nid species that are tended by ants but do not possess honeydew glands have been described (*15*). Like myrmecophilous staphylinid beetles (*16*) and other ant associates, certain lycaenid species such as *G. lygdamus* might simply be exploiting the ants which tend them.

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10. A χ^2 goodness of fit revealed no significant difference in parasitism rates of caterpillars retrieved from individual inflorescences of the same plant versus those retrieved from inflorescences of separate plants.
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15. In an alternative evolutionary pathway, there may have been selection for lycaenid larvae to aggregate, thereby providing a highly attractive and accordingly well-defended food resource for ants. This seems to be the situation for many species of Lycaenidae in Australia [I. F. B. Common and D. F. Waterhouse, *Butterflies of Australia* (Angus and Robertson, Sydney, 1972)].
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Dye Transfer Through Gap Junctions Between Neuroendocrine Cells of Rat Hypothalamus

Abstract. *Most magnocellular neurosecretory cells that terminate in the posterior pituitary secrete either vasopressin, oxytocin, or enkephalin. Intracellular injection of the fluorescent dye Lucifer Yellow into single magnocellular neurons in slices of rat hypothalamus resulted in dye transfer between these cells. Freeze-fracture replicas of these cells occasionally revealed gap junctions, which presumably contain channels that mediate the dye coupling. These two independent techniques strongly suggest that some mammalian neuropeptidergic cells are electrotonically coupled, providing a possible means for recruitment and synchronization of their electrical activity.*

Magnocellular neurosecretory cells (MNC's) in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the mammalian hypothalamus synthesize the neuropeptides oxytocin, vasopressin, and enkephalin (*1*, *2*) as three immunohistochemically distinct populations (*3*). In the unstimulated rat, MNC's fire action potentials irregularly at a low rate. Increased discharge in characteristic spike patterns is associated with neurohormone release from terminals in the neurohypophysis (*4*, *5*). Specifically, a pulse of oxytocin, which is released by a synchronous burst (30 to 80 Hz lasting 1 to 4 seconds) of action potentials, causes milk ejection in the lactating female (*4*). Under conditions requiring water conservation, vasopressin is thought to be released through a gradual recruitment of MNC's into a "phasic" bursting pattern (10 to 20 Hz lasting 30 to 60 seconds) (*5*). Several mechanisms underlying these bursting patterns can be suggested. Local circuit neurons or fibers from extrahypothalamic inputs might synaptically drive the neurosecretory cells. Alternatively, nonsynaptic mechanisms, such as endogenous oscillations in membrane conductance (*6*) or fluctuations in the concentration of extracellular ions (*7*), could cause changes in membrane potential and associated burst discharge. Finally, electrotonic coupling could mediate synchrony (*8*, *9*) and contribute significantly to burst activity within a population of cells (*8*).

The morphological substrate of electrotonic coupling between cells in a variety of tissues is the gap junction, which contains channels that permit dyes and other molecules of < 1000 daltons to pass between cells without entering extracellular space (*10*). Indirect physiological evidence for electrotonic coupling and ultrastructural observations of gap junctions in thin section have been reported for neurons in several areas of the mammalian brain (*11*). It is not known whether mammalian neuroendocrine cells (*12*) or any other hypothalamic cells possess electrotonic junctions. In the study reported here we used single-cell injections of the fluorescent dye Lucifer Yellow CH (457 daltons) (*13*, *14*) and the freeze-fracture technique and found that some MNC's are dye-coupled and possess gap junctions. This provides two independent lines of evidence for electrotonic coupling between these mammalian neuropeptidergic cells.

Since first histologically defined (*15*), the hypothalamo-neurohypophysial system has served as the primary model for examination of brain peptide synthesis, storage, and release (*2*). However, our understanding of the electrophysiology of this system has been limited by the difficulties associated with obtaining intracellular recordings in the hypothalamus of intact mammals. Recently it was shown that the brain slice is a suitable preparation for impaling neurons in the PVN of the rat hypothalamus (*16*). The elimination of vascular pulsation and stereotaxic problems permits the intracellular recording and dye injection essential for the study of cell-to-cell coupling.

