and that mutations in this region of Tsr result in so called "locked" or nonresponsive signaling output (*16*). An 80-residue sequence following the second transmembrane domain of EnvZ (residues 180 to 259) shows little sequence similarities to the linker regions of the Tar and Tsr chemoreceptors. Nevertheless, mutations in this region also appear to cause "locked" signal transduction: the *envZ*11 ($Thr^{247} \rightarrow Arg$) mutation results in a constitutive *ompC* phenotype (*17*). Furthermore, we have found that a mutation that changes His^{243} to Val resulted in unregulated *ompC* expression (*9*).

A switch region within the cytoplasmic signaling domain of Tsr has been proposed to modulate signaling conformations of this molecule (*16*). This region is between amino acids 371 and 420 of Tsr and is highly conserved between MCP molecules (*2, 3, 14*). A comparison between EnvZ and Tsr indicates a stretch of similar amino acids between residues 378 and 388 of Tsr (Leu-Ala-Leu-Asn-Ala-Ala-Val-Glu-Ala-Ala-Arg) and between residues 340 and 350 of EnvZ (Ala-Val-Ala-Asn-Met-Val-Val-Asn-Ala-Ala-Arg). This region in EnvZ is one of the conserved sequences shared by EnvZ-like molecules (*18*) and may serve a switching function.

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12. Strain RU1012 [Φ(*ompC-lacZ*) 10-25, Δ*envZ*::Km^r (kanamycin resistance)] was constructed by infection of strain MH225 [Φ(*ompC-lacZ*) 10-25] (*24*) with a P1 phage lysate of strain AT142 (Δ*envZ*::Km^r) [T. Mizuno and S. Mizushima, *J. Biochem. (Tokyo)* **101**, 387 (1987)] and selection of transductants that were white on MacConkey agar plates (Difco) containing kanamycin. The phenotype of strain AT142 is very low levels of OmpC and constitutive expression of OmpF. Strain K1108 was constructed from an *ompR* mutant strain W-2 [*ompR*, Φ(*ompC-lacZ*) 10-25] (R. Brissette and M. Inouye, unpublished data), which contained pAT2002 (*envZ*) (*11*; Mizuno and Mizushima above), by infecting with a P1 phage lysate of strain AT142, and the *ompR* and *envZ* double mutant was selected as white colonies on MacConkey plates containing kanamycin and ampicillin. Strain MH225 was transduced with P1 phage grown on one of these double mutants, and a white colony (K1108) was selected on MacConkey plates containing kanamycin. Strain K1108 was identified as the double mutant (*ompR*, Δ*envZ*::Km^r) because OmpC and OmpF were absent from outer membrane protein profiles, which is consistent with an $ompR^-$ and $envZ^-$ phenotype (*5*). In addition, K1108 containing plasmid pAT428 (*ompR*, *envZ*) was red on MacConkey plates but was white (that is, it could not be complemented) when the strain carried only the *envZ* gene (pAT2002). Strain TZ322 was constructed by infecting strain MM551 [strain RP437, Δ(*tar-tap* 5201)] [M. D. Manson, V. Blank, G. Brade, C. F. Higgins, *Nature* **321**, 253 (1986)] with a P1 lysate of strain AT142 and was selected for kanamycin resistance; transductants were then checked for their inability to swarm on low agar plates.
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Acetylcholine and GABA Mediate Opposing Actions on Neuronal Chloride Channels in Crayfish

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A central principle of neural integration is that excitatory and inhibitory neurotransmitters effect the opening of distinct classes of membrane ionic channels and that integration consists of the summation of the opposing ionic currents on the postsynaptic membrane. In tangential cells of crayfish optic lobes, a hyperpolarizing, biphasic synaptic potential is produced by the concurrent action of acetylcholine and γ-aminobutyric acid (GABA). Acetylcholine hyperpolarizes the cell and increases chloride conductance. GABA depolarizes the cell by closing some of the same chloride channels. Therefore, in this case integration is achieved by the antagonistic actions of two transmitters on the same ionic channel.

