mV in B, and 5 s and 2 mV in C.
FIGURE 10. Interaction between the 5-HT-evoked IPSP and the light-evoked IPSP. Top and bottom traces, as in Fig. 7. (A and B) Light-evoked IPSP's during and after long-duration 5-HT pulses of two intensities (50 and 90 nA). There was considerable iontophoresis current-induced noise recorded during the ejection pulses. (C) 5-HT-evoked IPSP's before and during light-evoked IPSP's. Two durations of 5-HT pulses, 2 and 3 s. Calibration, 5 s and 4 mV.

DISCUSSION

The earlier work on the inhibitory action of 5-HT on lateral eye responses strongly suggested its role as a putative inhibitory neurotransmitter (Behrens and Wulff, 1970; Adolph and Tuan, 1972). It not only had a direct inhibitory effect on spike firing but a blocking action on natural, presumably synaptically mediated, light-evoked lateral inhibition (Adolph and Tuan, 1972). In the present study, aspects of the synaptic mechanisms underlying the inhibitory actions of 5-HT have been examined using intracellular recordings of membrane potential in eccentric as well as retinular cells. In addition to a generalized perfusion method, the technique of highly localized drug application by microiontophoresis, selective synaptic blockade by LSD, and an initial study of the ionic basis for the postsynaptic membrane effects of 5-HT by varying extracellular ion concentrations, have been employed.

The results suggest that 5-HT is a highly potent agent that acts to increase membrane permeability to monovalent ions in a selective manner. This selective permeability increase favors those ions, e.g. K⁺ and possibly Cl⁻, having negative intracellular equilibrium potentials and possibly even further restricted to equilibrium potentials more hyperpolarized than the normal (?) resting potential.

The histochemical (Adolph and Ehinger, 1975) and ultrastructural and neurochemical studies (Adolph and Tuan, 1972) make 5-HT or a closely related
indoleamine the likliest candidate for the neurotransmitter subserving lateral, and most likely, self-, inhibition. Further evidence for this conclusion comes from the pharmacological and electrophysiological findings of Adolph and Tuan (1972), Adolph (1973), and the results of experiments reported in the present paper. A synthesis of all these findings suggests that lateral inhibition is mediated somewhat as follows: Spike activity invading collateral branches of eccentric (and retinular?) cell axons in the proximal neuropil, ultimately depolarizes presynaptic endings in the conventional manner. 5-HT, stored in presynaptic vesicles, is released from these vesicle stores into the intersynaptic space, possibly through an intermediary mechanism of increased presynaptic calcium ion concentration coupled to the spike-elicited depolarization process. 5-HT diffuses to adjacent postsynaptic membrane and interacts with a finite number of postsynaptic receptor sites. The interaction of 5-HT and the receptor sites produces the previously described membrane permeability changes and results, for membrane potentials at appropriate levels, in hyperpolarization. The effects of this hyperpolarization on the generation of spikes at the locus for such spike genesis, is an inhibitory modulation of spike activity which can range from slight to complete suppression.

The blocking effects of extrinsically applied 5-HT on naturally evoked, synaptically mediated inhibition may be explicable in terms of a competition, for the limited number of localized postsynaptic receptor sites, between synaptically liberated and extrinsically applied 5-HT. A similar mechanism may explain the action of LSD, since it too was found to have a direct inhibitory effect in the absence of lateral inhibition, in addition to an action blocking natural synaptic inhibition as well as locally applied 5-HT-evoked inhibition. Inactivation is hypothesized to occur by reuptake of extracellular 5-HT, probably by a presynaptic membrane process which would be a mechanism most efficient in terms of available 5-HT stores and the requirements for continued synthesis in the presynaptic cytoplasm, etc. Adolph and Ehinger (1975) have histochemically demonstrated an efficient uptake mechanism for 5-HT and its closely related indoleamines, 6-HT and 5,6-DHT. A reuptake rate sensitive to quantity of activated receptor sites as well as overall concentration may explain the prolonged recovery in response to perfused vs. iontophoretically applied 5-HT.

I would like to comment on two specific points concerning the experimental findings. First I will comment on the recovery of membrane potential and spike rate, even in the continued presence of the drug-containing bathing solution, during perfusion experiments. Although an objective explanation for the effect is not possible without a more extensive knowledge of the underlying membrane mechanisms, i.e., transmitter-receptor-membrane permeability modification, than is presently available, I offer the following speculations. (a) Inactivation (normal case): A small amount of transmitter (T) occurs either through natural synaptic release or iontophoretic pulse. Transmitter-receptor-membrane permeability mechanism (TRMP) remains unsaturated by ambient level of T. Inhibition is terminated through removal of T via reuptake into presynaptic cell. (b) Desensitization (gross excess of T): TRMP initially reacts in the normal manner, producing inhibition. Normal inactivation mechanism is ineffective in
reducing ambient level of T, which results in saturation and blockade of TRMP. The time scale of the resulting turn-off of inhibition is longer than normal inactivation turn-off. Prolonged desensitization of TRMP occurs, so that subsequent doses of 5-HT produce little or no activation of TRMP and a corresponding inhibitory effect.

