### I. Introduction

**A. Standard Synapse**

The standard synapse relies on regulated exocytosis (Fig. 1A). Vesicles, 30–60 nm in diameter, are first filled with transmitter molecules and docked at specialized “active zones” where they wait for a signal. The release of a vesicle’s contents commences when depolarization opens ion channels and, instead (as described in Fig. 4 and sect. II, A6 and B1), control the release of transmitter with both hyperpolarizing and depolarizing changes in voltage that last tens to hundreds of milliseconds. As I shall explain, transport-mediated synapses work best when controlled by slow changes in presynaptic voltage. Two types of transport-mediated synapses have been proposed. Here, I first describe the properties of the standard synapse and then the idealized properties of the transport-mediated synapses. Afterwards, I consider the evidence. The discussion includes the twists and detours necessary to understand both mechanism and function.

### A. Standard Synapse

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Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+} flows into the cytoplasm. Ca\textsuperscript{2+} is the immediate trigger for the fusion of vesicle and surface membranes. Transmitter molecules exposed to the extracellular space quickly diffuse to receptors on nearby neurons. The entire release process can be completed in \textasciitilde1 ms (52, 80). Subsequently, transmission is terminated as transmitter molecules diffuse from a release site, are chemically modified, or removed from the extracellular space by transporters. Although transporters help remove transmitter molecules from the synaptic cleft, their role is not essential for the rapid transfer of synaptic information.

Three properties are characteristic of the standard model. First, exocytosis is triggered by Ca\textsuperscript{2+} influx and a rise in intracellular Ca\textsuperscript{2+} concentration (37, 67). Second, each loaded vesicle contains a few thousand transmitter molecules (69). As a consequence, the concentration in the synaptic cleft can momentarily reach a relatively high concentration, i.e., \textasciitilde1 mM (24). Third, because all loaded vesicles contain a roughly equal number of transmitter molecules, sequential fusion events are of roughly equal size (31), i.e., release is “quantized.”

B. What Are Transporters?

Charged molecules, like transmitters, do not easily penetrate lipid membranes. Instead, channels, pumps, and transporters provide substrate-specific pathways through a membrane. Channels are aqueous pores that allow ions to move down an electrochemical gradient. Pumps utilize energy derived from the breaking of chemical bonds to do the work required to move a substrate up a concentration gradient. Transporters can couple the movements of several substrates. The entire process can be considered to be a chemical reaction that translocates reactants, transmitter and ions, across a membrane rather than altering chemical bonds. Some reactants will move down their electrochemical gradient while others are moved up, so long as the total movement is energetically favorable. The transport reaction is reversible. Equilibrium occurs when the forward and backward fluxes are equal. If the reactants are charged, zero current is observed at a reversal potential. Away from the reversal potential, the rate of transport is determined both by membrane voltage and the concentrations of all of the reactants.

Transporters can be grouped into gene families whose members share sequence similarity. Na\textsuperscript{+}-coupled transporters are a large family whose members move several transmitters across the surface membrane (96). In humans there are four GABA transporters, two glycine transporters, one for monoamines, and another for serotonin. A separate family of five transporters translocates glutamate (128). In addition, H\textsuperscript{+}-coupled transporters are preferentially expressed in synaptic vesicles (41, 45).

Transporters have distinctive ion requirements. Each Na\textsuperscript{+}-coupled transporter moves a substrate (transmitter) with Na\textsuperscript{+} and a select set of other ions across a cell’s surface membrane (see Ref. 117). The requirement for Na\textsuperscript{+} is stringent. No other ion can substitute. Consequently, the absolute dependence on Na\textsuperscript{+} can be used as a diagnostic test.

Each transport reaction moves only one or a small number of transmitter molecules across a membrane. The maximum reaction rate has been estimated as \textasciitilde10–100 s\textsuperscript{–1}. This estimate often comes from the time course of a
transient current observed in the absence of transmitter (82, 152). However, a reaction observed in the absence of transmitter may not indicate the rate of transport when transmitter is present. In fact, transporters may operate at rather brisk rates. For example, a Ca\(^{2+}\) transporter operates at rates of 5,000 s\(^{-1}\) (53). Likewise, a GABA transporter can cycle at a rate of \(\sim 1,000\) s\(^{-1}\) (unpublished observations; see also Ref. 4 for evidence that the partial reaction for influx through a glutamate transporter operates at \(\sim 1,000\) s\(^{-1}\)).

Transporters were initially viewed as molecular machines that moved substrate from the extracellular medium into the cytoplasm. “Reverse” transport, viz. efflux, was rarely encountered and considered to be inefficient. Why? Efflux is usually limited by the intracellular concentrations of Na\(^{+}\) and transmitter. The consequence can be emphasized with two examples. Transporters allow Müller glia to remove glutamate from the retina’s extracellular space. The efficient intracellular conversion of glutamate to glutamine keeps the cytoplasmic glutamate concentration \(<0.1\) mM (84, 85). Moreover, the intracellular cation is predominately K\(^{+}\) rather than Na\(^{+}\). Low concentrations of intracellular glutamate and Na\(^{+}\) make efflux unlikely during physiological conditions. Next consider a retinal horizontal cell that accumulates GABA. The intracellular GABA concentration may be 10 mM (72, 84). Moreover, Na\(^{+}\) enters continuously through synaptic receptors activated by the release of glutamate from adjacent photoreceptors. Thus a horizontal cell contains both GABA and Na\(^{+}\). As a result, the influx of GABA from horizontal cells is possible.

C. Transport Shuttle

The model transport-synapse incorporates a bidirectional shuttle whose balance point is set by ion concentrations and voltage (Fig. 1B). Intra- and extracellular transmitter concentrations are in balance during usual physiological conditions. Usually the intracellular concentration is high, perhaps 10 mM, while the extracellular concentration is low, \(<100\) µM. In addition, the intracellular volume is relatively large compared with the much smaller extracellular volume. A low concentration in a small extracellular volume is buffered by a high concentration in a large intracellular volume. Altering the balance between influx and efflux changes the concentration of transmitter in the extracellular space.

Two properties are characteristic of a transport shuttle. First, Na\(^{+}\) is required for both substrate influx (uptake) and efflux (release). Release occurs only when a neuron has adequate transmitter and Na\(^{+}\) concentrations. Second, transporters are less sensitive than voltage-gated channels to changes in membrane voltage. Consequently, a transport shuttle is likely to require a relatively large change in either presynaptic voltage or ion gradients.

