SYNAPTIC MECHANISMS FOR CODING TIMING IN AUDITORY NEURONS

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ABSTRACT
Neurons in the cochlear ganglion and auditory brain stem nuclei preserve the relative timing of action potentials passed through sequential synaptic levels. To accomplish this task, these neurons have unique morphological and biophysical specializations in axons, dendrites, and nerve terminals. At the membrane level, these adaptations include low-threshold, voltage-gated potassium channels and unusually rapid-acting transmitter-gated channels, which govern how quickly and reliably action potential threshold is reached during a synaptic response. Some nerve terminals are remarkably large and release large amounts of excitatory neurotransmitter. The high output of transmitter at these terminals can lead to synaptic depression, which may itself be regulated by presynaptic transmitter receptors. The way in which these different cellular mechanisms are employed varies in different cell types and circuits and reflects refinements suited to different aspects of acoustic processing.

THE TEMPORAL CODE IN AUDITION
In order to convey information about pitch, intensity, and location, the auditory system takes advantage of place, rate, and temporal codes. An anatomical map of acoustic frequency is generated in the cochlea by the tonotopic response of the hair cell epithelium (1). While the spatial pattern of activation of this array and the firing rate of its auditory nerve output encode information, the timing of action potentials is the lingua franca of auditory processing.
used by a wide variety of neurons to convey specific aspects of the acoustic environment.

The temporal code appears in different forms. Phase-locking occurs when the onset of an action potential appears reproducibly at a particular part of the cycle of the stimulating sound source (1a). While phase-locking is a typical feature of the response to low-frequency sound (\( \sim 1–2 \text{ kHz} \)), phase-locked firing of action potentials may also occur with respect to the cycle of amplitude modulation of a high-frequency carrier (2). In some cases, neurons are adapted to preserving the timing of action potentials with respect to action potentials in other circuits rather than to the sound cycle itself (3). For other neurons, the precision of action potential timing may be retained just in the earliest part of an acoustic response, such as when an onset must be encoded. Neural circuits, which use timing to extract the location and meaning of sounds, are discussed in this volume (3a).

Several principles are played out in the cellular adaptions that permit the conveyance of timing. The surety and consistent timing of the response are essential to transmitting the onset and frequency of an acoustic stimulus and to promote entrainment. As signals are passaged from synapse to axon to synapse, conduction and synaptic delays inevitably accumulate; the processing of temporal information, particularly when convergence is an issue, requires that these delays be highly uniform, despite the inherently probabilistic nature of ion channel gating and transmitter release. Uniform latencies are achieved in part by the large size of the excitatory synaptic potential (EPSP), which ensures that the variability or jitter in the timing of threshold crossing is kept short (4). The shape of the EPSP is fundamental to auditory timing circuits, since narrow EPSPs help minimize temporal summation and ensure a brief refractory period. In this review, we examine how the timing of action potentials can be preserved and transmitted through different synaptic levels in the auditory system, focusing on the mechanisms by which brief, well-timed EPSPs are generated and how their amplitude may be regulated.

SYNAPTIC MORPHOLOGY AND TIMING

In bushy cells of the mammalian ventral cochlear nucleus (VCN) and in their avian homologs of the nucleus magnocellularis (nMAG), as well as in principal cells of the mammalian medial nucleus of the trapezoid body (MNTB) and the ventral nucleus of the lateral lemniscus, somatic innervation by large calyceal or end-bulb terminals, which feature large numbers of functional synaptic release sites per axon terminal, facilitates reliable transmission (5–8). In spherical bushy cells, MNTB, and nMAG, a single stimulus liberates 100–200 transmitter quanta from each axon terminal, as indicated by quantal analysis (9–11). This
bolus of transmitter generates an excitatory postsynaptic current (EPSC) more than 30 times larger than that needed to drive an action potential (4, 11). The resulting EPSP reaches threshold quickly and reliably, despite considerable use-dependent rundown (see below). Somatic innervation avoids the slowing of the onset that dendritic innervation would produce (12). However, despite the apparent adaptive significance of somatic innervation, the octopus cells of the VCN (13), the neurons of the mammalian medial superior olive (MSO) (14), their avian homolog the nucleus laminaris (NL) (15, 16), and to a lesser extent the lateral superior olive (LSO) (3) and the stellate cells of the VCN (17) combine dendrites and good timing, possibly because of biophysical specializations that compensate for cable effects. Moreover, in these dendritic cells and in the globular bushy cells of the VCN, convergence of many axonal inputs is favored over the presence of only a few massive synapses.

