# A Direct Synaptic Connection Mediating Both Excitation and Inhibition

Abstract. *Neurons have generally been thought to produce only one synaptic action on any particular cell which they innervate. An identified interneuron in the abdominal ganglion of* Aplysia *mediates both direct excitation and inhibition to an identified follower cell. At low firing rates the interneuron produces excitatory postsynaptic potentials; however at higher firing rates these gradually diminish in size and eventually invert to inhibitory postsynaptic potentials. Electrophysiological and pharmacological evidence indicates that the connection between these cells is monosynaptic, and that a single transmitter, acetylcholine, mediates both actions. These opposite synaptic responses appear to result from the transmitter's acting on two types of postsynaptic receptors having different thresholds for activation and different susceptibilities for desensitization.*

Direct synaptic connections between two nerve cells have been assumed to mediate either postsynaptic excitation or inhibition, but not both. We have found a connection between two identified cells in the abdominal ganglion of the marine mollusc *Aplysia californica* which mediates both excitation and inhibition. The sign of the synaptic action is determined by the firing rate of the presynaptic element. This finding is of further interest because it contributes to an understanding of the topographical determinants of synaptic action in this ganglion.

The abdominal ganglion can be divided into quarters, each of which contains a number of cells that can be identified on the basis of morphological and electrophysiological criteria (Fig. 1A) (*1*). One identified cell, L10, is an interneuron which innervates many other identified cells. This interneuron produces inhibitory postsynaptic potentials (IPSP's) in identified cells in the left rostral quarter ganglion (LRQG) and excitatory postsynaptic potentials (EPSP's) in cells in the right caudal quarter ganglion (RCQG) (*2*). In the left caudal quarter ganglion (LCQG) the interneuron produces IPSP's in some cells and EPSP's in others. Cell L7, which receives both EPSP's and IPSP's from L10, lies in this quarter ganglion.

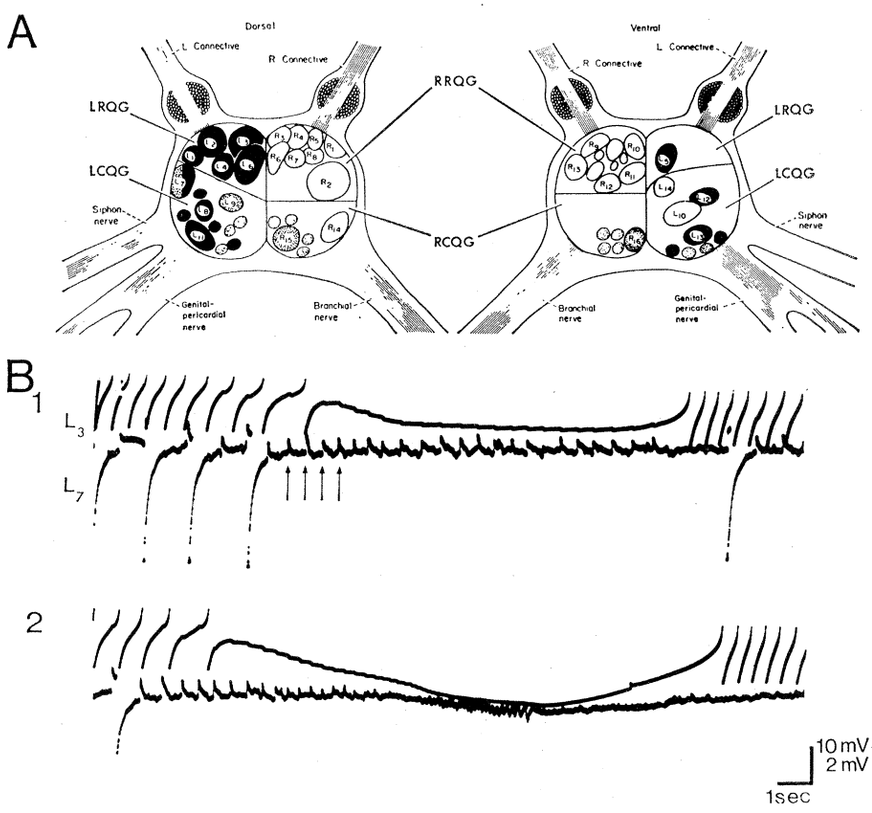


Fig. 1. (A) Schematic drawing of the abdominal ganglion of *Aplysia californica* showing the typical position of the 30 identified cells and of several cell clusters. Cells receiving an inhibitory connection from L10 are shown in black, those receiving an excitatory connection from L10 are stipled. Cell L7 receiving the dual connection is shown half black and half stipled. (B) Simultaneous intracellular recordings from L3 and L7, showing common postsynaptic potentials from the interneuron (L10) firing in bursts. The arrows in the upper pair of records indicate several EPSP's in L7 occurring simultaneously with IPSP's in L3. In the lower pair of records a very fast burst from the common interneuron causes the IPSP's in L3 to summate into a smooth hyperpolarization, while the EPSP's in L7 invert to IPSP's.