Transport-mediated release lacks the anatomical specialization of vesicles localized at a release site. Thus the identification of a transport synapse usually depends on physiology. Three observations can help in the identification. 1) Transport requires Na\(^{+}\) and does not depend on Ca\(^{2+}\). 2) The voltage dependence for transport does not match the voltage dependence of the Ca\(^{2+}\) current. 3) Transporters have a distinctive pharmacology. Selective and potent agents are available for catecholamine and indolamine transporters (14). A smaller pharmacopeia is available for GABA transporters (16). Unfortunately, equally good drugs are not yet available for glutamate transporters (30).

D. Transport Retrieval

A second type of transport synapse is a hybrid that depends on a balance between exocytosis and transport. Although exocytosis releases transmitter, it is voltage-dependent uptake that is essential for controlling the concentration of transmitter in the synaptic cleft. In an extreme case, a steady rate of vesicle fusion continuously adds transmitter to the synaptic cleft. The concentration of transmitter in the cleft is determined only by the rate of transmitter removal (Fig. 1C). Hyperpolarization speeds the transporter and lowers the concentration in the cleft, and depolarization has the opposite effect.

Standard and transport-retrieval synapses use exocytosis in different ways. The standard synapse relies upon voltage-gated Ca\(^{2+}\) entry to produce a rapid fluctuation in intraterminal Ca\(^{2+}\) concentration and a burst of exocytosis. The transport-retrieval synapse maintains a relatively constant intraterminal Ca\(^{2+}\) concentration and produces a steady stream of vesicle fusion. Continuous exocytosis is unlikely at a synapse with only a few fusion sites but can occur in retinal neurons with ribbon synapses (see below), which have hundreds or even thousands of vesicle fusion sites (112, 151).

Transport-retrieval requires the following: 1) a surfeit of vesicles and hundreds of fusion sites able to sustain high rates of continuous exocytosis and 2) a voltage-dependent transporter in the presynaptic membrane that can control the transmitter concentration in the synaptic cleft.

Transporters play different roles in the transport-shuttle and transport-retrieval synapses. A transport shuttle moves transmitter in both directions across the presynaptic membrane. The transporter at a transport-retrieval synapse needs to operate in only one direction, removing transmitter from the synaptic cleft. Accordingly, reverse transport is unnecessary, and the intracellular transmitter and/or Na\(^{+}\) concentrations can be relatively low.
E. Comparison

Regulated exocytosis is the explosive, one-way delivery of a few thousand molecules into the synaptic space. It is ideally suited to transmit the staccato information encoded by action potentials. The pulsatile mobilization of quanta produces brief and high transmitter concentrations (24). Repeated stimulation often produces a series of unequal responses that reflect a random fluctuation in the number of released quanta (see Ref. 5).

Transporters operate in a different quantitative regime. Each transport reaction moves only one or a few transmitter molecules across a membrane. The total flux depends on the density of transporters and the rate of translocation. Even at a high density, a transporter with a slow cycle time may not be able to make rapid changes in extracellular transmitter concentrations. Hence, transport-mediated release is best controlled by relatively slow changes in voltage or ion concentrations. Consequently, a transport synapse is more likely to be found when changes in presynaptic voltage persist for hundreds of milliseconds rather than 1–10 ms. Slow, small undulations in extracellular transmitter concentration are in stark contrast to the large, brief injections produced by exocytosis. Modest changes in transmitter concentrations must be coupled to postsynaptic receptors with a relatively high sensitivity and little desensitization. Postsynaptic receptors for GABA, catecholamines, and glycine (or D-serine) are likely targets. On the other hand, the lower affinity and rapid desensitization of ionotropic glutamate receptors makes them unlikely targets of a transport synapse (however, see sect. II B1).\(^1\)

II. EXAMPLES OF TRANSPORT-MEDIATED SYNAPSES

A. Transport Shuttle in Horizontal Cells

The synapse between horizontal cells and cones provides, perhaps, the best-studied case for transport-mediated release. The story might at first seem simple. Horizontal cells accumulate and release GABA. Postsynaptic cones have GABA receptors. The story would be humdrum except for the claim that transmitter release is transport mediated. This unusual proposal requires an extraordinary proof. Although many parts have been confirmed and indirect evidence is abundant, a rigorous demonstration that transporters mediate synaptic communication has not been accomplished. Therefore, all of the parts deserve a careful review to see what is known, what is speculated, and what is disputed.

1. An atypical synapse

Synaptic interactions between cones, horizontal, and bipolar cells create the first step in processing visual information. Horizontal cells form an electrical syncytium, which integrates input from photoreceptors over a large retinal area. This integrated activity is relayed back to cones and forward to bipolar cells as a “surround signal.” Horizontal cells and bipolar cells poke slender fingers into a cone’s synaptic ending. Each photoreceptor has 5–25 invaginations. The typical organization within each invagination is a triad (89, 131), a central bipolar cell process sandwiched between two lateral, horizontal cell processes (Figs. 2 and 3). The synaptic site is clearly marked by a cytoplasmic organelle, a proteinaceous sheet or “ribbon” that is anchored to the presynaptic membrane. Vesicles fill a cone’s cytoplasm and are tethered to the flanks of ribbons. Several rows of vesicle fusion sites dimple the surface membrane along the flanks of a ribbon (112). In contrast, the lateral (horizontal cell) process is either empty (Fig. 3B) or occasionally contains scattered vesicles (Fig. 3C). An active zone and fusion sites are absent from the horizontal cell’s surface membrane (112). In many species these empty processes are the only sites where cones and horizontal cells are sufficiently close to form a synapse.

The lateral processes of horizontal cells (Fig. 3) provide an exception to the nearly monotonous morphology of conventional synapses. The absence of both vesicle clusters and fusion sites has been a lump under the carpet on which discussions of synaptic mechanism have tripped for nearly 20 years. At first the anatomy was only puzzling. The notion that these were sites of a novel release mechanism began with the observation that the putative transmitter GABA could be released without Ca\(^{2+}\). A sheet of horizontal cells was isolated from the toad retina by killing other retinal neurons with a cocktail of toxins (124). The surviving horizontal cells released GABA when depolarized either by K\(^+\) or glutamate. The release of GABA was unchanged when Ca\(^{2+}\) was removed from the extracellular saline and replaced with Co\(^{2+}\), an ion that blocks voltage-dependent Ca\(^{2+}\) channels and does not support exocytosis.

