Functional localization of neurotransmitter receptors and synaptic inputs to mature neurons of the medial superior olive

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Neurons of the medial superior olive (MSO) code for the azimuthal location of low-frequency sound sources via a binaural coincidence detection system operating on microsecond time scales. These neurons are morphologically simple and stereotyped, and anatomical studies have indicated a functional segregation of excitatory and inhibitory inputs between cellular compartments. It is thought that this morphological arrangement holds important implications for the computational task of these cells. To date, however, there has been no functional investigation into synaptic input sites or functional receptor distributions on mature neurons of the MSO. Here, functional neurotransmitter receptor maps for amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA), N-methyl-D-aspartate (NMDA), glycine (Gly), and ionotropic γ-aminobutyric acid (GABA) receptors (Rs) were compared and complemented by their corresponding synaptic input maps. We find in MSO neurons from postnatal day 20–35 gerbils that AMPARs and their excitatory inputs target the soma and dendrites. Functional GlyRs and their inhibitory inputs are predominantly refined to the somata, although a pool of functional GlyRs is present extrasynaptically on MSO dendrites. GABA Rs responses are present throughout the cell but lack direct synaptic contact indicating an involvement in volume transmission. NMDARs are present both synthetically and extrasynaptically with an overall distribution similar to GlyRs. Interestingly, even at physiological temperatures these functional NMDARs can be potentiated by synthetically released Gly. The functional receptor and synaptic input maps produced here led to the identification of a cross talk between transmitter systems and raises the possibility that extrasynaptic receptors could be modulating leak conductances as a homeostatic mechanism.

NEURONS EXPRESS A MULTITUDE of ionotropic neurotransmitter receptors on their membranes. These can be highly mobile, often clustering only transiently at postsynaptic densities (Borgdorff and Choquet 2002; Meier et al. 2001). Characterizing and localizing receptors at the synapse therefore neglects the physiology of a large, extrasynaptic part of the functional pool of receptors. In contrast, UV “uncaging” of neurotransmitters can be used to develop subcellular maps of functional neurotransmitter receptors across the entire membrane (Eder et al. 2001; Kato et al. 2007). Such receptor distributions have provided insight into synaptic input location and relative efficacy (Pettit and Augustine 2000), as well as postsynaptic receptor distributions, integration mechanisms, and interactions between neurotransmitter systems (Eder et al. 2001, 2003).

Neurotransmitter systems interact on neurons at various levels. At the whole cell level, overall excitatory and inhibitory drive are often matched (Couchman et al. 2010; Dorrn et al. 2010; Haider et al. 2006; Sun et al. 2010). On the receptor level, coagonist neurotransmitters modulate responses to the major signaling transmitters (Farrant and Nusser 2005; Johnson and Ascher 1987; Li et al. 2009; Liu et al. 2010; Malenka and Nicoll 1993). These coagonists can originate from direct synaptic transmission (Kalbaugh et al. 2009), synaptic spill-over, or volume transmission (Farrant and Nusser 2005; Semyanov et al. 2004). Neurotransmitter accumulating from any of these sources can act on receptors across the entire surface of the neuron. These non-site-specific actions have profound effects on synaptic transmission and cellular computation (Chen et al. 2010; Semyanov et al. 2004). For example, extrasynaptic receptor activation can affect cell excitability (Ballo et al. 2010; Ciruela et al. 2010; Luscher et al. 1997; van Welie et al. 2004) and synaptic short-term plasticity (Chanda and Xu-Friedman 2010; Hassfurth et al. 2010; Magnusson et al. 2008).

Such extrasynaptic receptor-driven modulations also occur in the auditory brain stem. Here, for example, GABA receptors (GABARs) have been implicated in cell and circuit modulation at different stages of sound processing (Chanda and Xu-Friedman 2010; Hassfurth et al. 2010; Magnusson et al. 2008; Paolini et al. 1998; Takahashi et al. 1998). Recent evidence also suggests a functional role for presynaptic GABA Rs in the medial superior olive (MSO) (Hassfurth et al. 2010). Given the extremely minimal contribution of GABARs to synaptic currents in the cochlear nucleus (Lim et al. 2000), lateral superior olive (LSO) (Couchman et al. 2011), and MSO (Smith et al. 2000), it is likely that some of these effects are mediated by extrasynaptically located receptors. Knowledge of the functional expression of receptors in general and those that can mediate modulatory effects is therefore key to understanding circuit function in the intact animal.

Neurons of the MSO are of general computational interest as their cellular dynamics are closely linked to a well-defined behavioral task. The output from a single MSO cell is precise enough to code for the microsecond-scale differences in arrival times of sounds between the two ears, the interaural time difference (ITD) (Skottun 1998), as a basis for sound localization (Grothe et al. 2010). They achieve this feat via an extremely precise coincidence detection mechanism (Goldberg
and Brown 1969; Grothe and Sanes 1994; Yin and Chan 1990). This mechanism relies on the interaction between binaural excitatory and inhibitory inputs (Brand et al. 2002; Grothe et al. 2010; Pecka et al. 2008), a subset of large cation conductances (Khurana et al. 2011; Mathews et al. 2010), and is thought to be strongly influenced by unique anatomical adaptations (Agmon-Snir et al. 1998; Rautenberg et al. 2009; Zhou et al. 2005). Because of this tight structure-function relationship, a thorough survey of functional receptor and synaptic locations can provide insight into the in vivo behavior of MSO neurons and ultimately into ITD coding.

