DISTRIBUTION OF SEROTONIN-IMMUNOREACTIVE CELL BODIES AND PROCESSES IN THE ABDOMINAL GANGLION OF MATURE *APLYSIA*¹

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Abstract

Sensitization of the gill withdrawal reflex in *Aplysia californica* is an elementary form of learning, in part resulting from presynaptic facilitation of the LE mechanoreceptor neurons of the abdominal ganglion. It has previously been established that either application of serotonin or direct stimulation of a group of facilitatory neurons, the L29 cells of the abdominal ganglion, can simulate the effect of physiological stimulation in producing presynaptic facilitation. Because the evidence that serotonin serves as a facilitatory transmitter was indirect, we examined the distribution of serotonin-immunoreactive fibers and cell bodies in the abdominal ganglion in order to answer two questions: (1) do the sensory neurons receive serotonergic innervation and (2) are the L29 cells serotonergic? We observed two distinctive patterns of serotonergic innervation within the ganglion, sparse and dense. The sparse pattern is correlated with a serotonin-stimulated increase in cAMP in identified target cells, while the dense innervation is not. We found a sparse distribution of serotonin-immunoreactive fibers with varicosities close to both cell bodies and processes of identified LE sensory cells. It therefore is likely that the sensory neurons do receive serotonergic innervation. We also mapped the population of serotonergic neuronal cell bodies in the ganglion, and found five clusters of neurons. Cells in one of these clusters, the identified RB neurons, had previously been shown to synthesize serotonin from tryptophan and to contain the neurotransmitter in high concentration. Identified L29 facilitator cells marked by injection with Lucifer Yellow do not contain serotonin immunoreactivity and therefore evidently are not a source of serotonergic input onto sensory cells.

Pharmacological evidence, accumulated over several years, indicates that serotonin is a neurotransmitter capable of producing the presynaptic facilitation which underlies sensitization of several defensive withdrawal reflexes in *Aplysia* (Kandel and Schwartz, 1982; Walters et al., 1983a, 1983b). Application of serotonin prolongs the action potential in LE mechanosensory neurons, and thereby enhances transmitter release (Brunelli et al., 1976; Klein and Kandel, 1980). Intracellular stimulation of several neurons, including cells in the L29 cluster, also produces the facilitation (Hawkins et al., 1981b). It was therefore plausible to suggest that L29 uses serotonin or a closely related amine as a transmitter substance. This idea was supported by morphological evidence. The size and distribution of synaptic vesicles in endings of L29 (Bailey et al., 1981) resemble those in the serotonergic giant metacerebral neuron

(GCN) (Shkolnik and Schwartz, 1980). Moreover, as in GCN (Schwartz et al., 1982), [³H]serotonin intracellularly injected is localized to end stage lysosomes in L29's cell body (Bailey et al., 1981). Finally, L29 neurons show a specific, high affinity uptake of [³H]serotonin (Bailey et al., 1983).

Because the evidence for the role of serotonin in behavioral sensitization and as a transmitter of the L29 cells was indirect, we next approached these questions directly using immunocytochemical techniques. We first surveyed the ventral surface of the abdominal ganglion in mature animals (N. N. Osborne, R. D. Hawkins, E. R. Kandel, and J. H. Schwartz, unpublished), and then in whole mounts of juvenile *Aplysia* (Goldstein et al., 1984), and found no serotonin-immunoreactive cell bodies in the location of the L29 cluster. Similar results have now been obtained in whole mounts of adult ganglia by Ono and McCaman (1984). These observations are not definitive, however, because the position of L29 neurons is variable. In addition, cell bodies in vertebrates sometimes fail to react with antibodies against neurotransmitter substances, even though their axons and terminals are intensely stained with the same antibodies. Because of these problems, we thought it best to use immunocytochemical techniques on the cell bodies and processes of facilitator neurons which had been electrophysiologically identified and marked by intracellular injections of dye.

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We therefore examined serial cryostat sections of adult ganglia in which either individual L29 facilitator cells or LE mechanosensory neurons were identified physiologically and then marked by intracellular injection of Lucifer Yellow. Particularly, we sought to determine whether: (1) serotonergic processes innervate LE sensory neurons and (2) whether L29 cells contain serotonin immunoreactivity. We also looked for other serotonergic neurons in the abdominal ganglion that might serve as facilitator neurons.