TANGENTIAL (TAN1) CELLS ARE A ubiquitous class of centrifugal interneurons in the optic lobes of arthropods (*1*). TAN1 cells convey signals from dendrites in the second optic neuromere, the medulla externa, through a terminal arborization in the first optic neuromere, the lamina ganglionaris (Fig. 1A). The medulla is a rich source of acetylcholine (ACh) and GABA in several arthropods (*2*), including crayfish (*3, 4*). GABA-like immunoreactivity is observed in the axons of monopolar neurons (Fig. 1A) of the external chiasm, which project from the lamina to the medulla (Fig. 1B) and in columnar neurons within the medulla (Fig. 1C). The distribution of both transmitters is coextensive with the dendrites of TAN1 cells in the distal half of the medulla externa.

TAN1 cells respond to an increment of illumination with a graded, hyperpolarization [a postsynaptic potential (PSP)] of 10 to 20 mV, which decays to a smaller plateau (Fig. 2A) (*5*). The input conductance (G_n) of TAN1 cells change in parallel with the PSP; it increases at light onset (by 58 ± 18% SEM, $n = 10$) and then decreases toward a smaller value (17 ± 10% above resting G_n) (Fig. 2B).

Pharmacological analysis indicates that the light-elicited PSP and subsequent repolarization are mediated by ACh and GABA, respectively. Neuronal nicotinic antagonists such as pempidine and mecamylamine (*6, 7*) at micromolar doses reduce the PSP by 60 to 90%. Conversely, the GABA antagonist bicuculline (*8*) enhances the PSP and blocks the repolarization phase (Fig. 2C, lower trace). Bicuculline at 100 μ*M* increases the amplitude of the plateau phase by 245 ± 71% ($n = 6$) (±SEM).

ACh and GABA were pressure-injected into the medullary neuropile in the presence of 20 m*M* $CoCl_2$, which blocks synaptic

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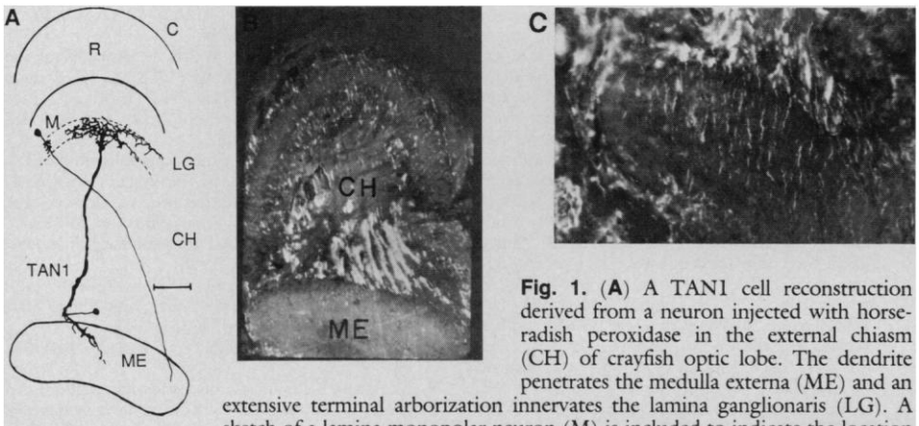

Fig. 1. (**A**) A TAN1 cell reconstruction derived from a neuron injected with horseradish peroxidase in the external chiasm (CH) of crayfish optic lobe. The dendrite penetrates the medulla externa (ME) and an extensive terminal arborization innervates the lamina ganglionaris (LG). A sketch of a lamina monopolar neuron (M) is included to indicate the location of a typical columnar cell with a decussating axon in the chiasm. The scale is 100 μm and also applies to (B) and (C). Cornea, C; retinular cell layer, R. Modified from Wang-Bennett and Glantz (*1*). (**B**) The distribution of GABA-like immunoreactivity in the crayfish optic lobe detected with antisera to GABA-glutaryl–bovine serum albumin conjugate (24-hour incubation) (Chemicon and T. Kingan) and visualized with goat antiserum to rabbit immunoglobulin G conjugated to fluorescein isothiocyanate (3-day incubation). Observable reactivity is entirely suppressed by absorption of rabbit serum with antigen. (**C**) Distribution of GABA-like reactivity in the fine axons of the medulla externa (incubation with primary and secondary antisera was for 3 and 9 days, respectively).