At mature stages MSO inputs are thought to be spatially segregated. Glutamatergic excitation is dendritically targeted and mediated by AMPA receptors (AMPARs) (Clark 1969; Kil et al. 1995; Smith et al. 1993; Stotler 1953), while the inhibitory input is glycinegic (Brand et al. 2002; Pecka et al. 2008; Smith et al. 2000) and targets the soma and proximal dendrites (Fig. 1A) (Couchman et al. 2010; Kapfer et al. 2002; Kuwabara and Zook 1992; Werth et al. 2008). NMDA receptors (NMDARs) and GABA<sub>A</sub>Rs are thought to be synaptically activated only during early postnatal development and fully downregulated at mature synapses (Smith et al. 2000). The highly compartmentalized synaptic input pattern is complemented by the simple, stereotyped morphology of MSO neurons (Fig. 1A). Typically bipolar, the dendrites of MSO neurons are short (~150 µm) and spineless and remain largely uniform in diameter along their length (Rautenberg et al. 2009). These neurons are therefore ideal candidates for functional neurotransmitter receptor mapping using single-photon UV uncaging.

**METHODS**

All experiments complied with institutional guidelines and national and regional laws. Animal protocols were reviewed and approved by the Regierung of Oberbayern according to the Deutsches Tier schutzgesetz for the AZ 55.2-1-54.2531.8-211-10. Slices were prepared from Mongolian gerbils (*Meriones unguiculatus*). Like humans, these animals use low-frequency sound cues and have a well-developed MSO (Klumpp and Eady 1957; Mills 1958; Ryan 1976). Animals of different postnatal days (P) were used. P10 animals were selected to represent the immature, prehearing stage of auditory development. Animals at P20–35 were used to represent a stage at which MSO neurons are reaching their adult morphology (Rautenberg et al. 2009). Animals were decapitated, and brains were removed in the Regierung of Oberbayern according to the Deutsches Tier schutzgesetz for the AZ 55.2-1-54.2531.8-211-10. Slices were prepared from Mongolian gerbils (*Meriones unguiculatus*). Like humans, these animals use low-frequency sound cues and have a well-developed MSO (Klumpp and Eady 1957; Mills 1958; Ryan 1976). Animals of different postnatal days (P) were used. P10 animals were selected to represent the immature, prehearing stage of auditory development. Animals at P20–35 were used to represent a stage at which MSO neurons are reaching their adult morphology (Rautenberg et al. 2009). Animals were decapitated, and brains were removed in

**Fig. 1.** Full width at half-maximum (FWHM) calibrations for UV uncaging and Picospitzer puff application. A: schematic of a transverse section of the auditory brain stem circuit encoding interaural time differences (ITDs). The medial superior olive (MSO) receives bilateral excitatory inputs from the antero-ventral cochlear nucleus (AVCN) and inhibitory inputs predominantly from the medial but also from the lateral nucleus of the trapezoid body (MNTB and LNTB). The lateral lemniscus (LL) and inferior colliculus (IC). Inset: reconstructed MSO neuron with schematic of input locations. Excitatory inputs target respective dendrites, while the inhibition is somatically targeted. B: schematic of experimental paradigm; uncaging pulses were delivered in 2.5-µm steps successively distant from the proximal dendrite of an MSO neuron. C: normalized peak AMPA receptor (AMPAR) and GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) responses to uncaging pulses delivered at locations as indicated in A. FWHM was calculated from doubling the distance at 50% (dashed lines) of peak from sigmoid fits to the data (solid lines). Filled circles represent averages. D–F: Picospitzer puff application FWHM calibration. D: fluorescence image showing the pipette and the full extent of a puff (4 ms at 4 ps) of 1 mM glycine, visualized by the inclusion of 100 µM Alexa 568 in the puff solution. Image of MSO dendrite is overlaid, indicated with a solid white line. Gray dashed line indicates the position at which the FWHM was calculated. Scale bar, 20 µm. E: normalized plot of fluorescence intensity from dashed line in D. Gray dashed line corresponds to the FWHM of this puff. F: line fit to FWHMs of 50 randomly selected puffs from 12 cells plotted against distance from the soma center. FWHM remained stable between experiments and along dendrite length.
loaded cells (filled via the recording pipette—see below) were visualized and imaged with either a TILL Photonics imaging system (TILL Photonics) or a TILL Photonics IR camera (VX 55) with a fluorescence lamp (Xcite, Olympus) under the control of custom-written visual acquisition and microscope control software (Bendels et al. 2008). Voltage-clamp whole cell recordings were performed with an EPC10/2 amplifier (HEKA Elektronik) on visually identified MSO neurons. Cells were clamped at $-60$ mV if not stated otherwise. Access resistance was compensated to a residual of 3 MΩ; data were acquired at 20 kHz and filtered at 3 kHz.

The intracellular solution used to record excitatory postsynaptic currents (EPSCs) contained (in mM) 130 Cs-glucuronate, 10 Cs-HEPES, 20 tetraethylammonium (TEA)-Cl, 5 Cs-EGTA, 3.3 MgCl₂, 2 NaN₃-ATP, 0.3 Na-GTP, 3 Na₂-phosphocreatine, and 5 QX-314-bromide with 50–70 μM Alexa 568 and for recording inhibitory postsynaptic currents (IPSCs) (in mM) 105 Cs-glucuronate, 26.7 CsCl, 10 Cs-HEPES, 20 TEA-Cl, 5 Cs-EGTA, 3.3 MgCl₂, 2 Na₂-ATP, 0.3 Na-GTP, 3 Na₂-phosphocreatine, and 5 QX-314 with 50–70 μM Alexa 568. Intracellular solutions were adjusted to pH 7.2 with CsOH.