A preliminary account of these results has been published previously (Kistler et al., 1983).

Materials and Methods

Aplysia californica (50 to 150 gm, obtained from Pacific Bio-Marine Supply Co., Venice, CA) or grown at the *Aplysia* Mariculture Facility at the Marine Biological Laboratory, Woods Hole, MA) were maintained at 15°C in circulating Instant Ocean (Aquarium Systems, Eastlake, OH). Before dissection, animals were anesthetized by injection of isotonic MgCl₂. Abdominal and pleural ganglia were removed and pinned to silicone plastic (Sylgard, Dow Corning, Midland, MI) in a supplemented artificial seawater (Eisenstadt et al., 1973). Seven abdominal ganglia and three pleural ganglia were sectioned and treated with the immunocytochemistry protocol described below, without further dissection; the connective tissue sheath was removed from other ganglia in order to inject single identified cell bodies with Lucifer Yellow CH (5% in water, supplied by Walter W. Stewart, National Institute of Alcoholism and Mental Diseases, National Institutes of Health, Bethesda, MD) by pressure or iontophoresis (0.5 to 5 nA of hyperpolarizing pulses lasting 0.5 sec at 1 Hz for 0.5 to 2 hr before processing for immunocytochemistry).

Identification of neurons. L29 cells were identified both by their electrophysiological properties (Hawkins et al., 1981a) and by production in sensory neurons of either spike broadening (Hawkins, 1981b) or a decrease in outward current under voltage clamp (R. D. Hawkins, in preparation). Abdominal and pleural mechanoreceptor sensory neurons were identified by their characteristic size, position, pigmentation, and occurrence of an antidromic spike produced in response to stimulating the siphon or pedal nerves (Byrne et al., 1974; Walters et al., 1983a, 1983b). R15 and RB cells were identified by electrophysiological and morphological criteria (Frazier et al., 1967). The heart excitor cell (RB_{HE}), in a preparation consisting of the abdominal ganglion connected to the heart by the pericardial nerve, was identified by its ability to excite the quiescent heart when stimulated (Mayeri et al., 1974).

Single identified L29 cells were injected with Lucifer Yellow into three ganglia, one or more LE sensory cells were injected into three other ganglia, and four pleural sensory cells were injected into one ganglion. RB cells were injected into two ganglia and RB_{HE} cells were injected into two ganglia.

Immunocytochemistry. Ganglia were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4) containing 30% (w/v) sucrose at 4°C. After 2 hr, the tissue was rinsed in PBS (10 mM Na phosphate, 0.9% NaCl, 0.3% Na azide) overnight at 4°C and serial 16 μ m cryostat sections were collected on subbed slides.

After they were air dried, sections were rinsed briefly in PBS-saponin (PBS containing 0.25% saponin), dehydrated by serial rinses in 50, 70, 80 and 95% ethanol, and then bleached with 0.3% H₂O₂ in methanol to reduce endogenous tissue fluorescence. Sections were rinsed with PBS-saponin, exposed to PBS-saponin containing normal goat serum for 1 hr at room temperature, and then incubated in rabbit anti-serotonin antiserum (1:700 to 1:1000 dilution in PBS-saponin) overnight at 4°C. Sections were rinsed in PBS-saponin, incubated 2 to 4 hr at room temperature in rhodamine-labeled goat anti-rabbit antiserum (combined IgG and IgF fraction, Cappel Laboratories, Westchester, PA, diluted 1:30 to 1:50 in PBS-saponin), and then washed and coverslipped under glycerol. Sections were viewed by epifluorescence optics on a Leitz microscope (Filter Pack N-2) and photographed with high speed Tri-X or Ektachrome film.

Controls. Absorption studies with the serotonin antiserum used (Steinbusch designation SER-7-6 and SER-7-7) have been described (Steinbusch et al., 1978). In addition, previous work on whole mounts of ganglia and peripheral tissues of juvenile *Aplysia* (Goldstein et al., 1984) has shown that immunoreactivity of the antiserum to serotonin is blocked by addition of the immunogen (serotonin conjugated to bovine serum albumin; Steinbusch et al., 1978; Steinbusch and Tilders,

1984). Sections incubated without specific serotonin antiserum showed no staining greater than background.