The argument for transport-mediated release depends on the following observations: 1) GABA is synthesized and accumulated in horizontal cells, 2) GABA is released from horizontal cells during darkness, 3) the release of GABA is Na\(^+\) dependent and Ca\(^{2+}\) independent, 4) horizontal cells express a GABA transporter that mediates reverse transport in both intact retinas and isolated cells, and 5) GABA receptors are expressed at postsynaptic sites. Results supporting these points have come
from many species. One problem is to separate consistent features seen in most retinas from variations and specializations that distinguish individual species. I first discuss results from four nonmammalian species. No single species provides a complete set of results. Instead, their contributions articulate like pieces of a jigsaw puzzle.

2. Turtle

Two hundred years ago Thomas Young (170) assumed that the response in a photoreceptor was determined only by the light it absorbed, an idea later incorporated into the Young-Helmholtz theory of trichromatic vision. Consequently, it was a surprise when Baylor et al. (12) reported that cones also receive a synaptic input from horizontal cells. The processing of visual information begins when each cone combines the hyperpolarization produced by the light it absorbs with a synaptic input from horizontal cells, which relays information about the average light intensity that falls on a large area of surrounding retina. The "surround signal" was first seen in the turtle retina.

The turtle has four types of horizontal cell (see Ref. 28). Only one type uses a transporter to accumulate $[^3H]$GABA (71). The same cells also express an enzyme required for GABA synthesis: glutamic acid decarboxylase (GAD) (55). GABA, unlike glutamate or glycine, is not an amino acid involved in intermediary metabolism. It is synthesized only in neurons and $\beta$-cells of the pancreas, where its presence usually indicates a role in intercellular communication.

Physiology almost implicates GABA in the synaptic communication between horizontal cells and cones. Exogenous GABA depolarizes horizontal cells and attenuates and slows responses elicited by flashes of light (104). Isolated cones, but not rods, have GABA receptors on their synaptic endings (64). These receptors were believed to be sites of synaptic input. However, inexplicably, GABA agonists and antagonists have been reported to have no effect on the synaptic signal transmitted from horizontal cells to cones (139). It is hard to understand how GABA agonists can alter the amplitude and time course of response in a presynaptic horizontal cell (104), and increase the conductance of a postsynaptic cone (64), but not alter the synaptic signal that passes from the horizontal cell to the cone (139). Because the results are inconsistent, they almost, but not quite, implicate GABA as a transmitter. Fortunately, results from the salamander help fill the gap.

3. Salamander

The salamander retina has two types of horizontal cells. One type expresses GAD (159) and accumulates $[^3H]$GABA (155). The presence of a GABA transporter is confirmed by the ability of a relatively specific inhibitor, NO-711, to block a GABA-activated current (161; but see Ref. 81). Transport and synthesis produce a high cytoplasmic GABA concentration (32, 77, 155, 159).

The combination of immunohistochemistry, physiology, and pharmacology has demonstrated that GABA mediates signals transmitted to cones, bipolar cells, and horizontal cells. GABA receptors decorate photoreceptor terminals and horizontal cell processes (153). The sur-
round signal transmitted to cones is suppressed by GABA_A agonists and antagonists (156). In a similar manner, surround signals transmitted to bipolar cells are inhibited by GABA (155) or a combination of GABA_A and GABA_B antagonists (49). In addition, physiology demonstrates autoreceptors on isolated horizontal cells (47), which shape responses to flashes of light in intact retinas (63). When GABA is added to saline superfusing an iso-

**Fig. 3.** A: low-power electron micrograph of a cone’s synaptic pedicle in the turtle (*Pseudemys scripta elegans*) retina. An arrow points to a ribbon with a halo of synaptic vesicles. Three ribbons are evident. The cone’s cytoplasm is filled with synaptic vesicles. B: higher power view of the invaginating complex in the turtle retina. C: the invaginating complex in the monkey (*Macaca arctoides*) retina. H, horizontal cell lateral process; B, bipolar cell dendrite. The lateral processes made by horizontal cells are usually empty (as in B) or occasionally contain scattered vesicles (as in C). There are no obvious active zones in the lateral processes. (Electron micrographs kindly provided by Elio Raviola, Harvard Medical School.)
lated retina, responses produced by flashes of light are attenuated and slowed (63, 162, 163). Because similar changes occur during dark adaptation, Yang and Wu (160) suggest that dark adaptation increases extracellular GABA concentration.

Although several details must still be resolved, the experiments described above indicate that GABA is actually used as a horizontal cell transmitter. Nonetheless, the salamander has provided little insight into the mechanism of GABA release. This part of the story has come from other species, particularly the catfish.

4. Catfish

The catfish retina has two types of horizontal cells. Rods selectively contact one type of horizontal cell, and cones contact the other type. Only the cone-driven horizontal cells accumulate GABA (72). The cytoplasmic concentration of GABA is estimated to be ~10 mM (73). Experiments with intact retinas provide a broad outline of GABA movements (73, 75). The intracellular concentration is highest during constant illumination, when horizontal cells are hyperpolarized, and decreases during darkness, when they are depolarized. Presumably GABA is transferred from the cytoplasm to the extracellular space, where it is used as a neurotransmitter. As expected, surround signals in cone photoreceptors are blocked by a GABA receptor antagonist (72, 75).

Information about the mechanism of release comes from isolated cells. The release of GABA can be measured with a sensitive detector. Goldfish bipolar cells have a large GABA_A current. When a catfish horizontal cell and a goldfish bipolar cell were isolated, placed in the same dish, and then pushed against each other, the activation of a current in the bipolar cell could be used to detect the release of GABA from the horizontal cell (127). Even after Ca^{2+} in both the external solution and the horizontal cell’s internal solution was buffered to 50 nM with a combination of EGTA and BAPTA, GABA was released when the horizontal cell was depolarized. Thus depolarization released GABA by a mechanism that did not require a horizontal cell was depolarized. Thus depolarization reaction of EGTA and BAPTA, GABA was released when the internal solution was buffered to 50 nM with a combined retina, responses produced by flashes of light are attenuated and slowed (63, 162, 163). Because similar changes occur during dark adaptation, Yang and Wu (160) suggest that dark adaptation increases extracellular GABA concentration.