Postsynaptic receptor currents through AMPARs, NMDARs, glycine receptors (GlyRs), GABAARs, and kainate receptors were isolated with an appropriate mix of DNQX (20 μM; Tocris), D-APV (50 μM; Tocris), R-CPP (10 μM; Biotrend), L-689,560 (10–20 μM; Tocris), strychnine hydrochloride (STR, 0.5 or 1 μM; Sigma), SR95531 (SR, 10 μM; Biotrend), and GYKI53655 (GYKI, 50 μM; Axon Medchem). For receptor mapping experiments, ZD7288 (ZD, 50 μM; Biotrend) was added to prevent desensitization. Picrotoxin (PTX, 100 μM) was used to distinguish between α-homomeric and β-heteromeric GlyRs (Bormann et al. 1993; Deleuze et al. 2005). D-Serine (100 μM) was added to saturate the glycine binding site of the NMDAR (Ahmadi et al. 2003).

Synaptic currents were evoked by stimulating afferent fibers with a monopolar glass electrode filled with incubation solution. We probed the vicinity (40–150 μm) of a patched MSO neuron with this electrode for stably activatable input sites. To stimulate fibers, an isolated pulse stimulator (model 2100; A-M Systems) was triggered though the EPC10/2 amplifier (HEKA Elektronik). For fiber stimulation, protocols were repeated at least three times. To allow for the full recovery of the response, repetitions of single pulses were delivered at 7–s intervals and stimulus trains with intervals of 15–20 s (Couchman et al. 2010). For UV uncaging, laser pulses were delivered at least 15 s apart to allow for reequilibration of the caged compound at the uncaging site. For receptor/synapse mapping experiments, all protocols were repeated four to eight times at each location.

**UV uncaging of glutamate and GABA.** Single-photon focal laser uncaging of caged MNI-caged-L-glutamate (MNI-Glu, 1 mM; Tocris) or O-CNB-caged GABA (CBN-GABA, 1 mM; Invitrogen) was carried out with a double-pumped solid-state UV laser shuttered with an acousto-optic modulator (DPSSL-355/1000; Rapp Optoelectronics) connected to the microscope using a 50-μm quartz light guide and spot illumination adaptor (OSI-BX; Rapp Optoelectronics). To elicit AMPAR and GABA$_A$R currents, a 300-μs laser pulse at ~1.6 mW was applied to visually identified neuronal segments. To reliably elicit NMDAR currents, a 0.5- to 1-ms pulse was required, possibly because of their slower single-channel kinetics and relatively sparse distributions. For all receptor distributions, a ×60 objective (NA 0.9, LUMPlanFl; Olympus) was used to minimize the effective uncaging spot size. In the experiment shown in Fig. 8, a ×40 objective (NA 0.8, LUMPlanFl; Olympus) was used to increase the uncaging spot size to encompass much of the somatic region as possible.

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**Fig. 2. Functional AMPAR and NMDA receptor (NMDAR) distributions.** A. top: MSO neuron filled with Alexa 568; arrows indicate MNI-Glu uncaging positions. Bottom: corresponding AMPAR currents elicited (left to right) at 20 μm, 40 μm, 59 μm, and 79 μm from the soma center. B: summary of peak amplitude (top) and 20–80% rise times (τ$_{20-80}$; bottom) of AMPAR currents plotted vs. distance from the soma center of the uncaging position. Individual cells in gray; average in 20-μm bins overlaid in black. n.s., Not significant. C. top: MSO neuron filled with Alexa 568; arrows indicate MNI-Glu uncaging positions. Bottom: corresponding NMDAR currents elicited (left to right) at 16 μm, 37 μm, 59 μm, and 84 μm from the soma center. Gray traces are individual trials; black average overlaid. White trace is single-exponential fit to rising phase used to calculate current peaks and τ$_{20-80}$. D: summary of peak amplitude (top) and τ$_{20-80}$ (bottom) of NMDAR currents plotted vs. distance from the soma center of the uncaging position. Individual cells in gray; average in 20-μm bins overlaid in black. Scale bars, 20 μm. DNQX (except in A and B), D-APV (except in C and D), STR, SR, ZD, TTX, 4-AP, cyclothiazide (CTZ), and TEA were present in the extracellular solution.
Because two different caging groups (MNI and CNB) with different properties (Sarkisov and Wang 2006; Wieboldt et al. 1994) were used in this study, a separate calibration of the effective uncaging spot size at full width of the half-maximal response (FWHM) was necessary (Fig. 1). The FWHM was determined as the half-maximal current response to uncaging pulses at successively distant points from the proximal dendrite of an MSO neuron under a $\times 60/0.9$ NA objective (Fig. 1B). Normalized peak current responses to uncaging pulses for both MNI-Glu ($n = 6$ cells) and CNB-GABA ($n = 6$ cells) were plotted as a function of lateral distance from the dendrite (Fig. 1C). The distance at the half-maximal response was then measured from a sigmoid fit to the peak responses and used to calculate the effective uncaging spot size, or FWHM (Fig. 1C). For MNI-Glu, a 300-$\mu$s laser pulse at 1.6 mW resulted in a FWHM of 6.8 $\mu\text{m}$ ($n = 6$); for the CNB-GABA, the FWHM was 16.8 $\mu\text{m}$ ($n = 6$). We mapped and binned the receptor responses for MNI-Glu in 15-$\mu$m bins, and for the CNB-GABA responses the data were binned in 30-$\mu$m increments. When the $\times 400/0.8$ NA objective was used (see Fig. 8), we estimate that the larger objective increased the uncaging spot size by about one-third to $\sim 10 \mu\text{m}$ FWHM.