In addition to these absorption controls, we dissected abdominal ganglia from three animals and incubated them at 15°C in aerated, supplemented artificial sea water containing 10⁻⁶ M reserpine (Serpacil, Ciba-Geigy Corp., Summit, NJ), a treatment previously shown to deplete endogenous serotonin (Goldman and Schwartz, 1974). When these ganglia were fixed, sectioned, and reacted, no serotonin immunoreactivity was seen.

Results

Cell bodies

The location of serotonin-immunoreactive cell bodies in the abdominal ganglion was established from seven complete series of serial cryostat sections. We localized five clusters of neurons (see map in Fig. 1). The previously characterized RB cluster consists of 12 to 14 large (150 to 200 μ m) neurons on the dorsal surface of the ganglion and 12 to 14 smaller (75 to 100 μ m) cells that lie just beneath the larger cells (Fig. 2A). A group of 20 to 24 immunoreactive neurons on the dorsal surface of the left hemiganglion resembles the RB group because it includes both larger surface cells as well as smaller underlying neurons. Three other clusters of immunofluorescent cells are also present in the ganglion: a group of 8 to 10 small (30 to 50 μ m) stellate neurons, located below the ventral surface of the right hemiganglion (Fig. 2B), a cluster of 5 to 6 cells measuring 75 to 100 μ m in diameter on the rostromedial margin of the left hemiganglion, and three cells located at the caudolateral margin of the left hemiganglion.

Only the cells of the RB cluster had previously been shown to be serotonergic (Eisenstadt et al., 1973; Liebeswar et al., 1975; Weinreich et al., 1973). Cells of the remaining four clusters have not yet been characterized. Although the distribution of all these immunoreactive cell bodies is similar to that described in ganglia of juvenile *Aplysia* (Goldstein et al., 1984), we found approximately twice as many reactive cells in each cluster in the adult ganglia. The increase in serotonin-immunoreactive cells takes place as the total number of neurons in the ganglion also increases (Coggeshall, 1967).

Distribution of immunoreactive processes

Cell body innervation. Most neuronal cell bodies in the ganglion are surrounded by a network of serotonin-immunoreactive fibers, arranged in two distinct patterns (Fig. 3). One distribution is typified by a relatively low density of fibers. In areas with this innervation, a few immunoreactive fibers seem to wander between the cell bodies and their processes. This first pattern is characteristic of the distribution of fibers that surround the LE sensory cells of the abdominal ganglion (Fig. 3A), the sensory cells of the pleural ganglion, R15, and the neurosecretory neurons in the bag cell clusters (Fig. 3B).

In contrast, the other distribution of serotonergic innervation is much denser. In this pattern, individual neurons appear to be wrapped by a dense net of processes that surround their cell body and initial segment. Among the cells so innervated are the large, putative peptidergic cells of the left and right upper quadrants (Coggeshall et al., 1966; Loh et al., 1977; Price and McAdoo, 1979) (Fig. 3C and D) and many unidentified cell bodies in the ganglion.

Central neuropil. The central neuropil of both the abdominal and pleural ganglia contains a variable density of serotonin-immunoreactive fibers. Some areas are rich in fibers, and there are distinct zones where the number of fibers is quite small. The sparse zones appear as tracts containing nonserotonergic fibers of passage. One example of a region poor in serotonergic fibers is the tract coursing through the abdominal ganglion between the left pleuroabdominal connective and the siphon nerve. The sparsity of immunoreactive fibers there starkly

Figure 1. Diagram locating immunoreactive neurons in the abdominal ganglion of *Aplysia*. Darkened profiles represent serotonin-immunoreactive cell bodies situated in four planes of the ganglion. Large clusters of cells are present on the dorsal aspect of both right and left hemiganglia. The previously identified RB cells form the dorsal right cluster composed of large surface neurons (in A) and smaller underlying cells (in B). This group extends along the medial edge of the right hemiganglion from the rostrocaudal midpoint of the ganglion to its caudal edge. On the caudal surface of the ganglion, two large cells of the RB cluster flank R15. The cell cluster of the dorsal left hemiganglion is located rostral to the dorsal right group and resembles the RB cluster in its composition of large surface neurons and smaller underlying cells. Cells of this cluster have not yet been behaviorally or physiologically characterized. Three additional clusters of unidentified cells are deep to the ventral surface of the ganglion. A cluster of small neurons that often appear stellate in shape is situated just beneath the larger cells on the ventral surface of the right hemiganglion. Slightly deeper, in the left hemiganglion, two other small groups of cells are located in the plane of the commissure. The left lateral group is a trio of cells consisting of one large surface cell and two smaller cells lying just beneath it. The cells of the ventromedial group, lying along the left medial surface, are distinct from the small subsurface cells of the dorsal surface. These ventral cells give rise to axons that form a tight bundle that enters the central neuropil coursing towards the genital pericardial nerve.

contrasts to the dense entanglement of serotonergic fibers in the surrounding region (Fig. 3E).