**CONTROL OF EPSC DURATION**

**Synaptic Recordings**

The duration of the EPSC is the starting point for controlling the time course of EPSPs in neurons (reviewed in reference 18). EPSPs in auditory neurons must be brief in order to accommodate rapid transmission and minimize temporal distortion through synaptic networks, and brief EPSPs can be achieved only by having short EPSCs. A short membrane time constant ($R_mC_m$), generally influenced by resting potassium conductances, is also important (see below); however, a large synaptic conductance will itself lower the membrane time constant (19), and so EPSPs may decay faster than the intrinsic membrane $R_mC_m$. It remains unclear if this plays a role in the auditory system.

Glutamate, or a related substance, and glutamate receptors mediate fast excitatory transmission in the cochlea and in brain stem auditory neurons. Hair cells and nerve terminals in the cochlear nuclei and trapezoid body exhibit glutamate-like immunoreactivity (20–22), and evoked release of glutamate can be demonstrated biochemically or by a bioassay (23, 24). While there remains some controversy about whether glutamate itself is the afferent transmitter (25), the role of glutamate receptors is clear. As discussed below, glutamate receptors have been localized to the subsynaptic membrane in the cochlea and cochlear nuclei by using immunohistochemical techniques. Recently, the development of a wide range of specific pharmacological reagents and their use on preparations maintained in vitro have permitted precise identification of receptor subtypes mediating transmission in particular cell types. Selective antagonists of $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors block fast excitatory synaptic transmission in rodent bushy cells (26), stellate cells (17, 26), fusiform cells (27), and octopus cells (13). Beyond the cochlear
nucleus, block by AMPA antagonists has been observed in the rodent LSO (28) and MNTB (28–30). In chick brain stem or brain slice preparations, auditory-nerve evoked responses in nMAG are completely blocked by AMPA receptor antagonists (4, 11, 31), as illustrated in Figure 1A and B. Excitatory transmission in the avian NL is also blocked by these agents (32, 33).

Overall, it seems that N-methyl-D-aspartate (NMDA) receptors contribute little to the excitatory drive for those neurons that transmit well-timed signals. In the chick nMAG, NMDA receptors contribute only slightly to the EPSP (4), and by hatching age only 10% of the peak EPSC is produced by NMDA receptors (11); even this occurs only at positive membrane potentials where magnesium block of the NMDA receptor is minimized. However, during repetitive synaptic stimulation, NMDA receptors may contribute to a small plateau potential (4). In bushy cells of newborn rats, a small NMDA component is apparent, but it has nearly disappeared by the beginning of the third week after birth (26). NMDA receptor EPSCs have also been observed in the MNTB of rats up to
2 weeks of age (29); however, it remains possible that these also disappear as the neurons mature. In all of these cases, the NMDA receptor-mediated EPSCs do not have a notably different time course from their counterparts in other brain regions.

While the duration of synaptic currents varies in different cell types, the very fastest currents have been found only in neurons of the auditory system. Single quantal currents and miniature excitatory post-synaptic currents (mEPSCs) in chick nMAG decay with an exponential time constant of just under 0.5 ms at room temperature, declining to 0.18 ms at 31°C (11, 34). Given the bird brain temperature of 40–41°C and a $Q_{10}$ for receptor kinetics of $>2$, it is likely that nMAG mEPSCs normally last less than 0.1 ms. Similar values have been reported for chick NL (35, 36) and nucleus angularis (NA) (35). In rodent bushy cells, mEPSCs decay with a time course identical to that in chick bushy cells (9, 35, 37), perhaps reflecting the high sequence homology of the genes encoding avian and rodent glutamate receptor subunit (38, 39). Figure 2 illustrates the remarkable speed in auditory synaptic kinetics by comparing mEPSCs recorded from a cell in the VCN and from a hippocampal neuron.