**Pressure application of glycine and high K$^+$.**
To determine the subcellular location of GlyRs, glycine pressure was applied. A patch pipette was loaded with 1 mM glycine and 50–100 $\mu$M Alexa 488 or 568 dissolved in water. A saturating glycine concentration ensures that the peak of any elicited currents reflects the maximal response of receptors in the vicinity of the stimulation and minimizes the relative effects of diffusion on the rise times of the currents. The application pipette was placed within 5 $\mu$m of the cell membrane, and a 4-ms puff at 4 psi was given via Picospitzer. The puff of solution was visualized by using a TILL Photonics imaging system (TILL Photonics) to ensure the tip did not become blocked and the puff was of a consistent size (Fig. 1D). With normalized images taken at the maximum extent of the puff, the average FWHM was estimated at $\sim 16 \mu\text{m}$ ($n = 12$ cells; Fig. 1, D–F). The glycine maps were pooled and binned in 20-$\mu$m increments.

To functionally locate synaptic inputs, a patch recording pipette was loaded with a solution containing (in mM) 40 KCl, 3 CaCl$_2$, 100 NaCl, 10 Na-HEPES, and 20 glucose with 50 $\mu$M Alexa 568. This pipette was placed within 10–50 $\mu$m of the cell membrane, and a 50- to 150-ms puff at 4 psi was given via Picospitzer. Given the large puff size ($\sim$ 50- to 100-$\mu$m effective diameter), this experiment was only used to identify differences between the somatic and distal dendritic regions of an MSO neuron. These puffs were visualized by using a TILL Photonics imaging system to ensure the tip did not become blocked.

**Peak-scaled nonstationary variance analysis.**
Peak-scaled nonstationary variance analysis was carried out on miniature IPSCs (mIPSCs) as described by Silver et al. (1996). Briefly, for each cell, mIPSCs with amplitudes of within $\pm 25 \mu$A of the mean mIPSC were selected and peak-aligned. The background variance was calculated from a time window immediately preceding each mIPSC. The mean mIPSC waveform was then scaled to the peak of each individual mIPSC and the two waveforms subtracted. The resulting difference waveform was then binned in time according to equally sized amplitude bins of the average mIPSC. An average overall variance was calculated for each time bin and the background variance subtracted. The remaining variance, corresponding mainly to channel noise during the mIPSC, was plotted versus the average mIPSC amplitude. The peak-scaled variance $\sigma^2_{p-s}$ is a parabola and is calculated from the equation

$$
\sigma^2_{p-s} = \bar{I} - \bar{I}^2/N_p + \sigma^2_b
$$

(Silver et al. 1996) where $\bar{I}$ is the average single-channel current of all channels opened during the mIPSC, $\bar{I}$ is the mean mIPSC amplitude, and $N_p$ is the number of channels open at the peak of the mIPSC. $\sigma^2_b$ is the background variance, set to zero as this is earlier subtracted from the variance bins. An estimate of the average single-channel conductance ($G_i$) can then be calculated from Ohm’s law by dividing the single-channel current by the ionic driving force ($V_d$):

$$
G_i = i/V_d
$$

**Data and statistical analysis.**
Image stacks were taken with a Leica SP confocal microscopy system (Leica) with a $\times 250/0.75$ NA with a zoom factor of 1.2, leading to a voxel size of 651 nm$^3$. These images are presented as a standard deviation z-projection. Currents were analyzed in IGOR Pro (WaveMetrics). Results are presented as means $\pm$ SE. Statistical significance was determined by Student’s t-test with a significance threshold of $P < 0.05$.

**RESULTS**

The functional distribution of pharmacologically isolated postsynaptic receptor currents on the dendrites of MSO neurons was determined by UV laser uncaging of glutamate and GABA and pressure application of glycine. As MSO dendrites are largely uniform in diameter along their length (Rautenberg et al. 2009), it was possible to average these maps between neurons and compare the relative distributions of different neurotransmitter receptors. All tested receptor types were present on MSO somata, but the data were not analyzed because of the large differences in surface area between soma and dendrites. Receptor maps were complemented with an investigation of synaptic input location using pressure application of a high-K$^+$ concentration ($150 \text{mM}$ K$^+$, 50 mM) solution. In addition, some of the functional implications of these receptor and synapse distributions were explored.

**Functional AMPA and NMDA receptor distributions.**
The excitatory input to mature MSO neurons is thought to be AMPAR mediated (Kotak and Sanes 1996) and dendritically targeted (Clark 1969; Stotler 1953). To see whether this anatomical arrangement is reflected in the functional AMPAR distributions, we uncaged MNI-Glu along the dendrites of MSO neurons from P30–35 animals. AMPAR currents were elicited along the dendrites of 12 MSO neurons (Fig. 2, A and B). At the proximal dendrite (10–20 $\mu$m from the soma center) the average peak of the AMPAR current was 705 $\pm$ 89 pA, compared with 497 $\pm$ 81 pA at distal locations (105–120 $\mu$m from the soma). This difference was not significant ($P > 0.05$), indicating that AMPARs were rather uniformly distributed on MSO neurons. Finally, we note that the addition of GYKI to block AMPARs abolished all glutamatergic currents, indicating that kainite receptors are not present in the mature MSO ($n = 7$ cells; data not shown).