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dependence of the influx and efflux currents). The net flux is controlled by membrane voltage.

Experiments on isolated catfish horizontal cells indicate that GABA transporters are expressed at a reasonably high density over a large part of the surface membrane (22, 36, 60, 136, 137). Consequently, transporters allow a depolarized horizontal cell to ooze transmitter from its entire surface. Although this is not a picture of selective communication restricted to specialized points of contact, the cloud of transmitter that surrounds a horizontal cell may be what is required to transfer information about the horizontal cell membrane potential to neighboring cells.

Catfish horizontal cells have provided an important piece of the jigsaw puzzle. The concentration of GABA in horizontal cells changes with illumination. A Na⁺-coupled transporter moves GABA in both directions across the membrane. The amplitude and direction of net flux is controlled by membrane voltage.

5. Goldfish

The model transport-mediated synapse incorporates the release mechanism observed in isolated, catfish horizontal cells and the surround signal mediated by GABA in salamander retinas. Experiments with goldfish retinas echo results from catfish and salamanders.

The goldfish has four morphological types of horizontal cells. One of these receives input from rods, and three types receive input from varying combinations of cones (132, 133). Only one type of cone-driven horizontal cell expresses GAD (10, 17) and accumulates GABA (84, 86, 94, 101, 169). GABA is accumulated in the cytoplasm and released during continuous illumination, when horizontal cells are hyperpolarized, and is released during darkness, when they are depolarized (86, 165, 168). In addition, release is Ca²⁺ dependent and increased when horizontal cells are preloaded with Na⁺ (164, 166). Experiments on isolated cells have demonstrated that a transporter is responsible for the efflux of GABA. Retinas were dissociated, and horizontal cells were separated from other cells by velocity sedimentation (6–8). GABA was released when a collection of several hundred isolated cells was depolarized with a high-K⁺ saline or glutamate. Most of the GABA efflux was Na⁺ dependent, Ca²⁺ independent, and controlled by voltage over a broad range. Only a small part was Ca²⁺ dependent. Voltage-dependent transport is the predominant mechanism controlling the movement of GABA between cytoplasm and the extracellular space.

Physiology and pharmacology experiments, similar to those already described for the salamander and catfish, indicate that GABA is used as a horizontal cell transmitter and contributes to the surround signal. GABA affects the responses to light of both cones and horizontal cells (157). In a related teleost, the carp, GABA mediates a surround signal transmitted from horizontal cells to cones (95, 142, 143).

6. Role of GABA in nonmammalian horizontal cells

All of the items necessary for transport-mediated release are present in nonmammalian horizontal cells. A phylogenetic survey of vertebrates demonstrates that GABA is synthesized and accumulated in cone-driven horizontal cells in virtually all nonmammalian retinas (83). This includes cartilaginous and bony fish, amphibians, reptiles, and birds. The intracellular concentration of GABA depends on membrane voltage. Light produces a change in membrane potential that is well suited for controlling a voltage-dependent transporter. Like other neurons in the outer retina, horizontal cells do not produce action potentials, but instead generate potentials that are continuously graded between −20 and −70 mV. Shifts in voltage occur on a time scale of ~100 ms. Large, graded potentials are ideal for controlling a transport mechanism. GABA is released when horizontal cells are depolarized. The voltage dependence of Na⁺-coupled GABA transporters is sufficient to control the flux of GABA. Once released, GABA activates receptors on cones and bipolar cells as well as autoreceptors on horizontal cells.

The first tendency was to believe that GABA was the whole story and able by itself to explain the genesis of surround signals and all lateral interactions in the outer retina. The following considerations, all based on observations made in the goldfish retina, provide a slightly different view.

Synaptic interaction between a horizontal cell and a cone was initially assumed to occur within the invagination. However, ultrastructural immunolabeling demonstrates few GABA_A receptors located within the invaginating complex. Instead, they are located on the lateral surfaces of the cone’s synaptic pedicle (169). GABA must diffuse in the extracellular space to reach these receptors. The release of GABA from the entire cell surface (see sect. A4) together with GABA receptors at a “peri-synaptic” location implies that GABA operates by volume transmission (for a discussion of volume transmission, see Ref. 43).

There is a gradient in GABAergic properties between central and peripheral retina. Horizontal cells in the periphery accumulate more [³H]GABA and label more intensely with GABA and GAD antibodies than centrally located cells (S. Yazulla, personal communication; see also Ref. 1). The function of horizontal cells in the retinal circuit may change with retinal position.

The activity of the horizontal cell’s GABA transporter may be modulated with a circadian rhythm. In teleosts, dopaminergic interplexiform cells cross from the inner to the outer plexiform layer and make abundant synapses on
cone-driven horizontal cells (141). The release of dopamine follows a circadian cycle (see Ref. 19). Exogenous dopamine inhibits the release of GABA from horizontal cells (167; see also Ref. 65). As a consequence, the release of GABA may be reduced during the day and increased at night.

These considerations suggest that GABA communication involves volume transmission, is accentuated in the periphery, and is modulated in a circadian cycle. Although GABA may still contribute to a surround signal, this more restricted picture of GABA action makes it unlikely that the transport-mediated release of GABA can explain all synaptic interactions mediated by horizontal cells.

7. Mammalian horizontal cells

Nonmammals have between two and five types of horizontal cells. Usually, only one type of cone-driven cell is GABAergic. In contrast, most mammals have two types of horizontal cells, and both are GABAergic. Our understanding of the role of GABA in mammalian horizontal cells has changed during the past 10 years. Mammalian horizontal cells usually contain less GABA than GABAergic amacrine cells in the same retina. In addition, horizontal cells in different species have very different apparent GABA concentrations. The rabbit and cat illustrate this situation. The rabbit typifies mammalian species in which GABA content is relatively low. It is now apparent that the concentration of GABA is developmentally regulated. At first, during an early phase of retinal differentiation, both types of horizontal cell accumulate [3H]GABA (113) and contain an appreciable GABA concentration (88, 101, 109). Afterwards, uptake and GABA content decline as Müller glia differentiate and become new sites of GABA uptake. Although adult horizontal cells continue to express the synthetic enzyme GAD (59, 93), the concentration of GABA in adult horizontal cells is relatively low (59), albeit greater than in non-GABAergic neighbors. The cat typifies a mammal whose horizontal cells contain a significant concentration of GABA. The expression of GAD has been demonstrated by immunolabeling (145) and by in situ mRNA hybridization (119). The concentration of GABA is moderate but still less than in amacrine cells (85, 107, 145, 154).