The time course of the EPSC represents the convergence of several factors. Isaacson & Walmsley (9) showed that the evoked EPSC in rat bushy cells could be described as the convolution of the time course of the mEPSC and the period over which quanta are released, the release time course. The latter represents a narrow, skewed function with a duration of less than 1 ms. Similar estimates were made by Borst & Sakmann (10) with rat MNTB. The duration of the EPSC is therefore longer than that of the mEPSC, with a dominant exponential

![Figure 2](image-url)
decay time of about 0.9 ms at room temperature. Although it is clear that a narrow release time course is necessary for auditory function, it is probably not unique to the auditory system. Although much broader release profiles have been observed in cultured hippocampal pyramidal neurons (40), the release time courses at excitatory and inhibitory contacts in the dentate gyrus are just as brief as in the calyceal auditory synapses (19).

In chick nMAG, while the major component of decay is essentially identical to that of mammals, the situation is more complex. First, at least two other, smaller components of decay are present, one of about 3 ms and one lasting several tens of milliseconds (41). Second, evidence indicates that in addition to mEPSC and release time course, transmitter clearance and desensitization help to shape the nMAG EPSC. Unlike in rat bushy cells (9), an increase in the quantal content broadens the EPSC, consistent with delayed clearance of transmitter (42). In the presence of drugs that block desensitization and prolong EPSCs, the effect of changing quantal content on EPSC duration is even more dramatic (34). The slowest component of the EPSC (41), whose amplitude is less than 1% of the peak current, is a consequence of transmitter pooling in the large synaptic cleft after glutamate release from many adjacent release sites.

Properties of AMPA Receptors

The brevity of EPSCs in auditory neurons reflects the intrinsic, biophysical properties of postsynaptic AMPA receptors (43). In outside-out patches from nMAG, the time course of current decay after a brief pulse of glutamate (which estimates the so-called channel deactivation time) and the duration of mEPSCs are virtually identical (41). This matching of synaptic and receptor kinetics is maintained over a range of membrane potentials that cause graded shifts in AMPA receptor gating kinetics (41, 44). The results imply that when one vesicle is released, it is the channel burst duration, rather than the receptor desensitization time course or the transmitter clearance rate, that determines the decay time of the postsynaptic response. Moreover, since the mEPSCs decay with a 0.5-ms time constant, the lifetime of the transmitter must be much briefer. Except for MNTB, in which deactivation is 0.9 ms (45), no other such measurements are yet available for mammalian auditory neurons. However, given the uniformly rapid mEPSC time courses among the cells described above, it seems likely that the kinetics of their AMPA receptors are also similar.

A broader survey of AMPA receptor kinetics in chick was made by Raman et al (35), focusing on the time course of desensitization to rapidly applied glutamate. The desensitization time constant was found to be similar in cells from nMAG, NA, NL, and cochlear ganglion. These values were all significantly shorter than the desensitization time constant of non-auditory neurons, including cerebellar Purkinje cells, granule cells, motor neurons, and cortical
cells. While cerebellar granule cells and cortical interneurons have quite fast AMPA channel kinetics (19, 45, 46), decay times identical to those for auditory cells are found only in patches excised from rod photoreceptor cells (47).