Magnocellular neurosecretory cells of the PVN (*17*) or SON were first injected with Lucifer Yellow and then identified by their minimum diameter of 15 μm and their large cytoplasm-to-nucleus ratio (Fig. 1). Of 32 well-filled MNC's, 14 were dye-coupled to a second MNC. The proportions of dye-coupled cells in the nuclei were similar (9 of 19 injected cells in the PVN, 5 of 13 in the SON). Of these, 4 of 9 injected cells were dye-coupled in males and 10 of 23 in females. The site of dye coupling appeared to be either dendrodendritic or dendrosomatic (Fig. 1A), although three pairs of MNC's had close cell body apposition (Fig. 1, B and C) suggesting somasomatic coupling

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sites. In two cases, a third MNC was faintly stained (not shown).

To control for nonspecific staining of nearby cells, ten ejections of Lucifer Yellow were made into extracellular space. No intracellular uptake of dye was observed under such conditions, and conversely, no extracellular dye was observed after single-cell injections. The occurrence of anode break spikes at the termination of each pulsed injection of hyperpolarizing current indicated that the impalement was maintained during dye injection. This observation, together with the occurrence of dendrodendritic and three-cell dye coupling, argues against the unlikely possibility of multiple-cell impalement during a single injection. Injury during impalement is an unlikely explanation for dye coupling because the spontaneous action potentials of dye-coupled cells (Fig. 2) were similar to those of noncoupled MNC's. It might be argued that the presence of Lucifer Yellow in multiple MNC's was due to leakage from the electrode tip during previous cell penetrations while searching for a stable impalement. However, these earlier penetrations were always brief (< 1 minute) and did not involve current injection; cellular staining was never observed under these conditions alone. Furthermore, dye-coupled neurons were usually found within 80 μm of each other (Fig. 1). Dye coupling has been documented in vivo between segments of crayfish septate axon and between neurons in *Hermissenda*, indicating that in these cases it was not a fixation artifact (*13*). Therefore, when Lucifer Yellow is injected into a single MNC, it selectively diffuses into other MNC's before fixation by a pathway isolated from extracellular space (*18*).