Innervation of sensory cells. Because previous work has established that facilitation of LE and pleural mechanoreceptor neurons can be produced by application of serotonin, we examined the distribution of serotonergic processes in the regions of the abdominal and pleural ganglia containing sensory clusters. In serial sections of these regions, sensory cells, recognized by their characteristic size and location, were surrounded by a sparse distribution of immunoreactive, varicose fibers (Fig. 3A). A similar sparse distribution of immunoreactive fibers was also observed in the neuropil just beneath the cell body layer on the ventral surface in the region of the ganglion containing axon processes of the sensory neurons. In some abdominal ganglia, we marked physiologically identified mechanoreceptors by intracellular injection of Lucifer Yellow (Fig. 4). In these ganglia also, varicose immunoreactive fibers are seen in the extracellular space surrounding sensory cell bodies and adjacent to

their axon hillocks (Fig. 4A, and B); in the underlying neuropil, they come in close contact with axon processes of cells marked with the dye (Fig. 4C, and D).

The L29 cells. Earlier studies had shown that facilitation could be produced by direct intracellular stimulation of L29 cells. These neurons share several morphological characteristics with known serotonergic neurons and take up exogenous serotonin with high affinity. It therefore seemed likely that the L29 cells are a source of serotonergic inputs onto sensory cells and thus were expected to be labeled by immunoreactivity.

The ventral surface of the left abdominal hemiganglion, the known location of the L29 facilitator neurons, contained no cells with serotonin-specific immunofluorescence. To examine these neurons definitively, single, physiologically identified L29 cells were injected with Lucifer Yellow and processed for immunocytochemistry. Neither cell bodies nor processes of these cells were found to contain serotonin immunoreactivity (Fig. 5A, and B).

Figure 2. Fluorescence photomicrographs of cryostat sections showing clusters of neurons with serotonin immunoreactivity. *A*, section near the dorsal surface of the right hemiganglion showing cells of the RB cluster. Included are large immunoreactive surface cells (*large arrows*), smaller subsurface cells (*small arrows*), and fibers of unknown origin containing numerous varicosities (*bar* = 100 μ m). *B*, cluster of immunoreactive neurons below the right ventral surface (*bar* = 100 μ m).

In control experiments, we showed that injection of Lucifer Yellow does not interfere with the demonstration of serotonin immunofluorescence in cell bodies or processes of serotonergic RB cells (two experiments with RB_{HE} and two with RB cells chosen from the cluster without further identification) (Fig. 5C, and D).

Discussion

The L29 facilitator cells do not contain serotonin immunoreactivity. It has previously been shown that direct stimulation of cells in the L29 cluster can facilitate release of transmitter from sensory neurons (Hawkins, 1981b; Hawkins et al., 1981b). Since the actions of L29 and serotonin on sensory cells are similar, it was attractive to propose that L29 cells use serotonin as neurotransmitter and make direct connections on sensory cells. Although our immunocytochemical observations indicate that the LE sensory neurons receive serotonergic innervation, the origin of these immunoreactive fibers is not likely to be the cluster of L29 facilitator cells. In seven serial series of cryostat sections, no immunoreactive cell bodies were seen on the ventral surface of the left abdominal hemiganglion, the known location of the L29 cluster. Similar observations have been made by Osborne, Hawkins, Kandel, and Schwartz (unpublished observations) and by Ono and McCaman (1984). More conclusively, L29 cells that were shown to produce facilitation

in sensory cells and which were marked by intracellular injection of Lucifer Yellow contained no serotonin immunoreactivity.