**Receptor Expression Studies**

Various studies of the types of AMPA receptors expressed in auditory neurons form a suggestive, but still incomplete, picture of the molecular underpinnings of the rapid EPSC. AMPA receptors are pentameric complexes composed of subunits that are the product of up to four different genes, termed GluR1 through GluR4 or GluRA through GluRD. Each subunit is subject to alternative splicing (the flip/flop splice cassette) and RNA editing, which further determines channel kinetics and permeability (48). Expression studies, examining the biophysical properties of receptors composed of different receptor subunits, have shown that receptors whose subunits are dominated by “flop” splice variants (49, 50), and in particular those composed mostly of GluR4\textsubscript{flop} and which lack GluR2 (45, 50), have the most rapid channel kinetics. RNA editing at a site just N-terminal to the flip/flop cassette produces channels that recover faster from desensitization (51). Moreover, AMPA receptors with a paucity of GluR2 have higher calcium permeability along with sensitivity to block intracellularly by polyamines and extracellularly by components of the venom of certain spiders and wasps (52).

In situ hybridization has revealed expression of mRNA for GluR2 through GluR4, but very little GluR1, in rodent spiral ganglion (53, 54) and VCN (55, 56). Initial studies of protein distribution used antibodies to GluR1, GluR2/3 (nonselective), and GluR4; in general, they demonstrated a pattern consistent with the mRNA analysis (53, 57, 58). A recent study of expression at the ultrastructural level in rat end-bulbs of Held has reexamined this issue by using antibodies specific for GluR2, as well as those for GluR2/3 and GluR4, concluding that this synapse uses primarily GluR3- and GluR4-containing receptors for transmission (59). Using the GluR1, GluR2/3, and GluR4 antibody set in owl, Levin et al (60) found high expression of GluR2/3 and GluR4 and low expression of GluR1, in cells of the nMAG and NL, with heterogeneous expression of GluR4 in NA.

Geiger et al (45) have examined subunit expression by the method of reverse transcriptase polymerase chain reaction (RT-PCR) in a variety of cell types, including the MNTB. They found that MNTB expressed a high level of GluR4, moderate levels of GluR1 and GluR2, but only a low level of GluR3. Significantly, virtually all the subunits in MNTB were of the flop splice variant. The prevalence of GluR4\textsubscript{flop} is consistent with the rapid channel kinetics found in that study. It will be important to determine if the fast kinetic receptors found in bushy cells, nMAG cells, and cochlear ganglion cells also express the flop variant. However, the expectation that GluR4\textsubscript{flop} is a general mediator of rapid
auditory transmission is tempered by the results of Angulo et al (61), who performed RT-PCR on a population of neocortical interneurons and found little correlation between AMPA channel kinetics and the splice variant.

Given the preponderance of GluR4 in fast auditory synapses, a central issue to address will be the mechanisms that cause GluR4 gene expression to be favored over other subunit genes, especially GluR1 and GluR2. One hypothesis is that since targets of the auditory nerve in general often express fast kinetic receptors in the cochlear nuclei, the auditory nerve itself may have control over postsynaptic subunit expression. In this regard, Rubio & Wenthold (62) found that fusiform cells of the dorsal cochlear nucleus (DCN), which are innervated by glutamatergic parallel fibers as well as by auditory nerve fibers, do express GluR4 but, remarkably, target it only to the dendrites that are contacted by the primary afferents. This result suggests not only that innervation plays an instructive role for gene expression but also that mechanisms for subcellular targeting of different GluR subunits are coordinated somehow by particular presynaptic neurons.

**Calcium Flux during Transmission**

The calcium permeability of AMPA receptors of auditory relay centers is an important issue for several reasons. Since auditory neurons are highly active, calcium loading of the cytoplasm by synaptic activity would constitute a severe metabolic challenge. Buildup of calcium may serve physiological functions, including activation of Ca\(^{2+}\)-sensitive ion channels and activation of second-messenger systems [although in some auditory neurons, calcium-activated channels are conspicuously absent (63)]. In principle, the presence of calcium-permeable transmitter-gated channels might serve to cause rapid control of extracellular [Ca\(^{2+}\)] in the synaptic cleft (64, 65). Circumstantial evidence is consistent with calcium loading of the cytosol. Neurons of the timing pathway in avians have high concentrations of the cytosolic calcium-binding proteins calretinin and calbindin (66–68). In mammals, neurons in the spiral ganglion, cochlear nuclei, superior olive, lemniscal nuclei, and inferior colliculus express high levels of a variety of calcium-binding proteins on a cell-type-specific basis (5, 69–72). Moreover, chick nMAG and NL cells express high levels of the sarcoplasmic/endoplasmic reticulum calcium uptake protein, SERCA (73), further suggesting that these cells have adaptations for coping with near-chronic influx of Ca\(^{2+}\).