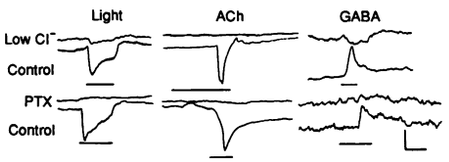

Fig. 3. (**Top half**) An 85% reduction in extracellular Cl^- concentration (top traces) diminishes light-, ACh-, and GABA-elicited responses. (**Bottom half**) Picrotoxin (PTX) at 100 μ*M* (top traces) reduces visual-, ACh-, and GABA-elicited responses. Scale is 5 mV and 1.0 s, except in the top left panel where it is 10 mV and 1.0 s.

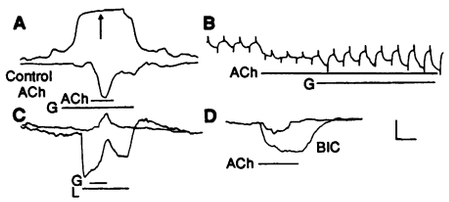

Fig. 4. Antagonist interactions. (**A**) The effect of superposing an ACh pulse on a longer GABA pulse, G (top trace). Arrow indicates the attenuated ACh response. (**B**) Conductance measurements during the superposition of a GABA pulse, G, on a longer ACh pulse. G_n was probed with −0.5-nA pulses. (**C**) The GABA (G)–elicited response (control, top trace) is enhanced during the hyperpolarizing light (L) response. (**D**) The ACh–elicited response is enhanced in 100 μ*M* bicuculline (BIC) (bottom trace). ACh and GABA are at 1.0 μ*M* and 10 μ*M*, respectively, throughout. Scale is 1.0 s and 10 mV.

function in the optic lobe (7). ACh (1.0 μ*M*) hyperpolarizes TAN1 cells (7) (Fig. 2D) and substantially increases G_n (159 ± 39%, $n = 11$) (Fig. 2E). The responses elicited with ACh and light have similar reversal potentials (7), and the ACh-elicited responses are also blocked by nicotinic antagonists.

GABA (10 μ*M*) depolarizes TAN1 cells by 5 to 15 mV (Fig. 2F) and reduces G_n (56 ± 6%, $n = 8$) (Fig. 2G). The GABA-elicited response is blocked by bicuculline (Fig. 2H, lower trace).

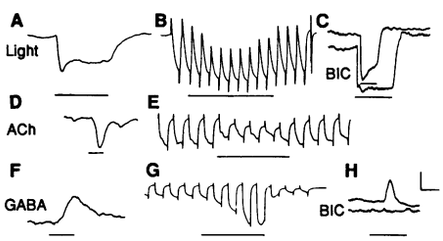

Fig. 2. Synaptic- and agonist-elicited potentials and conductances in TAN1 cells. (**A**) Light-elicited synaptic potential. Bottom trace indicates stimulus timing in this and subsequent panels. (**B**) Conductance changes during the light-evoked PSP, measured with −0.6-nA current pulses. G_n increases and then decreases during the synaptic response. (**C**) The effect of 100 μ*M* bicuculline (BIC, bottom trace) on the light-evoked response. Control PSP, top trace, shifted to the right for clarity. (**D**) Hyperpolarizing response to 1.0 μ*M* ACh. (**E**) Conductance increase elicited by 1.0 μ*M* ACh measured with −0.75-nA current pulses. (**F**) Depolarizing response elicited with 10 μ*M* GABA. (**G**) Conductance decrease elicited with 10 μ*M* GABA, measured with −0.5-nA current pulses. (**H**) GABA response in 100 μ*M* bicuculline (bottom trace). Control response to 10 μ*M* GABA, top trace. Scale, 10 mV and 1.0 s except in (H), which is 5 mV and 1.0 s.