Gap junctions contain the channels

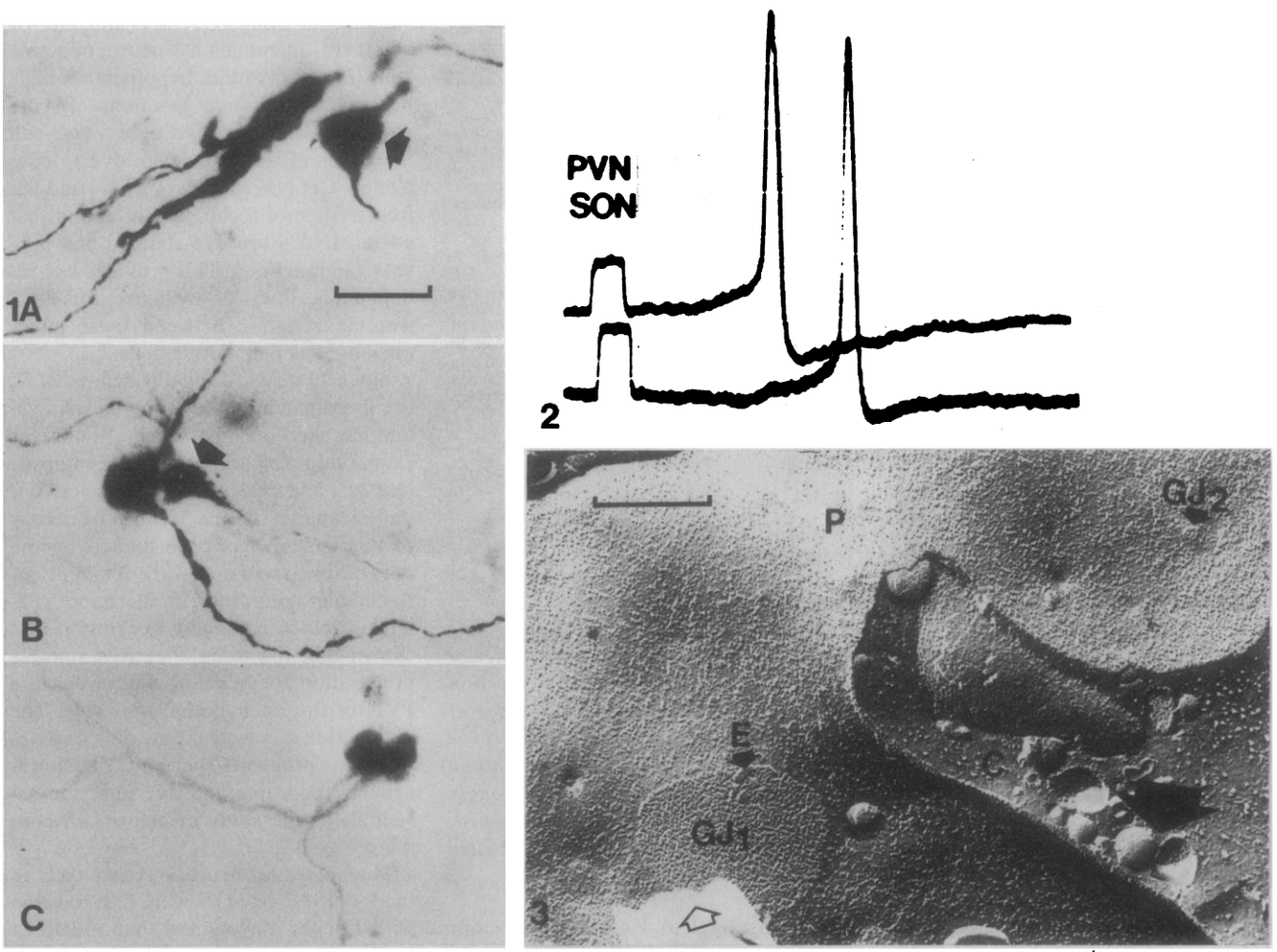

Fig. 1. Dye coupling between PVN and SON neurosecretory cells. Coronal hypothalamic slices were cut (500 μm) and single cells in the PVN or SON impaled and recorded from (*16*). Micropipettes were filled with 5 percent Lucifer Yellow in 0.33*M* lithium citrate. After cell impalement, dye was injected with constant hyperpolarizing current (7 nA, 30 to 180 seconds) interrupted every 15 seconds with brief hyperpolarizing pulses (7 nA, 50 msec, 5 Hz) to monitor anode break spikes of the impaled cells. At loss of response the injection was terminated. Only one cell per nucleus was injected. Ten dye ejections into extracellular space served as controls. Slices were fixed in 5 percent formaldehyde in 0.1*M* phosphate buffer (*p*H 7.3), sectioned (40 μm) on a freezing microtome, mounted on glass slides in methyl salicylate and viewed with a fluorescence microscope. Micrographs were reversed for clearer photographic reproduction. Injected MNC in (A) SON and (B) PVN is dye-coupled to a second MNC (arrow). In (C) there is close apposition of a dye-coupled pair in PVN. It is typical of such coupling that the dye diffused evenly throughout both cells. Calibration bar, 30 μm. Fig. 2. Spontaneous action potential from an MNC in PVN and in SON recorded in two different experiments. Each MNC was dye-coupled to another MNC. Calibration pulse, 10 mV, 5 msec. Fig. 3. Gap junction observed on neurosecretory cell in SON. For electron microscopy, PVN and SON were removed from freshly excised hypothalamus and fixed with 3 percent glutaraldehyde in 0.1*M* phosphate buffer (*p*H 7.3) for 1 hour at 3°C. After a buffer wash containing 2.5 percent glucose, the tissue was placed in 30 percent buffered glycerol for freeze-fracture in a Balzer unit. The gap junction (GJ_1) is on the freeze-fractured protoplasmic membrane face (*P*) of SON somata. Bits of exoplasmic membrane face (*E*) adhere. Another small gap junction (GJ_2) composed of about 15 particles is apparent. Cross fracture of the cell's cytoplasm (*C*) reveals typical neurosecretory vesicles (large arrow). Open arrow indicates direction of platinum shadowing. Calibration bar, 500 nm.

that electrotonically couple cells and underlie the phenomenon of dye coupling (*10*). If our hypothesis of coupling between MNC's is correct, we predict that gap junctions occur on the cell membrane of MNC's (*19*). In freeze-fracture replicas of the PVN and SON, MNC's were identified by the criteria of large cell body diameter (> 15 μm) and cytoplasmic content of neurosecretory vesicles (160 to 240 nm). Morphology typical of gap junctions in other mammalian nerve tissue (*11, 20*) was observed (Fig. 3). Aggregates of 15 to several hundred particles were occasionally (*21*) found on the protoplasmic face of membrane surrounding neurosecretory cytoplasm. Particle aggregates were surrounded by a partial halo of nonparticulate membrane. We conclude that gap junctions provide the channels by which MNC's are dye-coupled.