Several studies have indicated that in some cells of the auditory pathways, Ca\(^{2+}\) permeates AMPA receptor channels. Otis et al (65) measured inward current in nMAG generated by application of glutamate or stimulation of end-bulb synapses while in the presence of isotonic extracellular Ca\(^{2+}\) solutions, indicating that these AMPA receptors must pass Ca\(^{2+}\). These authors determined
a ratio of calcium versus cesium ($P_{Ca}/P_{Cs}$) of 1.2:3, depending on the assumptions made in estimating free calcium levels. As with low-GluR2-containing receptors, nMAG AMPA receptors exhibit block by intracellular polyamines (S Kriegler, J Lawrence & LO Trussell, unpublished observations). Zhou et al (74) also determined that the AMPA channel of nMAG and NL passes $Ca^{2+}$ by taking advantage of the observation that $Ca^{2+}$-permeable AMPA receptors pass $Co^{2+}$, which can be precipitated intracellularly and therefore used as a histological assay of divalent-ion permeability. In mammals, the AMPA receptors of MNTB neurons, which also feature rapid channel kinetics, have a $Ca^{2+}$ permeability close to that seen in chick (45). Whether the high calcium influx which must accompany acoustic signaling has any physiological role in central auditory timing neurons remains unknown.

POTASSIUM CURRENTS SHAPE THE SYNAPTIC RESPONSE

The response to transmitter is dependent on the complement of voltage-sensitive channels active during the synaptic potential. While some studies have focused on inward sodium and calcium currents in spiral ganglion and cochlear nuclear cells (75–79), we restrict our discussion here primarily to potassium currents, since these seem to play a prominent role in the ability of auditory neurons to relay spike timing.

The consequence of these outward currents is clear from the characteristic response profile of several cell types in the cochlear nucleus and olivary complex, in which outward rectification is so strong that prolonged depolarizing current steps generate only a single, short-latency action potential. This electrical behavior, shown in Figure 3A, is observed in spiral ganglion cells (77, 80, 81), bushy cells (26, 82, 83), octopus cells (13) and neurons of the MNTB (30, 84, 85) and MSO (86), and, in chick neurons of the nMAG (4, 63, 87) and NL (36, 87). The short time for onset of the increase in membrane conductance and outward current (4, 63, 79, 82, 88, 89) may provide a simple mechanism for ensuring that the latency to spike onset is brief. Very large EPSPs reach threshold quickly, before the onset of rectification. Smaller EPSPs take longer to attain an equivalent, near-threshold potential; during their rising phase, the ensuing rectification raises the threshold beyond their reach. Thus, the onset time of rectification makes certain that postsynaptic spikes, when they occur, always fire with a short latency.

Moreover, outward rectification near the resting potential shortens the membrane time constant so that synaptic potentials are brief (provided that the synaptic currents are also brief), and temporal summation of jittery subthreshold, converging inputs is minimized (83). Indeed, by this mechanism, jitter in
Figure 3  Effects of outward rectification on response properties of nMAG. (A) A set of voltage responses to inward and outward current stimuli ranging from −0.4 to 1 nA at 50-pA intervals. Note the decrease in membrane resistance upon depolarization and the single-spike response profile. (B) A series of brief 200-Hz, 1 nA-current pulses produces a train of well-timed postsynaptic action potentials. (C) mEPSPs (∗) occurring during steps to different potentials reveal the effects of outward rectification on the time course of synaptic potentials. Note that at potentials just above rest (−68 mV), the mEPSPs often terminate with a slight oscillation, due to rapid activation and deactivation of the low-threshold outward current.