Although their intracellular GABA concentration appears to be low or modest, there is reasonable evidence that mammalian horizontal cells use GABA as a neurotransmitter. For example, immunohistochemistry labels GABA_A receptors on rabbit cone synaptic terminals (90) and the shafts of bipolar cell dendrites adjacent to cone pedicles in the rabbit (48) and cat (146) retinas. Complementary evidence comes from physiology. GABA activates autoreceptors in isolated, rabbit horizontal cells (15), and both attenuate and slow responses produced in intact retinas (15). Likewise, GABA_A agonists have a similar effect on cat horizontal cells (42). Thus there is anatomical and physiological evidence for GABA as a horizontal cell transmitter in both nonmammalian and mammalian retinas.

8. A difference between nonmammals and mammals

There is a significant difference in the chemical organization of nonmammalian and mammalian retinas that involves both horizontal cells and Müller glia (see Ref. 83). Müller glia in nonmammalian retinas do not take up GABA. At the same time, virtually all nonmammals have one type of horizontal cell that synthesizes and accumulates a high concentration of GABA. The situation is altered in mammals, which have Müller glia that avidly scavenge GABA from the extracellular space and horizontal cells with only a low to moderate GABA concentration.

As expected, a Na^+-coupled transporter moves GABA into mammalian Müller glia. Unexpectedly, there is no indication of a Na^+-coupled transporter in mammalian horizontal cells. Johnson et al. (58) labeled rat retinas with specific antibodies that recognize three GABA transporters. Müller glia were labeled by two of these antibodies, and horizontal cells were unlabeled. A novel activity-dependent assay provided a similar result (108). The uptake of GABA transport agonists (γ-vinyl GABA, dianisobutyric acid, and gabaculine) was detected with specific antibodies. Amacrine and Müller cells were labeled in cat and monkey retinas, but horizontal cells remained unlabeled.

Nonmammalian horizontal cells control the GABA concentration in synaptic and nonsynaptic spaces. In mammals, these tasks appear to be divided between horizontal cells and Müller glia. Mammalian Müller glia use a Na^+-coupled transporter to control the extracellular GABA concentration in nonsynaptic regions. Mammalian horizontal cells may use another mechanism to focally release GABA at synaptic sites. Monkey horizontal cells express an H^+-coupled “vesicular” GABA transporter, VGAT. Light-microscope immunohistochemistry reveals a low density expressed in the cell surface and a higher density in dendrites that poke into the invaginating synapse (51). A similar distribution of VGAT is also seen in the rat, mouse, and rabbit retinas (29). Intense labeling of dendrites may reflect a high density in the surface membrane or an aggregation of synaptic vesicles. Electron-microscope immunohistochemistry will be needed to determine the site. Because the voltage and ion dependence of the H^+-coupled transporter are incompletely known, it is not yet clear whether this transporter could mediate the voltage-dependent release of GABA.

Both nonmammalian and mammalian horizontal cells appear to use GABA as a transmitter. A Na^+-coupled transporter releases GABA from the entire cell surface of
one type of cone-driven horizontal cell in nonmammalian retinas. Although mammalian horizontal cells appear to use GABA, another mechanism may restrict release to the tips of their dendrites.

9. Conundrums

The exact part that transport-mediated release of GABA plays in synaptic interactions between horizontal cells and cones continues to be a vexing question. The following observations indicate some of the difficulties and problems that must be overcome to understand GABA’s synaptic action.

Nonmammals have several subtypes of horizontal cells. Each subtype may use different transmitters or mechanisms for communicating with neighbors. Only one type of cone-driven horizontal cell accumulates GABA. For example, the goldfish has two types of cone-driven horizontal cells and a rod-driven horizontal cell that do not contain and release GABA. These cells must use other transmitters or mechanisms for synaptic communication.

An individual horizontal cell may have more than one mechanism for communicating with neighbors. For example, turtle (28), salamander (78), catfish (78), goldfish (78), and rabbit (103) horizontal cells contain a nitric oxide synthase. Presumably, these cells use nitric oxide to alter the behavior of neighboring neurons.

Horizontal cells make their characteristic synaptic connection in the invaginating complex. The typical lateral process has an empty cytoplasm (with few or no vesicles) and lacks an active zone. In addition, in some species, horizontal cells also make standard synapses at other locations. For example, catfish horizontal cells make conventional synapses (outside the invagination) onto cone telodendria, fine dendrites that interconnect neighboring cones (118). Because the catfish has only one type of cone-driven horizontal cell, transport-mediated and standard synapses must coexist in the same cell. Another example is in the salamander retina, which has two types of horizontal cells. One of these makes a conventional synapse with the second type (74). In this case, it is not known which subtype accumulates GABA. Although these synapses may be peculiar to the species cited, the examples illustrate that cone-driven horizontal cells (or at least some of them) can make standard synapses. Finally, in the human retina, lateral processes that contact rods (unlike those that contact cones) contain vesicles and an active zone (79). The function of this contact is unknown. Physiology has so far consistently failed to demonstrate a surround signal in both nonmammalian and mammalian rods.

The transport-mediated release of GABA is only one of several synaptic mechanisms that operate in the retina’s outer synaptic layer. Understanding the functional role of GABAergic transmission may require understanding the entire circuit.

10. An alternate mechanism for surround inhibition

Our perspective of GABA and transport-mediated release by horizontal cells will be altered if we discover other mechanisms that contribute to the surround signal. Hence, I include a detour to describe an ephaptic mechanism that has recently been suggested (61). No transmitter is involved. A horizontal cell dendrite that pokes into a cone’s synaptic base (see Figs. 2 and 3) is assumed to create an extracellular path with a high electrical resistance. The current that passes out the tip of a dendrite produces a voltage as it traverses the extracellular resistance. For example, 100 pA flowing out of a horizontal dendrite and into an extracellular space with 1-MΩ resistance would produce an extracellular voltage of 0.1 mV. As a consequence, channels in the cone membrane that are located opposite the current source would be depolarized by the shift in extracellular voltage, in this case 0.1 mV.