Figure 1B shows a simultaneous recording from L7 and L3, two follower cells of L10. Cell L10 generates a burst of postsynaptic potentials which is synchronous in both follower cells. When the frequency of the postsynaptic potentials in the burst is low, the unitary IPSP's in L3 are synchronous with EPSP's in L7 (Fig. 1, B1). Occasionally the burst reaches a higher frequency (Fig. 1, B2); when this occurs the IPSP's in L3 summate into a smooth hyperpolarization, whereas the EPSP's in L7 show a progressive decrement and finally invert to IPSP's.

To study the inversion more directly, we impaled L10 and controlled its firing by passing current through the microelectrode (Fig. 2). When the interneuron is fired slowly, every spike produces an EPSP in L7 (Fig. 2A). When the interneuron is fired rapidly, the EPSP's diminish in size and invert to IPSP's (Fig. 2, B1). If the firing of the interneuron is again slowed, the IPSP's persist for a number of spikes before the initial EPSP state is restored (beginning of Fig. 2, B2). A second and maintained increase in the firing rate of the interneuron produces a summated postsynaptic potential which is diphasic, consisting of an early and brief depolarization followed by a sustained hyperpolarization (end of Fig. 2, B2). The EPSP's produced by L10 can summate to increase the firing rate of L7, and the IPSP's inhibit firing (Fig. 2B).

The postsynaptic potentials in L7 follow the maximum firing frequency of L10. The EPSP does so initially; the IPSP, indefinitely. These data suggest that the double action is mediated monosynaptically. This was further examined with latency measurements. When we superimposed records (taken at high sweep speeds) of the action potentials of L10 and the postsynaptic potentials of L7 before and after their inversion we found that the latency for the two postsynaptic potentials was

equal (Fig. 3A). However, the latency for the postsynaptic potentials is at the upper limit of that generally encountered for presumed monosynaptic connections in this ganglion (*2*), and the possibility of two tightly interposed interneurons, one for excitation and the other for inhibition, could not be excluded from these findings alone. We tested whether the dual action results from the release of one or of more transmitters. If the connection is monosynaptic, the dual action is likely to be mediated by one transmitter in accordance with Dale's principles (*3*). The demonstration of a single transmitter in turn strengthens the arguments for a monosynaptic connection.

Anatomically verified monosynaptic connections between L10 and other follower cells (for example, L3, R15, and so forth) can be blocked by curare and appear to be cholinergic (*2, 4*). When we bathed the ganglion in a solution of $10^{-4}$ g of curare per milliliter of seawater, both the excitatory and inhibitory phases of the dual response in L7 were blocked (Fig. 3B). This finding suggests that both the EPSP's and the IPSP's result from cholinergic syn-

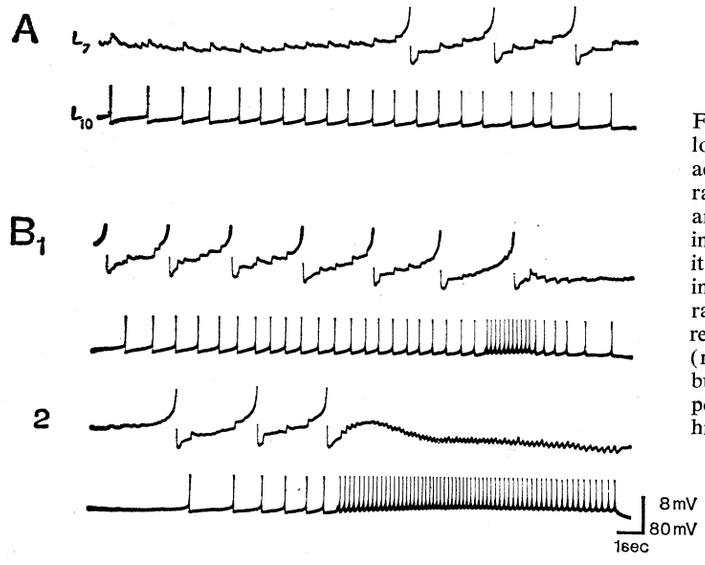


Fig. 2 (left). Simultaneous intracellar recordings from the follower cell L7 and the interneuron L10 showing a direct, double action, connection between them. (A) L10 is firing at a slow rate (about one per second), and every action potential produces an EPSP in L7. (B) B1 and B2 are continuous records. L10 is initially firing at a slow rate, producing EPSP's in L7, but when it is briefly speeded (B1) the EPSP's grow smaller and soon invert to IPSP's. For a short time after L10 returns to a slow rate the IPSP's persist, but gradually the initial EPSP state is restored (beginning of B2). When L10 is again speeded up (middle of B2) the EPSP's at first summate to depolarize L7 but then invert to IPSP's, which in turn summate to hyperpolarize L7. The IPSP amplitude remains stable as long as the high firing rate of L10 is maintained.

