Melatonin Action on Pineal Varies with Photoperiod

Abstract. *Administration of melatonin can prevent the diurnal rhythm of serotonin concentration characteristic of the pineal organ. Whether or not such a change is induced depends upon the point in the photoperiod when the hormone is injected. This observation indicates that the action of melatonin is affected by the photic environment of the recipient.*

A number of investigators have pointed out that, even now, assessment of the physiological role of the pineal organ is difficult. One reason may be that this gland appears to be periodically active and may be effective only when its active compounds (*1*), such as melatonin and 5-hydroxytryptophol, operate at a given time in the photoperiod. With this hypothesis in mind we have studied the effect of melatonin on the pineal organ in rats receiving carefully timed injections of this hormone at two particular point in the photoperiod.

Ninety-five young adult (initially 10 to 12 weeks old) female Sprague-Dawley rats were given daily subcutaneous injections of melatonin (50 μg in 0.2 ml oil), or the vehicle only, for 4 weeks (*2*). All animals were housed in rooms equipped with humidity and temperature controls and were exposed to 14 hours of light (*3*) (242 to 330 lumen/m^2 at cage level) followed by 10 hours of dark. As originally reported by Quay and later confirmed in this laboratory (*4, 5*), under these environmental conditions the pineal gland of the laboratory rat exhibits a diurnal rhythm of serotonin concentration. Its serotonin concentration reaches a maximum at the 8th hour of the light period, falls off rapidly with the onset of darkness, and reaches a minimum 4 hours later. Since the melatonin content of the pineal gland in the rat increases diurnally in the dark period and since the enzyme hydroxyindole-O-methyl transferase, necessary for the final step in the conversion of serotonin to melatonin, is suppressed by light, it appears that the serotonin rhythm reflects in an inverse fashion the production of melatonin (*6*).

We therefore injected melatonin at the 8th hour of the light period when little if any melatonin would normally be available to the rat, and during the 14th hour, just a few minutes before the onset of darkness when the rat's pineal seems to begin producing and releasing increased amounts of melatonin. At the end of the 28-day injection period the animals were killed, by a quick blow to the head, at any one of three specific points in the photoperiod. Their pineals were removed, individually extracted and, within 24 hours, they were assayed fluorometrically for serotonin by methods previously reported (see *5, 7*).

We found a well-defined serotonin rhythm in the oil-injected controls when their pineals were removed at the 8th hour of light or the 3rd hour of darkness and compared for serotonin content. Such a rhythm was not evident in rats receiving melatonin at the 8th hour of light although it was present and even appeared to be somewhat more marked in rats injected with melatonin as darkness fell (Table 1).

To check the possibility that melatonin given at the 8th hour of light was inducing a premature drop in serotonin which we were not detecting we killed 12 rats, given melatonin or oil daily at the 8th hour of light as previously described, three hours later, and analyzed their pineals for serotonin content. No drop in serotonin concentration was observed, an average of slightly more than 100 ng of serotonin per gland being found in both groups.

These results demonstrate that melatonin directly, or indirectly through the nervous system, shifts or blocks the usual serotonin rhythm of the pineal when the hormone is given at a specific time in the photoperiod; they indicate that other parameters affected by melatonin should be reexamined in animals receiving injections at different times in the photoperiod.

Table 1. Effect of timed injections of melatonin or oil on pineal serotonin concentration (nanograms per gland); S.E., standard error of the mean.

Removal of pineal		Treatment			
		Oil		Melatonin	
Hour	Cycle	Animals (No.)	Mean ± S.E.	Animals (No.)	Mean ± S.E.
		Animals injected 8th hour of light			
8	light	10	124 ± 15.6	13	103 ± 10.5
3	dark	14	65 ± 10.7	15	87 ± 11.0
			$P < .005$		$P < .4$
		Animals injected 14th hour of light			
8	light	8	107 ± 10.8	7	101 ± 9.1
3	dark	8	62 ± 9.7	8	36 ± 3.5
			$P < .025$		$P < .001$

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Long-Lasting Hyperpolarization after Activity of Neurons in Leech Central Nervous System

Abstract. *After a train of impulses, the membrane potential of sensory neurons may be increased for several minutes by an electrogenic pump. During this hyperpolarization, the sensitivity of the membrane potential to external potassium ions is increased, so that physiologically occurring increases in potassium concentration could influence synaptic processes.*