Although the ACh- and GABA-elicited responses are distinct, they share a common sensitivity to variations in the Cl^--concentration gradient and to the presumptive Cl^--channel blocker picrotoxin (*9*). When 85% of extracellular Cl^- is replaced by SO_4^-, the membrane depolarizes from −30 mV to −16 ± 3 mV. This is consistent with a modest resting Cl^- conductance (G_{Cl}). In low Cl^- concentrations, the visual and ACh- and GABA-elicited responses are each diminished by 75 to 78% (SEM < 10%, $n = 5$) (Fig. 3, top half, top traces) and each of the responses may be inverted (Fig. 3, GABA, top trace). The reduced ACh and GABA responses result from the positive shift of the Cl^- equilibrium potential (E_{Cl}) and are consistent with the participation of a Cl^- conductance in these responses. At the crayfish neuromuscular junction, picrotoxin is a noncompetitive GABA antagonist (*9*) that blocks Cl^- channels [but see (*10*) for exceptions]. In TAN1 cells, 100 μ*M* picrotoxin produces a 10- to 15-mV depolarization and a modest reduction in G_n. Picrotoxin also reduces the light, ACh, and GABA responses by 84 to 100% (Fig. 3, lower half, top traces). This result implicates a Cl^- conductance in both the ACh- and GABA-elicited responses.

Thus, the responses to ACh and the natural hyperpolarizing transmitter released by light share a common sensitivity to nicotinic antagonists, a similar reversal potential, and a sensitivity to extracellular Cl^- concentration and picrotoxin; both responses are associated with an increase in G_n. Similarly, the responses to GABA and the natural depolarizing transmitter share a common sensitivity to bicuculline and picrotoxin, and both responses are associated with a decrease in G_n. If the two transmitters act on independent channels, then the increased resistance and depolarization associated with the GABA-elicited response should enhance a concurrent ACh-elicited response. Exactly the opposite occurs, indicating that the two transmitters act, at least in part, on the same channels. GABA significantly reduces the ACh-elicited hyperpolarization (74 ± 7%, $n = 5$, $P < 0.01$) (Fig. 4A, top trace). A similar conclusion is supported by the agonist-elicited changes in G_n. The average resting G_n of a TAN1 cell is $(8.3 \pm 1.3)10^{-8}$ S ($n = 19$). ACh (1.0 μ*M*) increases G_n by $(13.4 \pm 3.3)10^{-8}$ S ($n = 11$), and 10 μ*M* GABA elicits a decrease of $(3.9 \pm 0.6)10^{-8}$ S ($n = 8$). If the two agonists were acting on independent conductances, then concurrent action would produce a measurable increase in G_n. In fact, simultaneous application of ACh and GABA produces a significant decline in G_n (42 ± 12%, $n = 7$, $P < .01$) (Fig. 4B). These results imply that GABA is closing at least some of the channels opened by ACh. Thus, the two transmitters exert opposing actions on the same or an overlapping population of ionic channels. Consistent with this inference, the GABA-elicited depolarizing

response is enhanced when additional Cl^- channels are opened during the PSP (Fig. 4C, lower trace). Conversely, in the absence of Co^{2+}, bicuculline enhances the ACh-elicited PSP by 86 ± 3% (n = 3) (Fig. 4D, lower trace). The latter result implies that GABA is acting on TAN1 cells in the absence of light. Bicuculline blocks the action of this "spontaneously" released GABA and thus enhances the action of ACh.

