analgesic activity as well as suppression of immune function. The PAG has been implicated indirectly in modulating certain aspects of immune function. Electrical stimulation of this region enhances metastatic tumor growth (*14*), presumably through alterations in immune function, particularly in NK cell activity. Indeed, electrical stimulation of the PAG produces an opiate-mediated analgesia in a variety of species including humans (*15*). Therefore, it is likely that this region of the brain may be critically involved in the regulation of immune effector functions that are important in resistance to neoplasms and infectious diseases and that are controlled, at least in part, by endorphinergic circuitry.

The precise neural mechanisms underlying the ability of opiate action in the PAG to alter NK cell activity are not entirely clear. Signals from the CNS to the immune system are relayed primarily through the hypothalamic-pituitary-adrenal axis (HPA) or via sympathetic innervation of lymphoid organs. Thus, opiate action in the PAG could be translated into activation of the HPA axis through hypothalamic efferents (*16*), or enhanced opiate activity in the PAG could cause increases in peripheral sympathetic output, either of which could have an effect on NK cell activity (*17*).

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Effect of Serotonergic Afferents on Quantal Release at Central Inhibitory Synapses

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Although most examples of modulation of synaptic transmission have been obtained from excitatory rather than from inhibitory connections, serotonin (5HT) is now shown to cause a presynaptic facilitation of release of the inhibitory neurotransmitter glycine. Brief local injections of this amine, or application of a 5HT uptake blocker, produce a long-lasting enhancement of both spontaneous and evoked inhibitory currents in the teleost Mauthner cell. Quantal analysis showed that the probability of release is increased. Focal recording indicated that 5HT acts directly on the inhibitory terminals, possibly reducing potassium conductances. Double staining with specific antibodies demonstrated a morphological substrate for this effect. Nerve endings that contain 5HT contact inhibitory terminals directly apposed to postsynaptic glycine receptors.

MODULATION OF TRANSMITTER RELEASE has been proposed as a key cellular mechanism for various forms of plasticity at excitatory synapses (*1, 2*). In contrast, although inhibitory networks do participate in long-lasting behavioral changes (*3*), studies of their presynaptic regulation are scarce and mainly involve peripheral systems (*4, 5*). To examine this question in a vertebrate brain, we chose the teleost Mauthner (M) cell, the command neuron of the escape reflex. The excitability of this cell is controlled by inhibitory afferents characterized morphologically (*6, 7*) and electrophysiologically (*8–11*). A quantal description of the spontaneous noise that these afferents produce permits straightforward access to their release parameters (*12*). We used this property to demonstrate that the inhibitory synapses receive presynaptic contacts that contain serotonin (5HT) and facilitate the release of glycine.

Experiments were conducted on curarized goldfish (*Carassius auratus*) anesthetized with 3-aminobenzoic acid ethyl ester (MS-222, Sandoz). Chloride-dependent inhibitory postsynaptic currents (IPSCs), produced by activity in two groups (*10*) of glycine-containing interneurons (Fig. 1A), were recorded at resting potential. Potassium acetate– or potassium chloride–filled microelectrodes (1.5 to 3 megohms) were used for single-electrode voltage-clamp recording (Axoclamp, Axon Instruments, Burlingame, California; sampling frequency, 18 to 25 kHz). Serotonin (2 to 10 m*M*, in a solution of 130 m*M* NaCl and 10 m*M* Hepes) was applied by pressure-pulses (30-ms duration) through a second microelectrode positioned 10 to 20 μm above the M cell and close to the recording site. As in other neurons (*13*), 5HT increased the inward rectification in the M cell (148% ± 34%, mean ± SD, $n = 20$). Once this occurred, the pipette was withdrawn to avoid leakage of the amine.

Evoked IPSCs were increased by 5HT. In an initial series of 14 experiments, it enhanced the collateral (Fig. 1B) and commissural (Fig. 1C) IPSCs by 136 ± 34% ($n = 14$) and 175 ± 77% ($n = 4$; that is, in all cases, when tested), respectively, because of an increase of glycine-activated conductances (Fig. 1B, inset).

In each experiment, the enhanced transmission was accompanied by an increase in inhibitory synaptic noise (Fig. $2A_1$), which dominates in the M cell (*14*). This augmentation started 2 to 5 min after a single 5HT application and generally persisted for at least 25 to 30 min. Its composition was systematically analyzed by comparing the amplitudes of individual components constituting noise on continuous recordings, according to a method (*12*) that distinguishes closely spaced overlapping events (Fig. $2A_2$). This analysis showed that 5HT did not alter the size of individual quanta, but that it increased the number of large responses that represent integral multiples of these basic units and that are produced, as are the evoked responses, by spike activities in presynaptic fibers (*12*) (Fig. 2, B_1 and B_2). Because the total number of responses was essentially the same in identical sampling periods (120 s in the illustrated case), these data indicate that 5HT increases the probability of release at the presynaptic terminal inhibitory active zones (*11*) close to the pipette.