In crustacean stretch receptors, snail neurons, and mammalian C-fibers, a train of action potentials is followed by an increase in membrane potential that lasts seconds or minutes (*1*). This after-hyperpolarization is due to an electrogenic pump, which extrudes the Na^+ that has entered the cell during activity. Our experiments show that a similar hyperpolarization occurs after activity

in the cell bodies and synaptic processes of neurons in the central nervous system of the leech. When a cell is in the hyperpolarized state, the effects of small changes in external K^+ on the membrane potential are accentuated. The possibility therefore arises that the increases in extracellular K^+ concentration that occur physiologically are sufficiently large to influence the integrative function of a neuron that is recovering from previous activity.

Intracellular recordings were made from the cell bodies of sensory neurons within isolated ganglia of leeches (*2*). Ringer fluid of variable composition continuously flowed past the preparation, and trains of impulses were initiated by stimulating the cell through a microelectrode, or by way of its peripheral axon by means of natural or electrical stimuli. Figure 1A shows the hyperpolarization following a train of action potentials. The size and time course of the effect vary with the number of action potentials in the train; trains of longer or higher frequency produced larger and more slowly declining hyperpolarizations. For example, prolonged activation of a touch sensory neuron by natural stimuli can lead to a hyperpolarization of over 25 mv which gradually declines over a 15-minute period. To test whether the hyperpolarization was produced by an electrogenic pump, we applied strophanthidin, a specific inhibitor of cationic pumps (*3*), to the neuron. This reversibly abolished the hyperpolarization (Fig. 1). We consider this good evidence that it is produced by an electrogenic pump. Other experiments, in which cell conductance was measured and Mg was used to block synaptic transmission, clearly indicated that the hyperpolarization was not caused by the action of a chemical transmitter agent.