the timing of each presynaptic action potential may actually be subtracted if postsynaptic spiking requires precisely coincident EPSPs (90). After EPSPs reach threshold, a short membrane time constant allows the membrane to return quickly to a ready state for the next stimulus (18). Thus, when the cell in Figure 3A is driven by brief current pulses instead of one long current, it can fire well-timed spikes at a high rate (Figure 3B). Estimates of the half-time of decay of the response to current injection in nMAG at 31°C gave half-times of
about 2.5 ms near the resting potential of −66 mV, declining to under 0.5 ms at −55 mV (4). The resulting effect on small synaptic currents is illustrated in Figure 3C, which shows mEPSPs (marked by asterisks) at different membrane potentials. Note that at the more positive potentials, activation and deactivation of rectification are so fast that each mEPSP terminates with an oscillation rather than a smooth exponential decline.

Voltage-clamp analysis of potassium currents that underlie outward rectification has been performed with rat bushy cells (82), chick nMAG (63, 79), and rat MNTB (89, 91). In these cases, profiles of depolarizing voltage steps from the resting potential activate primarily two outward currents with distinct voltage and pharmacological sensitivities. A high threshold current, which activates at potentials positive to −20 mV, is most sensitive to tetraethylammonium (TEA) (63, 79, 82, 88, 89, 91) and is weakly blocked by 4-aminopyridine (4-AP) (88). Dendrotoxin (DTX) and 4-AP, by contrast, are effective at blocking a conductance that activates only slightly depolarized to rest (63, 79, 82, 88, 89, 91). Spiral ganglion cells also exhibit a low-threshold outward current (75–77), but no pharmacological studies of these cells have been performed with 4-AP or DTX.

Current clamp recordings have illustrated the relative roles of high- and low-threshold currents in shaping the response properties of the neurons. The single-spike feature of the cells described above is altered profoundly by application of certain blockers of voltage-activated K⁺ currents. Application of millimolar concentrations of 4-AP or 0.1 µM DTX allows these neurons to fire repetitive action potentials in response to single, prolonged currents steps (4, 30, 79, 85, 89). Most significantly, DTX promotes multiple postsynaptic spikes in response to single, brief synaptic stimuli, without any apparent effect on the duration of transmitter release (89). Application of TEA, by contrast, does not allow for repetitive firing (4, 79) but, like application of 4-AP, broadens action potentials (4, 63, 79, 91). Thus, while the high- and low-threshold currents are likely to play a role in repolarizing the action potential, the striking single-spike firing characteristic of auditory neurons involved in the fast relay of signals may be attributed to the activation of the low-threshold current.

Recent efforts have focused on the molecular identification of the high- and low-threshold potassium channels described above. One likely candidate for the low-threshold current is Kv1.1 or Kv1.2, since these channels have a relatively low activation threshold and are highly sensitive to DTX (92). Moreover, Kv1.1/1.2 is expressed in juxtaparanodal regions of axons in the brain stem and in cell bodies of the AVCN and MNTB (93, 94). Homomeric Kv1.1/1.2 channels have a higher threshold than the DTX-sensitive current in MNTB and nMAG (88, 89), suggesting that these cloned subunits alone cannot fully account for the channels observed in vivo.
KV 3.1 is a TEA-sensitive potassium channel that has been proposed to account for the high-threshold, TEA-sensitive channel in auditory timing and relay neurons (91, 95, 96). Wang et al (91) in particular have shown a close match in kinetics of current activation and sensitivity to TEA for expressed KV 3.1 and mouse MNTB TEA-sensitive current. Computer simulations of responses of neurons containing classical sodium channels and high- and low-threshold K+ currents indicate that without the high threshold current, action potentials broaden and the responsiveness to trains of stimuli is degraded (91). Perney et al (95) have shown that the distribution of KV 3.1-immunoreactive cells is only partly consistent with a role in transmission of timing. High levels have been observed not only in bushy cells and MNTB but also in neurons not associated with fast relay of acoustic signals, such as DCN giant cells and granule cells. Most striking is the lack of KV 3.1 in the octopus cells, whose exceedingly fast and reliable EPSPs are well suited to precise relay of signal timing (13). It would seem that this channel and perhaps others not yet described are key players in fast spike repolarization, a necessary feature for the use of both temporal and rate coding in acoustic processing.