Ephaptic transmission within the cone invagination was originally suggested by Byzov and Shura-Bura (20). The most recent version, proposed by Kamermans et al. (61), emphasizes a special role for hemi-gap channels. An antibody that recognizes the gap-junction protein connexin-26 (Cx26) labels the tips of goldfish horizontal cell dendrites but does not label gap junctions that connect neighboring horizontal cells (57). Because electron microscopy fails to provide evidence for gap junctions within the invagination, Kamermans et al. (61) suggest that Cx26 forms hemi-gap channels. Normally, hemi-gap channels are kept closed by a negative membrane potential and extracellular Ca²⁺ (33). Kamermans et al. (61) suggest that Cx26 forms patent hemi-gap channels in horizontal cell dendrites. They buttress their claim with a physiological experiment. After retinas were continuously treated with SKF89976A, a potent inhibitor of GABA transport, a component of the surround signal survived. Carbenoxolone, an agent that closes gap junction channels, blocked the surviving surround signal. The anatomical and physiological correlation strikes one as more than a coincidence and is the essence of the argument for ephaptic transmission.

Ephaptic transmission through hemi-gap channels creates at least three problems.

1) A significant change in extracellular voltage requires either a very large current or an improbably large extracellular resistance (see above). Moreover, it is difficult to see how hemi-gap channels can be the source of a large extracellular current and glutamate receptors can simultaneously polarize a cell from −70 to −20 mV without violating Ohm’s law.

2) Hemi-gap channels are permeable to molecules
less than several hundred Daltons in molecular mass (33, see also Ref. 148) and should allow GABA (and other small molecules, like glutamate and ATP) to leak out of the cytoplasm and into the synaptic space. Thus hemi-gap channels might also be sites of transmitter release.

3) The surround signal generated in horizontal cells is transmitted to cones and hyperpolarizing bipolar cells at inhibitory synapses and transmitted to depolarizing bipolar cells at excitatory synapses. It is difficult to see how ephaptic transmission could produce opposite polarity signals in adjacent cells. In contrast, the polarity of responses produced by GABA receptors could be determined by differences in intracellular Cl− concentrations (see Ref. 147).

The existence of hemi-gap junction channels in the tips of horizontal cell dendrites needs to be confirmed. It is not clear if a GABA-mediated component of surround inhibition is eliminated by SKF89976A. The pharmacological specificity of carbenoxolone is uncertain. Nonetheless, the ability of carbenoxolone to inhibit a component of the surround signal emphasizes that GABA and GABA transport are not the whole story.

11. Summary of transport-mediated release by horizontal cells

One type of horizontal cell synthesizes, accumulates, and releases GABA in virtually all nonmammals. GABA is released during darkness when horizontal cells are depolarized and accumulated during illumination when they are hyperpolarized. A voltage-dependent transporter operates for both influx and efflux. The transporter can explain the release of GABA. However, the story is not complete. The problem that remains is to identify exactly what information is transmitted by the release of GABA. The existence of several horizontal cell types with multiple synaptic interactions has complicated the analysis. GABA transport is only expected in one subtype of horizontal cell. GABA has been reported both to be (49, 95, 142, 143, 155–157) and not to be (62, 81, 139) responsible for a component of the surround signal. A rigorous demonstration that transporters mediate synaptic communication will require identifying synaptic signals that directly result from transport action. This will require dissecting a GABA synaptic pathway from other mechanisms.

Although GABA is likely to be a horizontal cell transmitter in mammalian retinas, the role of transport-mediated release is less certain. Although the anatomy of the mammalian retina has been intensively studied, its physiology has been, unfortunately, less well explored. Nonetheless, it is already apparent that there are significant differences between nonmammalian and mammalian retinas.

B. Transport Retrieval in Cones

Cone photoreceptors provide another perspective on transport-mediated synaptic transmission. The machinery for exocytosis is obvious. The cytoplasm of a cone’s synaptic ending is filled with synaptic vesicles, and intracellular ribbons mark active zones (Fig. 3A). Nonetheless, two physiological observations in nonmammals suggest a problem. First, the voltage dependence of the cone’s Ca2+ current (see Fig. 4B and Table 1) is barely activated within the physiological operating range (76, 126). Second, a component of synaptic transmission is reported to persist after Ca2+ entry through voltage-gated channels is blocked with either Co2+ (125) or nisoldipine (115). These observations can be resolved by the discovery that a glutamate transporter plays a surprising and critical role at the nonmammalian cone synapse. A further twist appears when we compare the behavior of nonmammalian and mammalian cones. Thus it is necessary to discuss the difference.

1. Nonmammals

The Ca2+ current in nonmammalian cones appears to be ill suited for controlling the release of transmitter (see Fig. 4B). In the absence of light, the membrane voltage is between −35 and −40 mV. A brief flash produces a hyperpolarization that reaches a maximum of approximately −65 mV and lasts several hundred milliseconds. The entire presynaptic voltage range contains information that must be communicated to postsynaptic neurons. Nonmammalian photoreceptors express a relatively sim-

<table>
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<th>Species</th>
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<th>pH</th>
<th>V1/2 mV</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
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<td>−18 ± 6</td>
<td>9</td>
</tr>
<tr>
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<td>7.2–7.3</td>
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<tr>
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<td>−17.8</td>
<td>70</td>
</tr>
<tr>
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<td>7.4</td>
<td>−15.3 ± 2.3</td>
<td>115</td>
</tr>
<tr>
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<td>11</td>
</tr>
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<td>76</td>
</tr>
<tr>
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<tr>
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<td>−26.6 ± 1.1</td>
<td>134</td>
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<tr>
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<td>−31</td>
<td>150</td>
</tr>
<tr>
<td>Monkey</td>
<td>C</td>
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<td>−41.5</td>
<td>138</td>
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<tr>
<td>Squirrel</td>
<td>C</td>
<td>7.4</td>
<td>(−47)</td>
<td>34</td>
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</table>

Values in parentheses have been estimated from data that appear within figures of the cited references. Otherwise, values have been taken from the text in each citation. The calcium current in nonmammalian rods and cones activates at similar voltages. The activation voltage is shifted approximately −7 mV for a 0.4 increase in pH. The voltage for half-maximum activation of Ca2+ current in mammalian cones is −28 mV more negative than in nonmammalian cones.
ple mix of voltage- and Ca\(^{2+}\)-dependent currents (9, 87). The Ca\(^{2+}\) current activates when the potential is above −40 mV and has a half-maximum amplitude at approximately −20 mV (Table 1). Hence, there is only a small overlap between voltages produced during exposure to light (−35 to −65 mV) and voltages that activate the Ca\(^{2+}\) current (−40 to 0 mV). Two mechanisms have been suggested to resolve the paradox: 1) the voltage dependence of the Ca\(^{2+}\) current is shifted to overlap the functional range, or 2) Ca\(^{2+}\) enters the cytoplasm through another route.