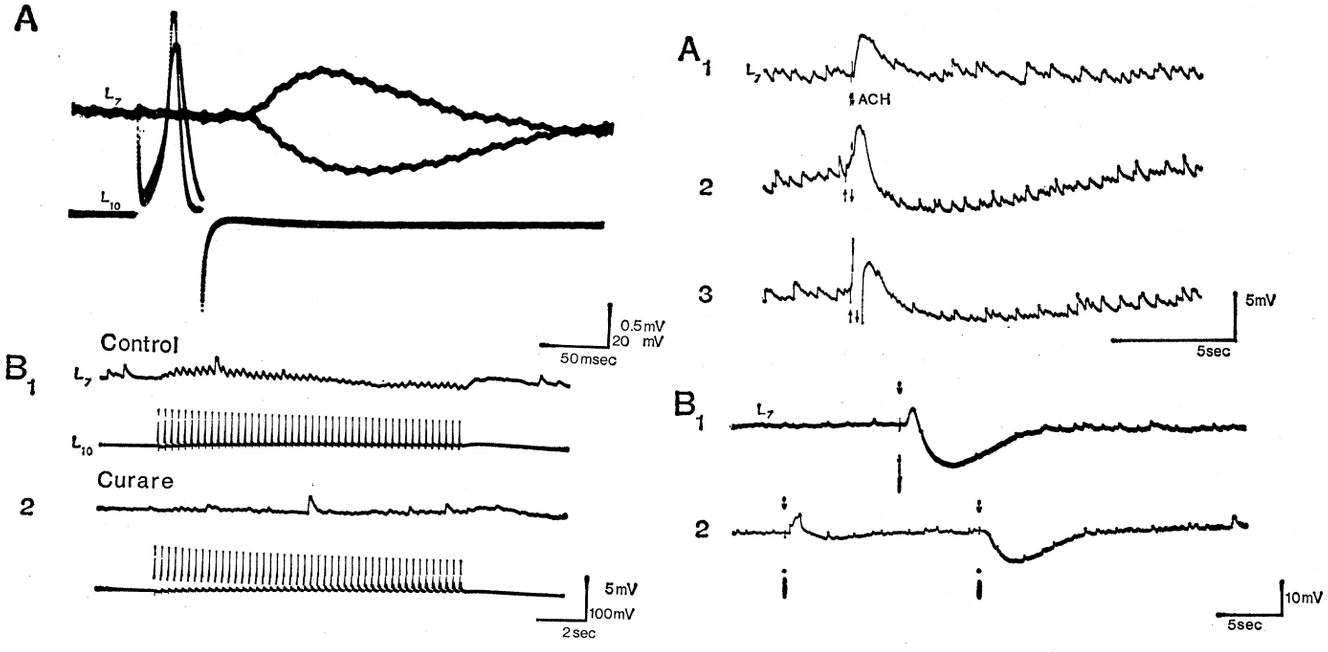


Fig. 3 (left). (A) Simultaneous high-speed recordings photographically superimposed to show a comparison of the latency of the EPSP and IPSP from L10 on L7. The IPSP is associated with the shorter and broader of the two action potentials in L10. The change in the configuration of the action potential of L10 results from the high firing rates needed to produce the inversion of the postsynaptic potentials in L7. (B) Simultaneous recordings from L7 and L10 showing the effect of curare. The control record shows both EPSP's and IPSP's in L7, produced by firing L10 at four spikes per second for 10 seconds. In the lower pair of records, taken 15 minutes after the application of *d*-tubocurarine ($10^{-4}$ g/ml), both the EPSP and IPSP have been blocked. Some other postsynaptic potentials produced in L7 by different interneurons are not blocked by curare. Fig. 4 (right). (A) Recordings from 17 showing the effects of iontophoretic applications of ACh. A very brief pulse of ACh (arrows) results in a pure depolarization (A1). A longer pulse of ACh produces a diphasic response consisting of an early, brief depolarization followed by a longer hyperpolarization (A2). The early depolarization is effective in producing an action potential (A3). (B) Recordings from L7 showing desensitization of the D component following repeated iontophoretic application of ACh. In B1 a single, strong application of ACh produces a diphastic response (D-H), whereas in B2 two consecutive weaker applications produce first a D response and then an H response. The separate responses (in B2) are similar to the constituent components of the diphasic response (B1).

aptic transmission and implies a single transmitter.