Functional lateral inhibition gradually disappears with intense, long-duration, inhibitory stimuli, i.e., inhibitory fatigue. Purple (1964), Lange (1965), and others have found that neither light-induced nor antidromic inhibition can be maintained at high stimulus frequencies (ca. 30–40/s) for extended lengths of time, greater than ca. 20–30 s. Fatigue was accompanied by a gradual decrease in membrane hyperpolarization and conductance during constant inhibitory stimulation. There was a parallel recovery of spike rate in cells which had been firing before inhibition, which could extend to the original firing rate in cells under antidromic inhibition for several minutes. The fatigue did not involve spike conduction failure in the optic nerve since the antidromically evoked spikes showed no decrease in size even during the period of inhibitory fatigue.

Secondly, concerning the effects on retinular cell response (e.g. Fig. 2): The stated effects of 5-HT and LSD on the membrane potentials of retinular cells are the results of several repetitions of carefully controlled experiments. They are not random fluctuations of resting potential since they (a) are always in the same direction, (b) are of about the same magnitude and time-course, and (c) do not occur in response to control ASW flushes. The peculiar morphology of the retinular cells in the lateral eye and their relationship to the second-order eccentric cells should be noted. The retinular “axons” branch profusely in the synaptic neuropil, interwoven extensively with the branches of eccentric cell axons in that tangled mass. A spike-conducting function has not been convincingly demonstrated for these retinular cell axons. In addition, Gerschenfeld (1973) reports that the extrasynaptic membranes of many invertebrate neurons are sensitive to localized application of putative neurotransmitters. In some instances (Aplysia cells) these extrasynaptic loci are on cell bodies devoid of any morphological signs of synaptic endings, and hundreds of microns distant from conventional synaptic neuropil.

Some of the concepts discussed here, although heretofore experimentally unrelated to inhibitory mechanisms in the Limulus lateral eye, have been applied to synaptic processes in other invertebrate and vertebrate animals, most notably in the work of Gerschenfeld (1973) and Kehoe (1972) on Aplysia, and Phillis (1970), Curtis (1964), and Aghajanian et al. (1972), in various mammals.

Supported by the Massachusetts Lions Eye Research, Inc. LSD and BOL kindly supplied by the Bureau of Narcotics and Dangerous Drugs.

Received for publication 18 August 1975.

REFERENCES

Adolph, A. R. 1966. Excitation and inhibition of electrical activity in the Limulus eye by neuropharmacological agents. In Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press, Inc., Elmsford, N. Y. 465–482.
ADOLPH, A. R. 1973. Thermal sensitivity of lateral inhibition in the eye of Limulus. *J. Gen. Physiol.* 62:392–406.

ADOLPH, A. R., and B. EHINGER. 1975. Indoleamines and the eccentric cells of the Limulus lateral eye. *All Tiss. Res.* 163:1–14.

ADOLPH, A. R., and F. J. TUAN. 1972. Serotonin and inhibition in Limulus lateral eye. *J. Gen. Physiol.* 60:679–697.

AGHAJANIAN, G. K., H. J. HAIGLER, and F. E. BLOOM. 1972. Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons in rat brain. *Life Sci.* 11:615–622.

BEHRENS, M., and V. J. WULFF. 1970. Neuropharmacological modification of response characteristics of sense cells in the Limulus lateral eye. *Vision Res.* 10:679–689.

BRADLEY, P. B., and J. M. CANDY. 1970. Iontophoretic release of acetylcholine, noradrenaline, 5-hydroxytryptamine and D-lysergic acid diethylamide from micropipettes. *Br. J. Pharmacol.* 40:194–201.

BROWN, J. E., and M. I. MOTE. 1974. Ionic dependence of reversal voltage of the light response in Limulus ventral photoreceptors. *J. Gen. Physiol.* 63:337–350.

BROWN, A. M., J. L. WALKER, and R. B. SUTTON. 1970. Increased chloride conductance as the proximate cause of hydrogen ion concentration effects in Aplysia neurons. *J. Gen. Physiol.* 56:559–582.

CURTIS, D. R. 1964. Microelectrophoresis. In Physical Techniques in Biological Research, vol. 5. W. L. Nastuk, editor. Academic Press, Inc., New York.

FAHRENBACK, W. H. 1969. The morphology of the eyes of Limulus. II. Ommatidia of the compound eye. *Z. Zellforsch. Mikrosk. Anat.* 93:451–483.

GERSCHENFELD, H. M. 1973. Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.* 53:1–119.

GUR, M., R. L. PURPLE, and R. WHITEHEAD. 1972. Ultrastructure within the lateral plexus of the Limulus eye. *J. Gen. Physiol.* 59:285–304.

HARTLINE, H. K. 1974. Studies on Excitation and Inhibition in the Retina. F. Ratliff, editor. The Rockefeller University Press, New York.

HARTLINE, H. K., F. RATLIFF, and W. H. MILLER. 1961. Inhibitory interaction in the retina and its significance in vision. In *Nervous Inhibition*. E. Florey, editor. Pergamon Press, London. 241–284.

KEHOE, J. 1972. Ionic mechanisms of a two-component cholinergic inhibition in Aplysia neurones. *J. Physiol. (Lond.)* 225:85–114.

LANGE, G. D. 1965. Dynamics of inhibitory interactions in the eye of Limulus. Ph.D. Thesis. The Rockefeller University, New York.

PHILLIS, J. W. 1970. The Pharmacology of Synapses. Pergamon Press, Oxford.

PURPLE, R. L. 1964. The integration of excitatory and inhibitory influences in the eccentric cell in the eye of Limulus. Ph.D. Thesis. The Rockefeller University, New York.

WOLBARSHT, M. I., and S. YEANDLE, 1967. Visual processes in the Limulus eye. *Annu. Rev. Physiol.* 29:513–542.