Receptor responses recorded at different cellular locations evoked by UV uncaging can be subject to distortion effects originating from the diffusion of liberated glutamate, variations in light scatter with stimulation depth, and space clamp errors. To rule out the differential influences of such distortion effects between proximal and distal dendritic sites, the rise time (20–80%) of the receptor currents was measured and correlated to the location of the response (Fig. 2B). The difference between the rise times of AMPAR currents at the proximal dendrite ($1.23 \pm 0.19$ ms, 10–20 $\mu$m from the soma) and the rise times at distal sites ($1.49 \pm 0.17$ ms, 105–120 $\mu$m from the soma) was not statistically significant ($P > 0.05$). Overall, no systematic effect of dendritic distance on current kinetics could be identified. This suggests that distortion effects do not differentially influence our results.
Although NMDARs are strongly developmentally down-regulated in the ITD circuit (Futai et al. 2001; Zhou and Parks 1993), recent work indicates that NMDARs still play a functional role at mature stages (Caicedo and Eybalin 1999; Porres et al. 2011; Steinert et al. 2010). To investigate a possible contribution at mature MSO cells, functional NMDAR expression was mapped along their dendrites with UV uncaging of MNI-Glu (n = 14, P30–35). Because the currents elicited were small, four to eight trials at each location were averaged (Fig. 2C), and the peak of the current was determined using the maximum of a single-exponential fit to the rise of the response (Fig. 2C). Overall, NMDARs appear somatically biased (Fig. 2D). This bias was significant: Proximal dendritic NMDA currents (10–15 μm from the soma center) were 99 ± 18 pA compared with 33 ± 8 pA at 75–105 μm (P < 0.05). The rise times (τrise) of the NMDAR currents were determined from the single-exponential function (Fig. 2C). The τrise of NMDAR currents were not significantly different (P > 0.05) at the proximal dendrite (10–15 μm, 25 ± 1.7 ms) compared with the distal dendrite (75–105 μm, 29 ± 3.8 ms; Fig. 2D). In four cells, the addition of D-APV abolished all evoked currents, indicating that they were mediated by NMDARs.

**Functional AMPA and NMDA synapse distributions.** To correlate the functional receptor distributions with synapse locations, local synaptic AMPAR-mediated responses were evoked with the focal pressure application of a high-[K+] ([K+] = 40 mM) solution for 50–150 ms. Given the limited resolution of such stimuli (FWHM ~50–100 μm) it was applied only to the somata (n = 8) and to dendritic sites (n = 21, P30–35) at 59–121 μm from the soma center (Fig. 3, A and E). Irrespective of location, all stimulations elicited bursts of fast postsynaptic AMPAR-mediated responses of varying sizes (Fig. 3A), reminiscent of asynchronous quantal release (Fig. 3B). In a set of control experiments, the addition of TTX reduced the apparent amplitude of these responses without affecting the overall distribution of synaptic inputs to AMPARs (Fig. 3E). High [K+] was applied to within ~30 μm of the dendrite ends, with distances ranging from 47 to 115 μm from the soma center. This might indicate that under TTX application a smaller subset of local inputs were activated, or that normally sodium currents directly in the nerve ending increase a local presynaptic depolarization. In one cell, DNQX was added to the external solution, abolishing the evoked currents. Together these findings indicate the presence of functional synaptic input to AMPARs at both somatic and dendritic locations on mature MSO neurons.

The unexpected functional expression of NMDARs led us to investigate possible synaptic activation patterns for these receptors. The local pressure application of high [K+], this time in Mg2+-free recording solution, was again used to probe for synaptic activation. In total, the somata of 10 cells and 16 dendritic locations ranging from 73 to 167 μm were stimulated (Fig. 3E; P30–35). A synaptic input to NMDARs could only be stimulated at the dendrites of 2 of 10 cells (1 such cell in Fig. 3C). In both cases these response was blocked by D-APV. Part of the response is magnified in Fig. 3D (from gray circle in Fig. 3C).

**NMDARs are activated during fiber stimulation.** The functional presence of NMDARs in neurons of the MSO from P30–35 animals is surprising, especially given the limited...
synaptic input apparent with high-[K\textsuperscript{+}] stimulation (Fig. 3). The contribution of NMDARs to excitatory currents was therefore measured with electrical fiber stimulation. The AMPAR and NMDAR components of excitatory currents were recorded at −60 mV and +50 mV, respectively, at near-physiological temperature (35–36°C). Single shocks to afferent fibers revealed substantial AMPAR currents in MSO neurons (Fig. 4A), with an average peak of 4.12 ± 0.76 nA (Fig. 4D; n = 13, P30–35). Once a reliable input site was located, AMPAR currents were blocked with either DNQX or GYKI. For single-fiber shocks, a slow NMDAR current was apparent in 9 of 13 cells after background subtraction, achieved by the subsequent application of CPP (Fig. 4, B and C). The \( \tau_{rise} \) and peak of NMDAR currents were calculated from a single-exponential fit to a trace averaged from four to eight trials (Fig. 4C). In the nine cells with an NMDA response, single-fiber stimulation produced an average NMDAR current of 36.3 ± 7.97 pA (Fig. 4D) with an average \( \tau_{rise} \) of 3.24 ± 0.43 ms (data not shown). Again, no contribution from kainite receptors was found during fiber stimulation in the presence of GYKI and CPP (Fig. 4D).