To further examine the possibility that one transmitter could produce opposite actions on a single follower cell, we applied acetylcholine (ACh) iontophoretically to the cell body of L7 with an external microelectrode. Iontophoretic application has the advantage of limiting the action of ACh to an area probably less than 50 $\mu$ in diameter (*5*) and provides the best experimental approximation of a single synaptic knob. Figure 4A shows the responses of L7 to pulses of ACh of increasing duration. A brief pulse gives a pure depolarizing response (Fig. 4, A1) while (at constant current) longer ones give a diphasic (depolarizing-hyperpolarizing) response (Fig. 4, A2 and A3), The diphasic ACh response is similar to the summated diphasic, synaptic response produced in L7 by a high-frequency discharge of L10 (Fig. 2, B2); the depolarizing phase of the ACh response causes L7 to fire, whereas its hyperpolarizing phase inhibits it. This response to ACh is quite distinct from the monophasic responses shown by other identified cells receiving single actions from L10. For example, L3, which receives only IPSP's from L10, responds to ACh by pure hyperpolarization; whereas R15, which receives only EPSP's from L10, responds with a pure depolarization. Indeed, diphasic responses have not been previously reported in surveys of the cells in this ganglion which categorized the response to ACh as being either purely depolarizing (D) or hyperpolarizing (H) (*6*). Our finding implies the existence of a new pharmacological cell type (D-H) with dual receptor properties (*7*). The ability to produce the dual response with highly localized applications of ACh also suggests that the two receptor types are spatially closely related.

With iontophoretic application of ACh we were also able to explore two alternative mechanisms—one presynaptic, the other postsynaptic—which could account for inversion of the postsynaptic potential: (i) The EPSP and IPSP might be mediated by independent branches of L10 ending separately on the two receptor patches, and the decrement of the EPSP could be caused by a presynaptic failure of the excitatory branch due to blocking at high rates of stimulation. Alternatively (ii) the EPSP and IPSP might be mediated by a single branch of L10 which ends on both receptors, and the decrement could represent a rapid desensitization of the excitatory receptor. With repeated iontophoretic application of ACh, the depolarizing component invariably decreased rapidly, providing support for the desensitization hypothesis. In addition, the hyperpolarizing component of the ACh response often increased with repeated applications. In the extreme case, we could demonstrate that the response properties of the two receptors were sufficiently different that the first of two closely spaced ACh pulses produced a pure depolarizing response, whereas the second pulse produced a pure hyperpolarizing response (Fig. 4, B2).

The simplest model to account for the diphasic response of L7 is that of a single branch ending on two separate (perhaps overlapping) postsynaptic receptors for ACh, one giving rise to excitation and the other to inhibition. The excitatory receptor has a low threshold and a rapid risetime and is quickly desensitized; the inhibitory receptor has a higher threshold and a slower risetime and is not readily desensitized. The existence of two different receptors to ACh is consistent with the recent finding of Chiarandini, Stefani, and Gerschenfeld (*8*) that the depolarization and hyperpolarization produced by ACh in different molluscan cells (H and DILDA) involve different receptor mechanisms producing permeability changes to $Na^+$ and $Cl^-$, respectively. It is likely that L7 has both the $Na^+$ and $Cl^-$ permeability mechanisms to ACh and that these generate the separate components of the dual response.

Cell L7 therefore appears to combine, in a single cell, the receptor properties of cells receiving either purely excitatory or purely inhibitory branches from the same interneuron (L10). These findings provide additional support for the hypothesis (*4, 6, 9*) that in this ganglion the sign of the synaptic action is determined, not by the chemical nature of the transmitter substance released by the presynaptic neuron, but by type of receptor and the number of receptor types of the follower cell. The presence of L7 in the only quarter ganglion to contain both excitatory and inhibitory follower cells of L10 also suggests that the distribution of the two types of receptors among different follower cells and the permissible combinations of receptors on a given cell are specified regionally within each quarter ganglion.

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## Mice: Individual Recognition by Olfactory Cues

Abstract. *Mice discriminated between two male mice of the same inbred strain on the basis of olfactory cues. Mice could also discriminate by olfactory cues between two different species, C3H* Mus musculus and Peromyscus maniculatus, *and between males and females.*

Recent investigations with mice have uncovered several important olfactory phenomena. Odors from mice have proved to act as pheromones (*1*) by modifying estrous cycles (Whitten effect), increasing the number of spontaneous pseudopregnancies (Lee-Boot effect), and blocking pregnancy (Bruce effect). There is also evidence of species and sex discrimination by mice on the basis of odor cues alone (*2*).

The well-known work of Bruce *et al.* (*3*) on blockage of pregnancy indicates that individual recognition within strains may occur: they report that females may distinguish between familiar and unfamiliar males of the same strain. Other investigators have argued,