There are several possible explanations for the absence of serotonin-immunoreactivity in L29 facilitator neurons. (1) Serotonin might not play a physiological role in sensitization, but act as a pharmacological analogue of some endogenous transmitter substance. The observation that serotonergic fibers come in close contact with sensory cell processes in the neuropil, however, suggests that serotonin does participate in sensitization. We are currently using the immunocytochemical maps of serotonergic neurons to locate cells that might be direct facilitatory interneurons in the abdominal ganglion as well as in other ganglia.

(2) L29 cells, which are not serotonergic, might produce their facilitatory effects on sensory cells indirectly by exciting as yet unidentified serotonergic interneurons. Glanzman et al. (1984) have found that extracts of L29 cells do produce facilitation in sensory cells, however, suggesting that they could act directly, in addition to any indirect actions that they might also have.

(3) L29 cells, using some other facilitating transmitter substance, might act in parallel with an unidentified serotonergic facilitating pathway. Physiological evidence indicates that the L29 neurons cannot account for all of the facilitation produced by stimulation of the skin or connectives (Hawkins et al., 1981b;

Figure 3. Fluorescence photomicrographs showing types of serotonergic innervation. **A**, cell bodies of the left ventral surface. This cluster of immunologically unreactive cells (*n*) corresponds in size and position to the LE mechanoreceptor cells (*n*). Note the sparse distribution of varicose immunoreactive fibers (*arrows*) in the extracellular space (*bar* = 50 μ m). **B**, the cells (*n*) of the bag cell cluster are also sparsely innervated by varicose immunoreactive fibers (*arrows*) (*bar* = 50 μ m). **C**, tangential section through the surface of a left upper quadrant neuron (probably L5) surrounded by a dense tangle of immunoreactive, varicose fibers (*bar* = 50 μ m). **D**, section showing three right upper quadrant cells. Immunoreactive fibers densely enwrap cell bodies (*n*) and axon hillock regions (*arrow*) (*bar* = 50 μ m). **E**, section through the central neuropil of the left hemiganglion. Immunoreactive axons can be seen entering the ganglion in the left connective, siphon nerve, and the commissure. These fibers form a dense plexus within the central neuropil (*bar* = 100 μ m).

Figure 4. Fluorescence photomicrographs of cryostat sections of abdominal ganglia showing unidentified serotonin-immunoreactive processes in apposition to LE mechanoreceptor neurons. Pairs of photomicrographs are presented from identical fields. One photomicrograph of each pair (taken through a Leitz I-2 filter cube, excitation between 450 and 490 nm; suppression barrier, 515 nm) reveals Lucifer Yellow-labeled cells and their processes, and the other (taken through a Leitz N-2 filter cube, excitation between 530 and 560 nm; suppression barrier, 580 nm), reveals rhodamine fluorescence associated with immunolabeling of serotonergic processes. Photomicrographs of Lucifer Yellow fluorescence taken through the I-2 filter cube are contaminated slightly by leakage of rhodamine fluorescence through the filter system. The ventral surface of the left hemiganglion: *A* and *B*. *A*, a portion of a Lucifer Yellow-marked initial segment of an injected sensory cell (*). *B*, branching, varicose, rhodamine-labeled fibers are apparent in this region. Putative serotonergic fibers containing varicosities (arrows) come close to the initial segment of the sensory cell (bar = 25 μ m). The central neuropil adjacent to the LE sensory cell cluster: *C* and *D*. *C*, a portion of an axon marked with Lucifer Yellow from an injected sensory cell (*). *D*, two rhodamine-labeled processes (arrows) apposed to the labeled process seen in *C* (bar = 25 μ m).

Hawkins, 1981a). Two other classes of facilitating neurons have been identified (Hawkins et al., 1981b), and there probably are others that have not yet been identified. Some of these might be serotonergic.

(4) There is the unlikely possibility that the L29 cells may

belong to a previously unappreciated class of neurons that do not synthesize serotonin, but which are capable of taking it up (in amounts too small to be detected immunocytochemically) for subsequent release.