In about 20% of crayfish neuromuscular junctions, GABA (*11*) and glycine (*12*) mediate a decrease in G_{Cl} as we have observed in TAN1 cells. Furthermore, there are several reports in which two or more neurotransmitters appear to act on the same receptor–ion channel complex. In each instance, however, the results imply a facilitating or mimicking action. Thus, glutamate, ACh, and glycine mimic the action of GABA at some GABA receptors at crayfish neuromuscular junction (*13*). Glycine is a cofactor for the action of glutamate at mammalian *N*-methyl-D-aspartate–type glutamate receptors (*14*), and glutamate potentiates the action of GABA in hippocampal neurons (*15*).

An important principle of synaptic integration is that excitatory and inhibitory postsynaptic events are mediated by distinct and independent ionic channels, which in turn gate currents driven by well-separated equilibrium potentials. Integration is achieved by the summation of the opposing currents on the postsynaptic membrane (*16*). The significance of our finding is that the antagonistic actions of two transmitters, ACh and GABA, are expressed by opposing effects on identical or an overlapping subset of ionic channels. This inference implies that either the receptor contains binding sites for both ACh and GABA (possibly on different subunits from within the receptor superfamily) or that two populations of receptors are coupled to the same population of Cl^- channels. It is also possible that one of the two transmitters affects the binding of the other. Our results also suggest a mechanism of synaptic integration that is independent of the cable properties and the spatial distribution of synapses on dendrites.

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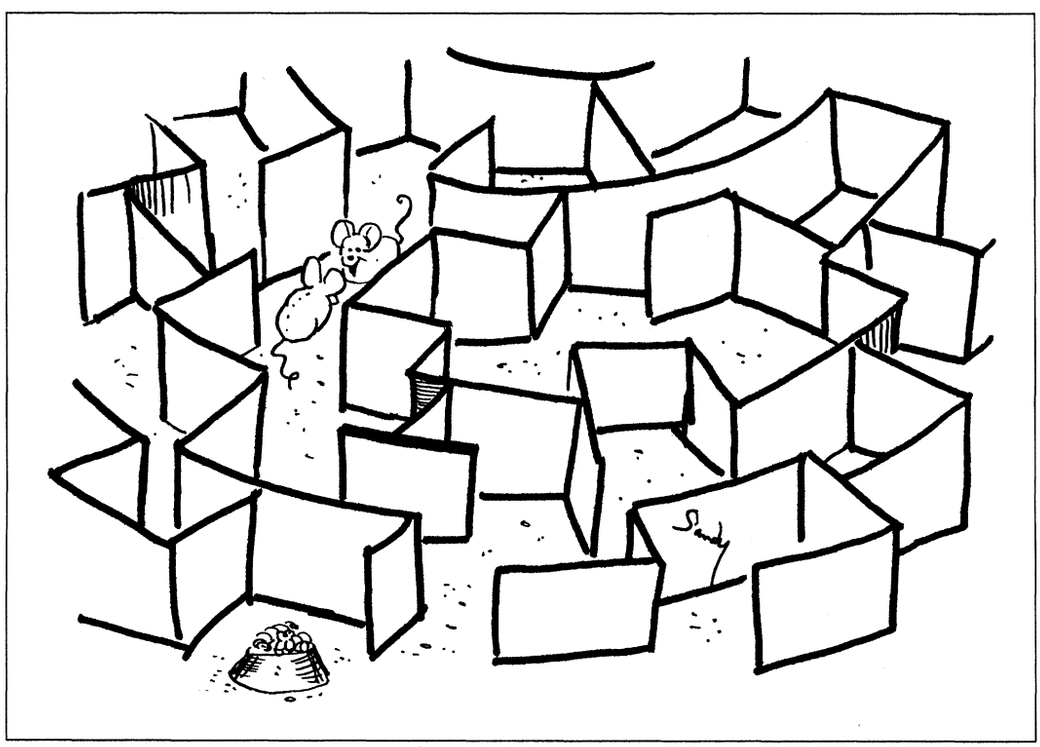

"It is a silly way to get food but you can't fight City Hall."