It remains unclear which subtypes of potassium currents are expressed in the membranes of presynaptic nerve terminals in the cochlear or olivary nuclei. Nerve terminal recordings in MNTB have shown that the terminals and associated axonal membrane support repetitive firing, unlike the cell bodies of the bushy cells that give rise to these axons (97). Single action potentials exhibit a pronounced after-depolarization in the nerve terminal in MNTB, also unlike the cell bodies in the VCN (98). It is intriguing that the terminals support a different pattern of firing than the cell body, from which excitation originates. These differences from the somatic response most probably reflect the lack of expression of low-threshold potassium currents in the synapse, an adaptation that may prevent shunting of the conducted action potential and facilitate its invasion throughout the fingers of the calyceal terminal.

Ih AND ITS POTENTIAL ROLES IN THE AUDITORY PERIPHERY AND BRAIN STEM

Ih is an inwardly rectifying, slowly activating and deactivating, mixed cation current, which is expressed in wide variety of neurons in the auditory pathway. Ih is also a prominent component of spiral ganglion cells in rodents, apparent either as a sag in the response to hyperpolarizing current steps or as a slow inward current during hyperpolarizing voltage steps (80, 99). This current is not apparent in voltage clamp recordings from chick cochlear ganglion (75). Ih is a prominent current in bushy cells (83, 100) and in cells of the MTNB (101), MSO (86), dorsal nucleus of the lateral lemniscus (102), and chicken nMAG.
(4). Golding et al (13) showed that no depolarizing sag is apparent in octopus cells of the VCN; indeed, the input resistance of the cells is so low that it is practically impossible to measure membrane time constants to either depolarizing or hyperpolarizing current stimuli. However, they interpreted this leakiness to be due in part to a very large \( I_H \) active at rest, because bath application of 10 mM Cs\(^+\) (but not TEA) markedly increased the input resistance and time constant, but only to hyperpolarizing current steps.

What is the function of this slow current in transmission of timing of brief signals? One possibility for some of the cells described above is that it helps define the resting membrane properties; in particular, that the membrane potential and input resistance may represent a balance between the depolarizing \( I_H \) and the hyperpolarizing low-threshold K\(^+\) current. These resting values should have profound effects on the duration of synaptic potentials and action potential threshold in response to rapid depolarizing currents. Inasmuch as the low-threshold K\(^+\) current is partially inactivated at potentials near rest (89), \( I_H \) may control the availability of the low-threshold current during a depolarization. Moreover, the voltage-dependence of \( I_H \) is modulated by protein kinases (80, 99, 101); such metabolic control may be a point of longer-term regulation in response to ongoing activity levels.

**SHORT-TERM SYNAPTIC PLASTICITY**

In response to stimuli delivered at rates of several hertz or higher, EPSCs in bushy cells and in cells of nMAG and MNTB exhibit pronounced synaptic depression (11, 34, 37, 103, 104). Examples of depression are shown in Figure 1D for an nMAG neuron. Note in Figure 1C that EPSC depression does not necessarily lead immediately to spike failure, since the safety factor for transmission is usually quite high. Several studies have focused on the cellular mechanisms of depression in these cells. von Gersdorff et al (105) concluded that depression seen with 10–20-Hz stimuli in MNTB was presynaptic, i.e., was due to a reduction in transmitter release. This conclusion is supported by recordings of presynaptic calcium current, which inactivates during repetitive stimulation, although not enough to account for depression of the EPSC (106). Trussell and colleagues have shown that in nMAG, depression observed with high-frequency stimuli may be due in part to AMPA receptor desensitization (34, 107), although the extent to which this may occur in mammals remains unclear. In any case, depression in response to high-frequency stimuli is characteristic of calyceal/end-bulb synapses studied under voltage clamp or current clamp (4, 98). By contrast, earlier current-clamp studies in VCN or MNTB with microelectrodes and recording at higher temperatures did not describe such pronounced depression (28, 108, 109). These differences may be attributed to the
combined effects of nonlinear summation of EPSPs in current clamp and the pronounced reduction (but not elimination) of depression seen at physiological temperatures (104). Depression of EPSPs has also been observed in hair cells of the goldfish lateral line (110, 111); here, quantal analyses indicate that depression resulted largely from a reduction in the number of available release sites.