Two sets of evidence demonstrate that endogenous 5HT acts in the same manner. First, topical application of 20 μ*M* imipramine, which blocks 5HT uptake, induced similar effects on the collateral and spontaneous IPSCs within 7 to 10 min, in seven

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other experiments. Second, the M cell axon cap and soma are innervated by thin (less than 0.2 μm in diameter) 5HT-containing varicose fibers with terminal side endings that run 10 to 20 μm from the postsynaptic membrane (Fig. 3A), an architecture found in the central nervous system of other vertebrates (*15*).

Endings in contact with the M cell soma, or its dendrites, which can be unambiguously identified because they are the only dendrites present in the axon cap (*6, 16*), exhibited distinct presynaptic differentiations (Fig. 3B). Some of them issued a small side expansion (Fig. 3C) at 100 to 200 nm from the M cell membrane. This appendage invaginated in adjacent terminals, which were typical of M cell inhibitory inputs (*6, 7*) and which were apposed to postsynaptic

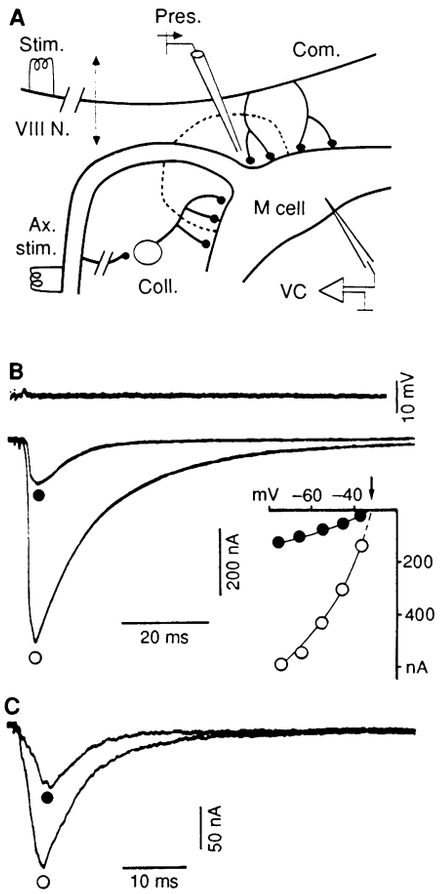

Fig. 1. Enhancement of glycinergic synaptic transmission by 5HT. (**A**) Experimental design indicating that inhibitory postsynaptic currents were recorded in the voltage-clamped (VC) M cell in response to activation of the recurrent collateral network (Coll.) or of the commissural pathway (Com.) by stimulation of the M cell axon (Ax. stim.) or of the contralateral vestibular nerve (VIII N.), respectively. Serotonin was applied by pressure injection (Pres.) in the axon cap (dashed line), a specialized neuropile that surrounds the initial segment and part of the M cell soma (*6*). Vertical arrow indicates midline. (**B** and **C**) Superimposed traces (n = 4) of (B, above) voltage recording and of (B, below) collateral- and (C) commissural-evoked IPSCs before (●) and after (○) 5HT administration. [In (B) and (C), inhibitory currents are inward-going because of Cl^- loading of the M cell.] (**Inset**) Current-voltage plots of the collateral current, indicating that the Cl^- reversal potential (arrow at −30 mV) remained unchanged and that the postsynaptic conductance was increased [holding potential (HP), −70 mV].