The results of the immunocytochemical experiments re-

Figure 5. Lack of serotonin immunoreactivity in L29 cells. Fluorescence photomicrographs from a cryostat section of an abdominal ganglion containing an L29 facilitator neuron injected with Lucifer Yellow. **A**, L29 cell, (*) viewed through fluorescein filter system (see legend to Fig. 4). **B**, the same field under rhodamine excitation for serotonin immunoreactivity. While numerous immunolabeled fibers are present (arrows), the facilitator (L29) contains no detectable immunofluorescence (bar = 50 μ m). Fluorescence photomicrographs from cryostat sections from a ganglion in which identified RB neurons were injected with Lucifer Yellow. **C**, the location of the dye-marked RB cell (*). **D**, the dye-marked cell (RB) and adjacent cells (+) react positively with the serotonin antibody, while neighboring cells (o) were immunologically unreactive (bar = 100 μ m).

ported here fail to confirm the predicted serotonergic nature of L29 cells, and therefore call into question the diagnostic value of a characteristic synaptic vesicle morphology, the localization of transmitter to end stage lysosomes in the cell body, and the ability to take up [3 H]serotonin with high affinity. Further

characterization of L29 cells, including identification of their neurotransmitter, should be useful in explaining these discrepancies. L29 cells might release a serotonin-like transmitter that, while not recognized by the antibody, is nevertheless capable of producing presynaptic facilitation of sensory neurons

by interacting with receptors sensitive to serotonin. Alternatively, L29's transmitter might be unrelated to serotonin. Castellucci et al. (1984) have fractionated extracts of abdominal ganglia by high performance liquid chromatography and tested which fractions produce presynaptic facilitation when applied to sensory neurons. The major peaks of facilitatory activity had retention times similar to serotonin and two peptides, SCP_A and SCP_B. In addition, at least one smaller, yet unidentified, peak of facilitatory activity was observed. The two peptides have been found to act in a fashion similar to serotonin, both on several peripheral tissues in *Aplysia* (Lloyd, 1982; Lloyd et al., 1984) and on siphon sensory neurons (Castellucci et al., 1984). Preliminary immunocytochemical studies provide no indication that L29 cells contain these peptides, however (Kupfermann et al., 1984).

Possible correlation between patterns of serotonergic innervation and physiological response. The presumption that serotonin is a facilitatory transmitter prompted us to determine whether the LE sensory cells are innervated by serotonin-immunoreactive processes. We found serotonin-immunoreactive processes with varicose swellings in the extracellular space surrounding LE sensory cell bodies. These fibers come close to the cell bodies and neuritic processes of these cells in the central neuropil of the ganglion. The resolution of light microscopy does not permit us to conclude that these varicosities make direct synaptic contact with sensory cells. Electron microscopic immunocytochemical studies, now in progress, are necessary to answer this question.

The sparse pattern of serotonergic innervation that we found on sensory neurons is characteristic of one of two distinct patterns in the abdominal ganglion. Similar sparse patterns of serotonergic innervation were also seen around the pleural sensory cells, the bag cell clusters, and R15. The distribution of immunoreactive fibers in the region of the ganglion containing the axon processes of sensory neurons also is sparse. In addition, Goldstein et al. (1984) found a similar and possibly analogous sparse distribution of serotonergic fibers in heart, body wall, and buccal muscle. A common feature of all sparsely innervated cells and their processes is that they respond to neuronal stimulation or to the application of serotonin by increased synthesis of cAMP (Bernier et al., 1982; Drummond et al., 1980; Mandelbaum et al., 1979; Ocorr et al., 1983; Pollock et al., 1982; Weiss et al., 1979). In contrast, cells that are not endowed with a serotonin-sensitive adenylate cyclase (Bernier et al., 1982) receive the other, distinctively dense type of innervation. These include L₂ to L₆ and R₃ to R₁₃. With these distinctions in mind, we examined the innervation to R2, the giant cholinergic neuron of the abdominal ganglion. This neuron responds to serotonin with only a small increase in cAMP (Cedar and Schwartz, 1972; Bernier et al., 1982). Interestingly, we found that the density of fibers surrounding R2 is moderate in comparison with that of the adjacent right upper quadrant cells or with those of the left.

The correlation between pattern of innervation and type of response in the target cells is likely to be physiologically significant. How the two distributions relate to function is not understood, however, because the kind of receptors that might respond to the dense innervation is unknown. Perhaps more serotonin must be released to activate the receptors postsynaptic to the denser type of innervation. In this situation, more transmitter might be required because the receptors are few in number or low in affinity. Alternatively, the serotonin released might be rapidly lost from this type of synapse. The release sites at these targets may be more numerous than in those inputs whose action is mediated by receptors linked to adenylate cyclase which amplifies the signal within the cell by synthesis of the second messenger.

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