What is the role of depression in auditory processing? Use-dependent changes in synaptic strength in auditory neurons might determine the duration for which timing is transmitted effectively. The output of a neuron would initially reflect the information content of a single input, shifting to that of a group of convergent inputs. Such a shift in emphasis from single to many inputs would probably degrade the entrainment of a synaptic relay (i.e., reduce the one-for-one relation between input and output) but could still preserve or improve (90) phase-locking. Comparing firing rates in vivo with those that produce depression in vitro indicates that many auditory synapses may always be at least partially depressed. Spontaneous firing rates of auditory nerve and fibers in the trapezoid body vary from several hertz to nearly 200 Hz and can approach 500 Hz when driven by intense acoustic stimuli (112–115). By contrast, EPSCs in MNTB neurons are depressed by about 70% with stimuli at 5 Hz at room temperature (105), while EPSCs in nMAG are depressed by this amount at about 80 Hz at 36°C (104). Thus, depression is likely to occur during acoustic stimuli. Moreover, inasmuch as auditory nerve activity is relatively high in the absence of sound, mechanisms that induce depression may be potent determinants of synaptic strength even early in the response to sound.

MODULATION OF GLUTAMATE RELEASE BY PRESYNAPTIC RECEPTORS

Selective agonists of metabotropic glutamate receptors (mGluRs) markedly reduce the amplitude of EPSCs in MNTB by a presynaptic mechanism (103). Takahashi et al (116) have shown that these receptors are negatively coupled to calcium channels that elicit release at this synapse. It remains uncertain, however, whether synaptically released glutamate might feed back onto these receptors and contribute to depression. In their study of MNTB, von Gersdorff et al (105) found that antagonists of mGluRs only slightly altered depression to stimuli up to 10 Hz. Thus, these data question whether the transmitter can reach very far beyond the area near the active zone. Otis et al (107), in a study of nMAG synapses, found that when separate nerve terminals contacting the same cell body were sequentially activated, glutamate from one synapse did not apparently reach and desensitize the other nearby synapse. Moreover, block of glutamate transporters in nMAG markedly prolonged EPSCs (41). Thus, it may be that under most conditions, synaptically released glutamate is effectively
taken up by glutamate transporters, preventing autoreceptor activation. However, further work should be done to explore possible functions of mGluRs. For example, it will be of interest to determine if synaptic activation of these autoreceptors can occur in response to higher-frequency stimuli or when a large number of synapses in a group of cells are activated in concert.

Both baclofen, an agonist at GABA\(_B\) receptors, and GABA (γ-amino-butyric acid) itself (in the presence of GABA\(_A\) receptor antagonists), markedly inhibit EPSCs in nMAG and MNTB through a presynaptic mechanism (104, 117). Brenowitz et al (104) explored the effects of combining repetitive stimulation with application of baclofen and found that EPSCs elicited at high rates were actually larger in the presence of baclofen. Additionally, high-frequency EPSPs in baclofen reached the spike threshold more reliably than in control solutions. This occurred because the strong frequency dependence of depression was removed by the action of baclofen. The mechanism of this effect is not clear, but the authors propose that reduction of release probability by GABA\(_B\) receptors may prevent the depletion/desensitization that normally occurs during high-frequency activity. Because nMAG neurons receive GABAergic innervation from the superior olive (118), which is in turn innervated by the NA and NL, it may be that GABA serves to minimize depression in response to high-intensity sounds (104).

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