Various factors have been suggested to modulate the voltage dependence of the photoreceptor’s Ca\(^{2+}\) current: protons (11), anions (140), divalent cations (106), nitric oxide (70), dopamine (134), somatostatin (2), and lipids (149). All of these may produce small shifts (5–10 mV) in voltage dependence. It is not clear that any agent or combination can produce the larger shift (20–30 mV) needed to align the voltage dependence of the cone’s Ca\(^{2+}\) current with the physiological operating range.

Rieke and Schwartz (115) have identified an alternate path for Ca\(^{2+}\) entry. Salamander cones have a cGMP-gated conductance in their somata and synaptic endings. The location of cGMP-gated channels in the synaptic ending has been confirmed by pulling patches from the pedicle of a lizard cone (120). Ca\(^{2+}\) enters through cGMP-gated channels and triggers exocytosis (measured as an increase in cell capacitance). Because these channels are relatively voltage independent within the physiological range, voltage does not modulate Ca\(^{2+}\) entry and exocytosis. Instead, the cGMP-gated conductance provides a steady influx of Ca\(^{2+}\), which supports a continuous exocytosis. The concentration of glutamate in the synaptic space is controlled by another mechanism.

Salamander cones express a glutamate transporter in their synaptic endings (105). A series of experiments (44), described below, indicate that the transporter controls the concentration of glutamate in the synaptic cleft. These experiments relied on selectively blocking the activity of the transporter with dihydrokainate (DHK), blocking Ca\(^{2+}\) entry and exocytosis with extracellular Mg\(^{2+}\), and using light to change the cone’s membrane voltage.

The first experiment determined the relationship between the extracellular glutamate concentration and the membrane potential of horizontal cells in a retinal slice. Glutamate was added to the bath after both release (by exocytosis) and uptake (by the transporter) were blocked. The membrane potential of horizontal cells depolarized from −70 to −20 mV as the glutamate concentration was changed from 1 to 80 μM (EC\(_{50}\) = 32 μM). The observed correspondence between glutamate concentration and membrane voltage was used in subsequent experiments to translate horizontal cell voltage into the apparent glutamate concentration in the synaptic cleft.

Release (by exocytosis) and uptake (by the transporter) were separately inhibited to determine their individual contributions to controlling the glutamate concentration in the synaptic cleft. As expected, exocytosis added glutamate to the synaptic cleft. Transporters controlled the rate of removal. The steady-state concentration in the synaptic cleft was determined by a balance between the ability of exocytosis to add glutamate and the ability of a transporter to remove glutamate from the synaptic cleft.

The following experiment demonstrates that the voltage dependence of the transporter was sufficient to control the concentration of glutamate in the synaptic cleft. The release of glutamate was first blocked with 20 mM Mg\(^{2+}\). As a result, horizontal cells hyperpolarized, and flashes of light failed to produce synaptic responses (see also Ref. 38). Next, glutamate was added to the bath and allowed to diffuse into the synaptic cleft. As expected, horizontal cells depolarized. But now, the surprising observation was that flashes of light produced relatively normal responses even though Ca\(^{2+}\) entry and exocytosis remained blocked. Changes in presynaptic voltage (without Ca\(^{2+}\) entry) changed the concentration of glutamate sensed by postsynaptic receptors from 70 to 2 μM.

Two factors allow glutamate to act at a relatively low concentration. First, “on” bipolar cells use metabotropic receptors, which have a relatively high glutamate sensitivity (see Refs. 121, 130). Second, ionotropic receptors in horizontal and “off” bipolar cells are desensitized by the continuous release of glutamate (34, 39). Desensitized receptors produce small macroscopic currents but, paradoxically, operate with a high glutamate sensitivity (see, for example, Ref. 50). The transporter modulates the extracellular glutamate concentration within a range that effectively controls the activation of metabotropic and desensitized ionotropic receptors.

The voltage-gated Ca\(^{2+}\) current is activated in only a small part of the physiological range, when a cone depolarizes above −40 mV. Within this narrow range, the Ca\(^{2+}\) current triggers exocytosis and should accentuate a transient off depolarization that occurs when a light is extinguished (123). The presence of two systems for Ca\(^{2+}\) entry, voltage-gated channels and cGMP-gated channels, may allow two pathways for modulating transmitter release. The relative contribution of the two mechanisms is uncertain. Indeed, the ratio may not be fixed. Small shifts in either the voltage dependence of the voltage-gated Ca\(^{2+}\) current or the cGMP concentration may have a marked effect.

In the standard synapse, a burst of transmitter is rapidly released and then removed by diffusion and the steady activity of transporters. Membrane voltage controls Ca\(^{2+}\) influx, vesicle fusion, and transmitter release. Transporters maintain a slow and constant recovery effort. Nonmammalian cones may reverse this situation. The balance between delivery and removal determines...
the concentration of transmitter in the synaptic cleft. A steady influx of Ca\(^{2+}\) through cGMP-gated channels sustains a relatively constant rate of exocytosis (115). The voltage dependence of transport-mediated uptake appears to be sufficient to control the concentration of transmitter in the synaptic cleft (44).

2. Mammals

The Ca\(^{2+}\) current in mammalian photoreceptors is well suited for controlling transmitter release (Fig. 4B, blue trace). Like other L-type Ca\(^{2+}\) currents, it is blocked by nisoldipine and has little voltage-dependent inactivation. Unlike the L-type currents expressed in cardiac and somatic muscle, which have a half-maximum activation at \(-120\) mV, the current in mammalian photoreceptors is half-maximally activated at a relatively hyperpolarized potential, approximately \(-47\) mV (Table 1). The remarkable leftward shift of Ca\(^{2+}\)-channel activation in mammalian photoreceptors may be due to the expression of a unique channel protein. Genetic and molecular studies have identified a unique L-type channel as the cause of "congenital stationary night blindness" (CSNB2) (13, 135). The channel protein is expressed in photoreceptors near synaptic ribbons (91, 92).