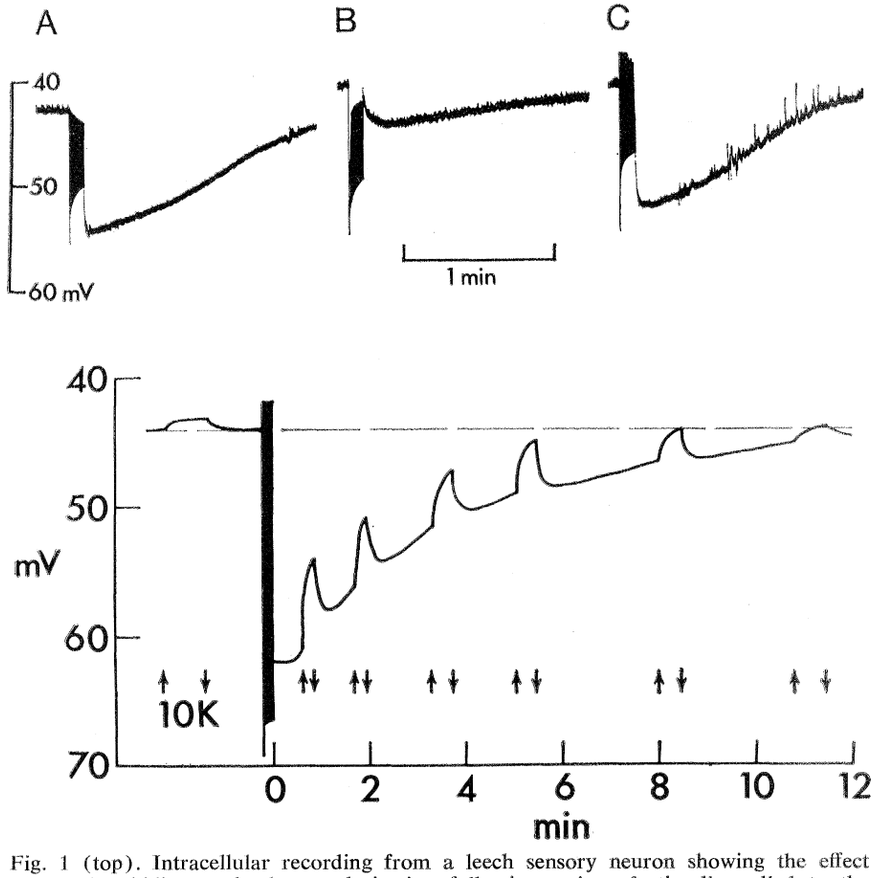

Fig. 1 (top). Intracellular recording from a leech sensory neuron showing the effect of strophanthidin on the hyperpolarization following trains of stimuli applied to the axon at 40 per second for 6 seconds. The action potentials are not seen, but their undershoots appear as heavy vertical lines. (A) Control response. (B) Two minutes after application of $1.3 \times 10^{-4} M$ strophanthidin. Note the reduction in the amplitude of the hyperpolarization. (C) Recovery 15 minutes after being returned to normal Ringer fluid. The decline in the amplitude of the undershoots is due to K^+ accumulation during the trains of action potentials (see text). Synaptic potentials can be seen as brief deflections in the record. Fig. 2 (bottom). Tracing of an intracellular recording to illustrate the effect of K^+ on the membrane potential of a leech neuron. The heavy vertical bar indicates the train of action potentials elicited by stimulation as in Fig. 1 at 20 per second for 8 seconds. Arrows mark the periods during which K^+ concentration in the bathing fluid was increased from 4 to 10 mmole/liter. At the resting potential (above left), 10 mM K^+ depolarizes the cell by only 2 mv. After the train of impulses K^+ has a more pronounced effect, the magnitude of which varies with the extent of the hyperpolarization.

The hyperpolarization does not occur only in the neuronal cell body, where the recording microelectrode is placed; it also involves the fine processes in the neuropil of the ganglion, where the sensory cells are in pre- and postsynaptic relation to other neurons. The evidence for hyperpolarization of these regions is the following: the touch sensory neurons are known to receive inhibitory synaptic inputs from cells which can be driven by electrical stimulation of their axons. At the normal resting potential the touch cells are hyperpolarized by the inhibitory transmitter (*2*). In our experiments we have shown that a sufficiently large hyperpolarization following activity reverses the inhibitory postsynaptic potentials so that they depolarize. This occurs because the voltage across the subsynaptic membrane of the sensory cell has exceeded the equilibrium potential for the inhibitory transmitter. In addition to this alteration in the synaptic currents generated by the sensory cells, a hyperpolarization of their presynaptic terminals might affect information transfer onto cells of higher order by modifying transmitter release.

The hyperpolarization confers on a neuron an enhanced sensitivity to the K^+ concentration in the bathing fluid. Figure 2 illustrates the effect of increasing the K^+ concentration around a sensory neuron from 4 to 10 mmole/liter before and after a train of impulses. At the normal resting potential the depolarization is only about 2 mv; the change is small because the resting potential of leech neurons is relatively insensitive to K^+ and does not obey the Nernst equation in this range. After a train of impulses the neuron becomes hyperpolarized by 18 mv and recovers with a half-time of about 4 minutes. Now the same increase in K^+ concentration leads to a considerably greater reduction in membrane potential (8 mv). In other experiments an increase of only 3 mmole of K^+ per liter has been observed to produce a similar but smaller effect. We have no direct evidence at this time for the mechanism by which

K^+ affects the hyperpolarized cell.

Increases in K^+ concentration sufficient to reduce the electrogenic hyperpolarization occur physiologically as the result of neuronal firing. A reliable estimate can be made of the buildup in K^+ concentration in the immediate vicinity of the neuronal membrane, the undershoot of the action potential being used as an indicator (*4*). With this technique it can be shown that when a single cell is stimulated at 45 stimuli per second for a few seconds the K^+ concentration around it may increase by 3 mmole/liter. When a whole nerve root is stimulated, the average concentration within the extracellular spaces can rise still further, to more than 10 mmole/liter, as estimated from the change in the membrane potential of the surrounding glial cell (*5*). Under such conditions, hyperpolarized neurons will be much more depolarized by K^+ than if their membrane potential had been at the normal resting level.

A speculation that can be made on the basis of these results is that K^+ liberated by active neurons might differentially affect adjacent cells depending on whether they had previously been active and were therefore hyperpolarized. Thus a cell which had conducted impulses would be more depolarized by a given increment of external K^+ produced by its neighbors than one which had been quiescent. If such a mechanism did operate, the glial compartmentation of neurons might influence the patterns of interaction around synaptic regions. For example, a common compartment might enclose one group of neurons, allowing them to interact by means of K^+ while reducing the effect of K^+ liberated from other cells.