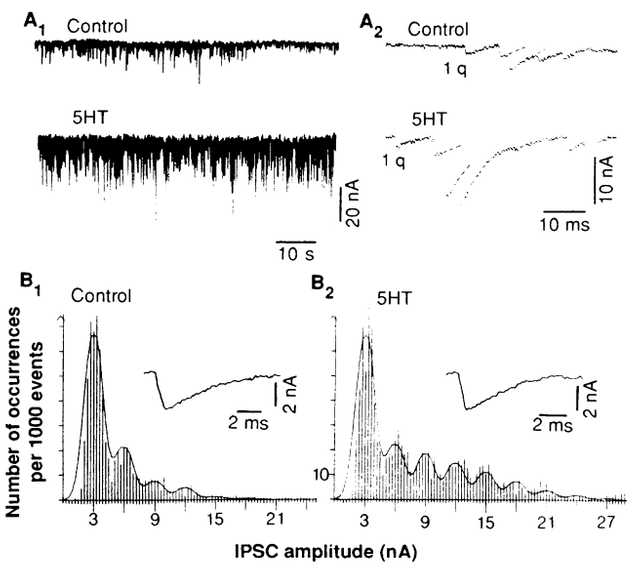

Fig. 2. Increased inhibitory synaptic noise in the presence of 5HT. (**A_1** and **A_2**) Spontaneous IPSCs in control conditions and after ejection of the amine (5HT) (HP, −72 mV). (**B_1** and **B_2**) Distributions of amplitudes of individual IPSCs contributing to the noise [same cell as (A_1) and (A_2)]. In (B_1), there were 2495 events; in (B_2), there were 2424 events. Each IPSC was measured from its peak to the projected tail of the previous event (dashed lines in A_2). There is an increased number of larger peaks after 5HT, suggestive of an enhanced probability of release. The insets in (B_1) and (B_2) are averaged sweeps (n = 41 in each) of isolated events within the first peak, with their means equal to 3.04 ± 0.68 nA and 3.00 ± 0.70 nA, respectively. Thus, individual quanta [1 q in (A_2) and averaged traces] are the same. Gaussian fits of the histograms indicate regular spacing of successive peaks; σ, the SD of the IPSC amplitudes, was 0.8 nA and was close to that of instrumental noise (0.6 nA), indicating a coefficient of variation of individual quanta (*12*) of about 17%.

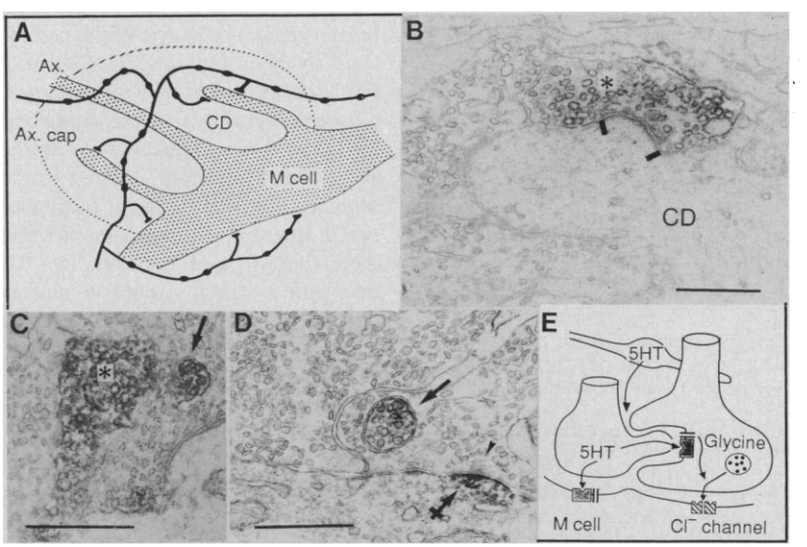

Fig. 3. Morphological features of serotonergic fibers innervating the M cell soma. (**A**) Schematic drawing of beaded profiles near the soma and cap dendrites (CD) in the axon cap (Ax. cap). (**B** through **D**) Electron micrographs of 5HT-positive endings. (B) Synaptic contact (*) with round vesicles and an active zone (between bars) on a small cap dendrite. (C) Protrusion (arrow) of another terminal (*) into an adjacent bouton containing pleiomorphic vesicles. (D) Double labeling with specific antibodies to 5HT and the glycine receptor, revealing a 5HT-positive side appendage (arrow) within a bouton that contains an active zone with a presynaptic dense projection (arrowhead) facing glycine receptors on the M cell (crossed arrow). Bar in (B) through (D), 0.5 μm. (**E**) Diagram of the identified pathways by which 5HT acts on the M cell system. To increase accumulation of 5HT in terminals, the goldfish (n = 11) were treated (*23*) with pargyline (a monoamine oxidase inhibitor) and L-tryptophan (a precursor of serotonin). Two hours later, and after anesthesia with MS-222, they were perfused with fixative (*24*). Immunochemical reactions were carried out on floating Vibratome sections with the Vectastain (Vector Laboratories, Burlingame, California) indirect avidin-biotin–horseradish peroxidase complex for immunoenzymatic labeling (*25*). Serotonin was detected with an antibody directed against it (Immunotech, Marseille, France). In four of these experiments, glycine receptors were identified with the antibody GlyR7a, which recognizes a receptor-associated 93-kD protein (*24, 26*). Separate omission of each primary antibody eliminated the corresponding staining.

glycine receptors (Fig. 3D). This design accounts for the pre- and postsynaptic effects of 5HT (Fig. 3E) observed in our study.