To measure any increase of the NMDA peak currents during ongoing activity, a 10-pulse stimulus train at 200 Hz was applied (n = 10; 5 of these cells were also used in the single-stimulus pulse recordings, P30–35; Fig. 4E). The average peak AMPAR current from the first pulse of the train was 3.91 ± 0.63 nA (Fig. 4F). In 4 of 10 of these cells, an NMDAR current built up over the course of the stimulus train (Fig. 4, F and G). The peak of this current was calculated from a fit to the average of four to eight trials recorded after DNQX application (Fig. 4F), subsequently baselined via the subtraction of an averaged trace recorded in the presence of CPP (Fig. 4G). An average peak NMDAR current of 61.6 ± 2.5 pA (n = 4) was elicited by the stimulation train (Fig. 4H). Thus ongoing activity can generate a twofold increase in the NMDAR-mediated response.

**Development of glycine receptor distributions.** Anatomically, the developmentally driven restriction of inhibitory inputs to the soma and proximal dendrites of MSO neurons is well documented (Couchman et al. 2010; Kapfer et al. 2002; Kuwabara and Zook 1992; Werth et al. 2008). To date, though, there has been no functional investigation to confirm this morphological arrangement. The distribution of functional GlyRs on the dendrites of MSO neurons was therefore measured with focal pressure application of glycine (Fig. 5). To track the developmental refinement of GlyRs, this experiment was carried out on animals both before hearing onset (P10) and at more mature stages after hearing onset (P20–35).

Already at P10, a sharp decrease in the GlyR-mediated response from soma to dendrite was evident (Fig. 5, A and B; n = 6). Proximal dendritic GlyR currents (10–20 μm from the soma center) were on average 16.40 ± 1.23 nA; dendritic currents (80–120 μm) were significantly smaller (\( P < 0.05 \)), on average 873 ± 56 pA. GlyR currents also decreased significantly (\( P < 0.05 \)) along the dendrites of P30–35 MSO neurons (Fig. 5, C and D; n = 10). At this mature stage, currents elicited at the proximal dendrite (10–20 μm from the soma center) were on average 10.44 ± 1.05 nA compared with 643 ± 67 pA at distal sites (120–150 μm) (Fig. 5D). Despite this sharp gradient, a sizeable distal dendritic current of up to

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**Fig. 4.** NMDARs are recruited during fiber stimulation. A: example traces of excitatory currents to MSO neurons elicited by electrical stimulation. Currents are recorded in whole cell voltage clamp at +50 mV and −60 mV. AMPAR currents (dark gray traces) are blocked completely with DNQX (light gray traces). B: extended time scale of average traces extended time scale of average traces recorded at +50 mV. A slow NMDAR component is blocked by CPP (light gray trace). C: isolated NMDAR current revealed after subtraction of the average traces in B. White trace is a single-exponential fit to the rising phase of the current used to calculate the peak of the NMDAR response. D: summary of AMPAR, kainite (kain), and NMDAR peak currents. Circles are average currents from single cells; filled circle represents average. E: example traces of excitatory currents to MSO neurons elicited by 200-Hz electrical stimulation for 10 pulses. Stimulus artifacts have been removed for clarity. Currents are recorded at +50 mV and −60 mV. AMPAR currents (dark gray traces) are blocked completely with DNQX (light gray traces). F: extended time scale of average traces recorded at +50 mV. A slow NMDAR component is blocked by CPP (light gray trace). G: isolated NMDAR current revealed after subtraction of the average traces in F. White trace is a single-exponential fit to the rising phase of the current used to calculate the peak of the NMDAR response. H: summary of peak AMPAR and NMDAR currents. Circles are average currents from single cells; filled circle represents average. Squares indicate stimulation pulses. All currents were recorded at near-physiological temperature (35–36°C). STR and SR were present in the extracellular solution.
abolished by the addition of STR (n/H11005).

Characterization of glycine receptors. In other systems, the composition of extrasynaptic and synaptic GlyRs differs between those consisting of α-homomers and αβ-heteromers, respectively (Deleuze et al. 2005). To test whether such heterogeneity exists in MSO neurons, the differential sensitivity of α-homeric and αβ-heteromeric GlyRs to PTX was exploited (Pribilla et al. 1992; Schofield et al. 1996). When added to the bath solution at 100 μM, PTX has significant antagonistic effect only on α-homomeric GlyRs. In three cells from P30–35 MSO, distal pressure application (106–147 μm from the soma center) of 1 mM glycine evoked GlyR-mediated currents with an amplitude of 300 ± 89 pA (Fig. 7, A and B).
The addition of 100 μM PTX did not significantly affect the currents (P > 0.05), which were on average 345 ± 100 pA (Fig. 7B). The subsequent bath application of 0.5 μM STR fully abolished the glycine-evoked response in all cases (Fig. 7A). This indicates that distal extrasynaptic GlyRs are normal synaptic-type αβ-heteromers.

To further characterize the composition of GlyRs in the MSO, peak-scaled nonstationary variance analysis was performed on mIPSCs recorded from eight cells (P20–35; Fig. 7, C and D). This analysis provides an estimate for the average single-channel current and average conductance state of all receptors activated during the mIPSCs. Glycinergic mIPSCs were recorded and extracted before thresholding to select those with peak amplitudes of within ±25 pA of the mean mIPSC (Fig. 7C). The variance during the decay phase of these selected mIPSCs was then calculated, binned, and plotted against the average mIPSC amplitude for each cell (Fig. 7D). The resulting data were fit with a parabolic function to determine the average single-channel current and the corresponding conductance as well as provide an estimate of the number of receptors activated at the peak of the mIPSC. The average single-channel current of 2.39 ± 0.12 pA (n = 8) corresponded to an average single-channel conductance of 53 ± 2.7 pS, with an estimated 32 ± 2.3 receptors activated at the peak of the mIPSC. This conductance estimate is similar to that reported in the cochlear nucleus (Balakrishnan et al. 2003), for spinal (Beato and Sivulotti 2007) and hypoglossal (Singer and Berger 1999) motoneurons, and for heterologously expressed GlyRs (Takahashi et al. 1992).