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Lateral Giant Fibers of Crayfish: Location of Somata by Dye Injection

Abstract. *Using a new technique for injecting fluorescent dye, we have analyzed the intraganglionic architecture of the lateral giant interneuron in crayfish. Each lateral giant interneuron comprises a giant axon, an ipsilateral dendritic arborization of fairly constant form, and a contralateral soma. Antidromic axon spikes produce only small, electronic potentials in the soma; direct depolarization there fails to produce electrically excitable membrane responses and cannot discharge the main axon.*

The lateral giant fibers (LG) of crayfish are among the best-known of the giant cells in invertebrate nervous systems. Extensive electrophysiological (*1*) and morphological (*2*) studies have shown the LG to be separate segmental interneurons connected end-to-end by unpolarized electrical septa. Each cell also (i) makes synaptic contacts in the neuropil of its own ganglion (*3*); (ii) forms a rectifying electrical junction with a "motor giant" neuron (*4*); and (iii) joins with its contralateral partner in a nonrectifying commissural connection (*1*). Although the principal electrical junctions have been studied thoroughly, the major branches have not been mapped, nor have the somata been found. This information is needed for three purposes. First, the morphology of ganglionic branches will be helpful in interpreting the motor output produced by single-cell stimulation (*5*, *6*). Second, some invertebrate giant fibers are formed from the processes of many cells. Until the LG soma is found, its status as a single element is in doubt. Third, the relationship between the electrical activity of an interneuron soma and its connection with the axon could explain the puzzling fact that microelectrode records from somata in crustacean abdominal ganglia have revealed no interneurons (*6*). We here report the application of a new intracellular injec-

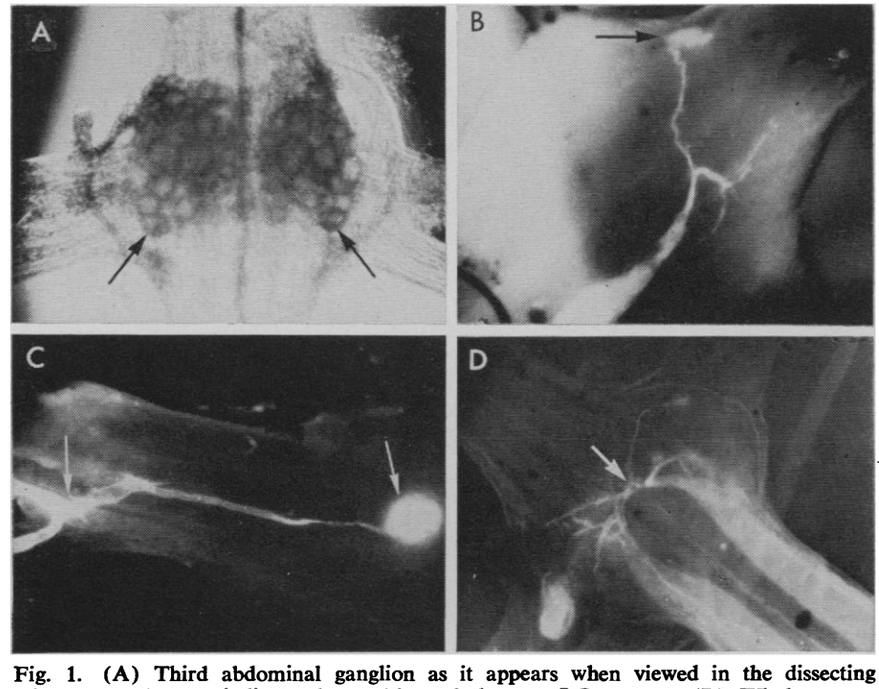

Fig. 1. (A) Third abdominal ganglion as it appears when viewed in the dissecting microscope. Arrows indicate the position of the two LG somata. (B) Whole mount of a cleared ganglion photographed with fluorescence microscopy after injection of the LG axon with Procion Yellow. The cell body is visible on the contralateral side (arrow); the dendritic branches are in the ipsilateral neuropil. (C) Electrical junction (left arrow) between fast flexor motoneuron and LG axon. The fluorescent dye crossed this junction, but on the other side remained confined to the motor giant cell whose soma (right arrow) is filled. (D) Double injection of LG axons to illustrate the commissural synapse (arrow). The lower cell body was broken off during the mounting procedure. For all figures, the width of the ganglion (1.0 mm) provides a size reference.