Neuronal populations that fire action potentials synchronously (*8, 9*) and endocrine populations stimulated to secrete hormone in unison (*22*) are usually composed of electrotonically coupled cells (*23*). Our results show that some neuroendocrine cells in the rat hypothalamo-neurohypophysial system are dye-coupled and possess gap junctions. Since ionic current can flow through gap junctions that mediate dye coupling (*10*), some mammalian neuroendocrine cells should also be electrotonically coupled. Two hypotheses are suggested. First, the recruitment of vasopressinergic MNC's into a phasic firing pattern in the rat could be enhanced by electrotonic coupling. Second, the spike burst of oxytocinergic MNC's before the milk ejection reflex could be synchronized by electrotonic coupling. Studies with immunohistochemical techniques (*24*) should reveal the identity of coupled neuropeptidergic cells.

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17. A percentage of somata in dorsomedial PVN innervates extrahypothalamic centers [T. Ono *et al.*, *Neurosci. Lett.* **10**, 141 (1978)]. We did not impale this area.
18. Our findings with Lucifer Yellow do not support the suggestion [U. Kuhnt, M. J. Kelly, R. Schaumberg, *Exp. Brain Res.* **35**, 371 (1979)] that intracellular Procion Yellow crosses chemical synapses, since we found secondary staining only in MNC's and not processes with abundant synapses on MNC somata.
19. Thin sections show close membrane apposition between some MNC's (*8*) without glial intervention.
20. We observed many gap junctions with similar morphology between ependymal cells of the third ventricle, medial to the PVN. We found them less commonly between glial cells within the PVN and SON.
21. Gap junctions are difficult to find on MNC's, possibly because only 1 to 2 percent of the soma membrane directly contacts other MNC's (*7*). However there may simply be a low incidence of identifiable channels. For example, regenerating hepatocytes still display dramatic electrotonic coupling and faint dye coupling even after gap junction numbers are greatly reduced, suggesting that electrotonic coupling can be mediated by very few channels [D. J. Meyer, S. B. Yancey, J. P. Revel, *J. Cell Biol.* **83**, 84a (1979); for further discussion of this point, see C. Peracchia, *Int. Rev. Cytol.* **66**, 81 (1980), p. 98].
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23. Spiking populations are not restricted to nerve cells. Some exocrine cells such as those in salivary gland of *Helisoma* [S. B. Kater, J. R. Rued, A. D. Murphy, *J. Exp. Biol.* **72**, 77 (1978)] generate action potentials that propagate through the gland by electrotonic synapses.
24. Difficulties with impalement and dye injection of single hypothalamic neurons of mammals in vivo have prevented correlation of single-cell physiology with neuropeptide content. In the preoptic nucleus of the goldfish, Lucifer Yellow has been injected into antidromically identified MNC's that were subsequently shown by immunohistochemistry to contain isotocin [T. A. Reaves and J. N. Hayward, *Cell Tissue Res.* **202**, 17 (1979)] and enkephalin [T. A. Reaves and J. N. Hayward *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6099 (1979)]. Dye coupling was not apparent. A similar approach in the rat should indicate the content of dye-coupled MNC's.
25. We are grateful to R. R. Shivers of the University of Western Ontario for assistance with the freeze-fracture technique, W. W. Stewart for numerous gifts of Lucifer Yellow, W. G. Filion for providing fluoresence microscopy facilities, and G. Weir for technical assistance. This research was supported by grants from the Connaught Foundation, National Science and Engineering Research Council of Canada (NSERC) (A0395), and NIH (NS 16683) to F.E.D.; by NIH grant NS 01940 to G.I.H.; and by Canadian fellowships from MRC and NSERC to R.D.A. and B.A.M., respectively.
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Unique Eye of Probable Evolutionary Significance

Abstract. *The eyes in the brain of a larval flatworm studied by electron microscopy are dissimilar. Light-sensitive structures in the right eye are microvilli; those in the left eye, the unique one, are both microvillar and ciliary. Perhaps this is evidence for the origin of a microvillar line of photoreceptoral evolution from a more primitive ciliary line.*

Larvae of certain marine flatworms have two symmetrically situated cerebral (that is, brain associated) eyes, called ocelli (*1*). In the larva of *Pseudoceros canadensis* studied by us (*2*) the two eyes are unlike each other. The right eye (Fig. 1) is composed of one cup-shaped pigmented cell (eyecup), whose cavity is directed laterally, and three sensory cells. Each sensory cell extends an array (rhabdomere) of straight, cylindrical, tightly packed microvilli into the

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