From these results, a crude estimate of the average density of GlyRs in the mature MSO can be made. These calculations were based on the average current peaks elicited by puff application of glycine at proximal and distal dendritic sites (Fig. 5B). The number of receptors affected by the average glycine puff was determined from the single-channel conductance for mature GlyRs (53 pS; Fig. 7) and the measured driving force for GlyR currents (45 mV; data not shown). The surface area affected by the average glycine puff was estimated from the measured FWHM of simultaneously ejected Alexa 568 (16 μm; Fig. 1E) and the precise quantification of MSO dendrite diameter (Rautenberg et al. 2009). As the single GlyR channel conductance is likely similar at P10 (Takahashi 2005), the approximate receptor density at this age is 57 receptors/μm² at the proximal dendrite and 5 receptors/μm² at the distal dendrite. Taking the developmental increase in dendrite diameter into account (Rautenberg et al. 2009), the approximate density of GlyRs is 29 receptors/μm² at the proximal dendrite and 2 receptors/μm² on the distal dendrites of cells from P20–35 animals. It should be noted that the relatively scarce GlyR density at dendritic locations at P10 is sufficient to support functional synapses. Furthermore, consistent with anatomical observations (Kapfer et al. 2002), this calculation indicates an absolute decrease in the GlyR number at dendrites during development.

Interplay between synaptically released glycine and NMDA receptors. Given the similar distribution profiles of GlyRs and NMDARs on MSO neurons, an interaction between the glycinergic and glutamatergic (NMDAR) transmitter systems on the receptor level (Ahmadi et al. 2003; Johnson and Ascher 1987; Li et al. 2009) is possible. We therefore sought to potentiate somatic NMDAR responses to uncaging pulses with synaptically released glycine. To do so, MSO neurons of P30–35 animals were recorded at a holding potential of +50 mV at 35–36°C. Afferent inputs containing glycinergic fibers...
were stimulated in the presence of DNQX with a 10-pulse train at 200 Hz with a glass fiber stimulation electrode (n = 12; Fig. 8A). Five milliseconds after the final stimulus pulse of the train, MNI-Glu was uncaged at the soma of the neuron with a ×40 objective for 0.5–1 ms (Fig. 8A, inset). To correct for background currents, interleaved recordings of either a glycinergic input alone or a simple voltage step to +50 mV were subtracted from those with uncaging pulses (Fig. 8B). NMDAR responses elicited by uncaging were potentiated significantly in both peak amplitude (88.0 ± 16.7 to 102.8 ± 20.0 pA) and charge (7.7 ± 1.8 to 9.6 ± 2.2 pC; P < 0.05, paired t-test) when paired with the stimulation of glycinergic inputs. Overall, these results represent an 11.6% increase in peak amplitudes and a 13% increase in charge through NMDARs after the liberation of synaptic glycine in the MSO (Fig. 8C). The addition of d-serine, to saturate the glycine binding site of NMDARs, blocked any increase in NMDAR peak amplitudes (n = 5, P > 0.05, paired t-test; Fig. 8C). The NMDAR response was completely abolished by the addition of CPP (n = 3) or L-689,560 (n = 2), an antagonist of the glycine binding site of NMDARs. Together, these findings show that the observed amplification of the NMDAR current is a specific action of synthetically liberated glycine. This amplification is likely an underestimate given that glutamate liberated during stimulation may partially occupy NMDARs, competing and thereby reducing the uncaging response.

GABA<sub>A</sub> receptor and synapse distributions. GABA<sub>A</sub>-mediated synaptic transmission is downregulated during the development of MSO neurons (Smith et al. 2000). However, a modulatory role for presynaptic GABA<sub>B</sub>Rs in the mature MSO has recently been suggested (Hassfurther et al. 2010), and there is indication of the presence of GABAergic fibers close to MSO neurons in mature animals (Korada and Schwartz 1999). To identify a postsynaptic GABA effect, the presence of GABA<sub>A</sub>Rs was probed with UV uncaging of CNB-GABA on the dendrites of mature MSO neurons (n = 13, P30–35). GABA<sub>A</sub>R currents were consistently elicited along the entire extent of the cell. Again, four to eight trials per location were averaged and the rising phase of the current fit with a single-exponential function to estimate τ<sub>rise</sub> and current peaks (Fig. 9A). Proximal to the soma (10–30 μm from the soma center) an average peak GABA<sub>A</sub>R current of 39.27 ± 5.27 pA was elicited, and at distal dendritic segments (90–150 μm) only 18.18 ± 4.26 pA. This drop in GABA<sub>A</sub>R current along the dendrite was significant (P < 0.05; Fig. 9B, top). Importantly, the average τ<sub>rise</sub> of these GABA<sub>A</sub>R currents were not significantly different (P > 0.05; Fig. 9B, bottom) between proximal (5.42 ± 0.81 ms) and distal (8.50 ± 1.50 ms, 90 - 150 μm) dendrites. The application of SR in two cells completely blocked the response, confirming the presence of functional GABA<sub>A</sub>Rs on mature MSO neurons.