Evolution appears to have provided nonmammalian and mammalian cones with different solutions for the control of exocytosis and the concentration of transmitter in the synaptic cleft. Mammalian cones use a unique voltage-gated Ca\(^{2+}\) channel, which activates over the entire physiological voltage range. Synaptic transmission from cones to bipolar cells is entirely Ca\(^{2+}\) dependent (34). Moreover, there is no indication of a cGMP-gated conductance in the synaptic ending of squirrel cones (personal observation). In contrast, nonmammalian cones use a more prosaic voltage-gated channel and supplement Ca\(^{2+}\) entry with a cGMP-gated channel. The voltage dependence of the transporter determines the concentration of transmitter in the synaptic cleft.

C. Other Synapses?

I have presented the evidence for a transport shuttle in nonmammalian horizontal cells and transport retrieval in nonmammalian cones. Does transport-mediated release only occur in slimy animals, viz., amphibians, reptiles, and fish, or is it also important for understanding the mammalian brain? Our slimy cousins have bigger cells, which are relatively easy to study. Slow responses in mammalian dendrites are difficult to study. Transport-mediated release is evident in the mammalian brain during pathological conditions and pharmacological intervention. Ischemia releases glutamate by reverse transport (3). Amphetamines stimulate the reverse transport of dopamine (54). These are interesting phenomena, but the more important question is the role of transporters in normal communication. Is there a transport-mediated synapse in the mammalian brain?

Amacrine cells are retinal interneurons with processes restricted to the inner synaptic layer. Starburst amacrine cells, named for their distinctive anatomy, are the only retinal neurons that use acetylcholine as a transmitter. Thus it was puzzling when immunohistochemistry revealed a high GABA concentration in their cytoplasm (18, 68, 144). Starburst amacrine cells contain both acetylcholine and GABA. Acetylcholine is stored in synaptic vesicles, and its release is Ca\(^{2+}\) dependent (100). In contrast, GABA is stored predominantly in the cytoplasm, and its release appears to be Ca\(^{2+}\) independent (100). The mechanism of GABA release is uncertain but may involve a transporter. An individual neuron might use exocytosis and transporters to release different transmitters. The two mechanisms would have different sensitivities to presynaptic voltage and ion concentrations and convey different temporal signals.

And what about the rest of the brain? Are there transport-mediated synapses outside the retina? The substantia nigra provides an opportunity to briefly discuss another transmitter, dopamine. Dopaminergic cells in the pars compacta project axons to the striatum, where they release dopamine at conventional synapses. Back in the substantia nigra, the cell bodies and dendrites receive inputs and also release dopamine (26, 46, 54, 97, 114). Somatodendritically released dopamine may diffuse tens of microns (see Ref. 27) before reaching receptors at nonsynaptic sites (129) and producing an autoinhibitory feedback (110). The mechanism of release is uncertain. Although quantal release has been detected at the cell body (56), electron microscopy reveals few conventional synapses along dendrites (98). It seems unlikely that the observed changes in extracellular dopamine could be sustained by a sparse distribution of conventional synapses. On the other hand, the abundant expression of the dopamine transporter (98) makes reverse transport possible. Axon terminals and dendrites may use different mechanisms for dopamine release. Release from axon terminals in the striatum is Ca\(^{2+}\) dependent, whereas release from dendrites in the substantia nigra is largely Ca\(^{2+}\) independent (23, 54). Falkenberger et al. (40) have recorded from cells with patch pipettes and detected extracellular dopamine with amperometry. They blocked the dopamine transporter with a specific inhibitor and blocked exocytosis by removing extracellular Ca\(^{2+}\). An autoinhibitory current was attributed to reverse transport. Unfortunately, blocking the transporter or removing Ca\(^{2+}\) was slow and required 60–90 min. During this interval much can change. It is not clear that the signal seen after superfusing a slice for 90 min with EGTA also operates in a normal brain. Thus the role of transport-mediated release remains uncertain. The problem is still to identify
the exact contribution of transport-mediated release during physiological conditions.

III. QUESTIONS AND CONCLUSIONS

The role that transporters play in transmitter release has been a topic for 30 years (102, 111, 124). Despite its long history, relatively few transport synapses have been identified. Is this because 1) transport synapses are rare, or 2) transport synapses are difficult to identify and we are reluctant to acknowledge their role without overwhelming evidence? The past decade has produced the cloning and electrophysiological characterization of transport proteins. As a consequence, the mechanism of transmitter translocation is roughly understood. Now, the problem is to determine the exact functional role that transporters play in synaptic communication. Are transporters sometimes important in the moment-to-moment determination of information flow, or are they always part of a support staff, which tidies up after a transmitter is released and corrects small changes in transmitter concentration? Even in the nonmammalian retina, questions remain concerning the exact role of GABA transporters in communication by horizontal cells. How do other transporters and non-GABAergic cells contribute to the surround signal and adaptation?

Why is transport-mediated communication difficult to study? First, reverse transport must be separated from the release of transmitter by exocytosis at both synaptic and nonsynaptic sites. Second, we may be mesmerized by action potentials, propagation, and rapid communication. As a consequence, we incorrectly expect transporters to emulate or participate in similar events. Presently, the standard synapse can account for communication between most neurons. However, the nervous system may operate simultaneously on several temporal and spatial scales. In addition to the rapid transfer of information from one brain part to another, slower signals may wax and wane in local regions (see, for example, Ref. 122). Extracellular recording detects only the timing of action potentials. Patch pipettes and sharp electrodes usually record from somata and do not see activity in distant dendrites, where transport-mediated release may operate. Our view of brain function may be limited by the same techniques that have successfully emphasized action potentials, propagation, and rapid communication.

The standard synapse and transport shuttle are models for synaptic function. Standard synapses are optimized for rapid communication. Transport-mediated synapses are expected to operate on a slower time-scale. Both mechanisms are likely to be embellished and altered when expressed in real neurons. Real synapses elaborate features that make them suited for individual tasks. Synapses are not all the same, and individual synapses have different tasks. Despite great success, our understanding of synaptic communication is still rudimentary.

Address for reprint requests and other correspondence: E. Schwartz, Dept. of NPP, MC0926, Univ. of Chicago, 947 E. 58th St., Chicago, IL, 60637 (E-mail: eas@drs.bsd.uchicago.edu).

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