Various mechanisms—for example, a decrease in K^+ conductances (*1*) or an increase in Ca^{2+} conductances (*17*) and in intracellular Ca^{2+} contents (*18*)—could account for these observations. Several lines of evidence, from 12 additional experiments, point toward an involvement of K^+ channels. These results showed that 5HT has opposite effects on (presumably different) K^+ channels in the M cell and in the presynaptic terminals. Postsynaptically, 5HT opened K^+ conductances that showed inward rectification (*19*) and were blocked by Cs ions (2 to 5 m*M*; $n = 7$). At the presynaptic terminal, 5HT, as well as K^+ channel blockers such as Cs (2 to 5 m*M*; $n = 7$) and tetraethylammonium (1 m*M*; $n = 5$), increased synaptic noise.

Facilitation of excitatory transmitter release by 5HT (*5, 20*) has been implicated in sensitization in *Aplysia* (*1*), whereas in other systems this amine may have a net inhibitory effect, such as with the restraint inhibition of the crayfish escape reflex (*21*). Our results define a structural locus for this type of modulation that is restricted to the presynaptic terminals and distant from the soma of the interneuron (*7*). They thus extend the notion of facilitated release to inhibitory networks and suggest that a reinforcement of glycine transmission might underlie habituation (*22*) of M cell–mediated escape reactions.

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Neural Connections Between the Lateral Geniculate Nucleus and Visual Cortex in Vitro

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Neural connections were established in cocultures of rat visual cortex (VC) and lateral geniculate nucleus (LGN), which were isolated in early infancy. Morphological and electrophysiological studies showed that the cortical laminar organization of afferent and efferent connections in the coculture preparations was similar to that in the adult VC. The results indicate the existence of intrinsic mechanisms in VC and LGN that guide the formation of synaptic connections with the appropriate targets.

THE QUESTION OF HOW SPECIFIC neural connections in neocortical structures are established during development has been one of the central problems in neurobiology. The visual cortex (VC), characterized by well-developed laminar organization of neural connections (*1, 2*), is the optimal material for studies that address this question. Because the laminar organization is immature at birth and develops in the early postnatal life of the animal, there should be mechanisms in infant VC that guide axonal extension and synapse formation with appropriate targets to produce stereotyped laminar structures (*3–5*). We investigated the mechanisms underlying the specific neural connections between VC and lateral geniculate nucleus (LGN) by using a coculture technique (*6*). Our results demonstrate that neural connections with laminar specificity can be established in LGN-VC cocultures, suggesting the existence of intrinsic mechanisms that control axonal growth and synaptogenesis.

Whole blocks of LGN (0.8 mm by 0.6 mm by 0.3 mm) were dissected from 16- to 17-day-old rat fetuses, which were removed from the mother (Sprague-Dawley) under pentobarbital anesthesia (*5, 7*). VC slices (0.3 to 0.5 mm thick) were dissected from 1- to 3-day-old rats. A pair of the LGN and VC were plated on a culture dish with a collagen-coated membrane (Coster, Transwell-Col) in serum-free, hormone-supplemented medium (*8*). The LGN explant was placed in contact with or at a distance of <0.5 mm to the ventral surface of the VC explant. The cultures were maintained at 37°C in humidified 95% air and 5% CO_2.

Massive neurite connections developed between the LGN and VC explants in 37 of 59 cocultures (Fig. 1A). Morphological and electrophysiological studies were usually conducted after 2 to 3 weeks in culture. Nissl-stained sections of the VC explant demonstrated laminar and columnar organization composed of pyramidal or granular cells (Fig. 1B), which resembled that of normal rat VC (Fig. 1C). There was not much difference in the cell size (10 to 20 μm) between the VC explant and normal VC. However, the entire cortical thickness of the VC explant was commonly slightly smaller than that of the normal VC (Fig. 1, B and C). This seems to be attributable to thinner supragranular layers, suggesting retardation in development of these layers.

Neuronal connections between the LGN and VC explants were studied in 13 cocultures by antero- and retrograde labeling with horseradish peroxidase (HRP) or a fluorescent dye (DiI, Molecular Probes) (*4, 9, 10*). HRP-anterograde labeling of the LGN axons, with very few retrogradely labeled VC cells is shown in Fig. 1D. Most of the labeled axons extended along the columnar structure in the VC and branched in and around the granular layer. Anterograde labeling with DiI showed the axonal arboriza-

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