The presence of functional GABA<sub>A</sub>Rs together with the histochemical evidence of GABAergic fibers in the MSO (Hassfurther et al. 2010; Korada and Schwartz 1999) is suggestive of a direct GABAergic synaptic input to these cells. As before, the pressure application of a high-[K<sup>+</sup>] solution at both somatic and distal dendritic locations was used to stimulate synaptic inputs, this time to GABA<sub>A</sub>Rs (Fig. 9C). A GABA<sub>A</sub>-mediated synaptic input could not be elicited at any of the locations tested (9 somata and 15 dendrites, P30–35), including in one case where SR was added to the extracellular solution in an attempt to unmask slower currents (Fig. 9C). Next an attempt was made to record pharmacologically isolated GABA<sub>A</sub>R-mediated mIPSCs (Fig. 9E). In these recordings, the addition of DNQX, D-APV, and STR abolished all mIPSCs, indicating a lack of functional synaptic input to GABA<sub>A</sub>Rs in the mature MSO (n = 5; Fig. 9E). In addition, trains of fiber stimulation (10 pulses at 200 Hz) failed to elicit pharmacologically isolated GABA<sub>A</sub>R-mediated responses in mature MSO neurons (data not shown; n = 8). We hypothesize therefore that these receptors respond to ambient GABA levels and are not activated during direct, fast synaptic transmission.

DISCUSSION

This work provides a thorough functional map of neurotransmitter receptors and their synaptic input sites onto mature neurons of the gerbil MSO. Single-photon UV laser uncaging is a useful tool for investigating functional morphology in terms of the distribution of neurotransmitter receptors. Neurons of the MSO are ideal to investigate receptor distributions with this method given that they have short, spineless dendrites (typically ~150 μm in length) aligned in a single focal plane with a relatively uniform diameter (Rautenberg et al. 2009). The suitability of these neurons was confirmed by an analysis of current rise times, which indicated that experimental distortions such as space clamp and light scatter appear not to interfere substantially with our results. Given the scarcity of biologically inert caged glycine compounds, GlyRs were mapped with a minimal pressure application paradigm. The
uniformity of the stimulus (Fig. 1F) makes this technique a fair match for comparison with UV uncaging methods.

AMPARs and GlyRs are the main receptor types expressed on mature MSO neurons, and they form the synaptic basis for exquisitely precise coincidence detection. For both receptor types the functional maps and the estimated synaptic input locations are largely in accord with anatomical data (Couchman et al. 2010; Kapfer et al. 2002; Kuwabara and Zook 1992; Stotler 1953; Werthat et al. 2008). AMPARs are evenly distributed throughout the whole MSO cell. In contrast to the LSO of immature animals (Vitten et al. 2004), there appears to be no functional expression of kainate receptors in the mature MSO. The excitatory synapses that activate AMPARs are also present at the soma and provide a strong input to the dendrites. Stimulation of excitatory fibers reliably elicits large, fast AMPAR currents. GlyRs are strongly biased to the soma, as are their functional inhibitory synaptic inputs. However, there are clearly GlyRs on MSO neurons that are not restricted to inhibitory synapses. A substantial αβ-heteromeric GlyR current was present along the full extent of MSO dendrites. This indicates that GlyRs are functional NMDARs. A: currents elicited by electrical stimulation of inhibitory inputs and/or glutamate uncaging on MSO neurons during a step to +50 mV. Solid traces contain a 500-μs uncaging stimulus on the soma of the neuron; in the recordings in black the uncaging pulse was preceded by a 10-pulse train of glycnergic inputs at 200 Hz. Inset: enlargement of the area indicated by the gray box. tr, Glycinergic train; p, uncaging pulse; n, simple step to +50 mV to subtract background currents. B: NMDAR currents resulting from the subtraction of traces in A; dashed traces were subtracted from respective solid traces to remove background leak currents, glycnergic currents, and stimulus artifacts. Gray trace (p) is the NMDAR current elicited by an uncaging pulse alone; black trace (tr+p) is the same current elicited 5 ms after a 200-Hz glycnergic train. Dashed line indicates the onset of the uncaging stimulus. C: summary of normalized peak amplitudes of the response to the uncaging pulse without (p) and with a preceding glycnergic train (tr+p) and in the presence of DNQX and SR (control) and after the addition of d-serine. Open circles are results from individual cells; bars represent the average. Significance calculated with a paired t-test. All recordings were made at 35–36°C.

Fig. 8. Synaptically released glycine acts on somatic NMDARs. A: currents elicited by electrical stimulation of inhibitory inputs and/or glutamate uncaging on MSO neurons during a step to +50 mV. Solid traces contain a 500-μs uncaging stimulus on the soma of the neuron; in the recordings in black the uncaging pulse was preceded by a 10-pulse train of glycnergic inputs at 200 Hz. Inset: enlargement of the area indicated by the gray box. tr, Glycinergic train; p, uncaging pulse; n, simple step to +50 mV to subtract background currents. B: NMDAR currents resulting from the subtraction of traces in A; dashed traces were subtracted from respective solid traces to remove background leak currents, glycnergic currents, and stimulus artifacts. Gray trace (p) is the NMDAR current elicited by an uncaging pulse alone; black trace (tr+p) is the same current elicited 5 ms after a 200-Hz glycnergic train. Dashed line indicates the onset of the uncaging stimulus. C: summary of normalized peak amplitudes of the response to the uncaging pulse without (p) and with a preceding glycnergic train (tr+p) and in the presence of DNQX and SR (control) and after the addition of d-serine. Open circles are results from individual cells; bars represent the average. Significance calculated with a paired t-test. All recordings were made at 35–36°C.

Fig. 9. GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) and synapse distributions. A, top: MSO neuron filled with Alexa 568; arrows indicate CNB-GABA uncaging positions. Bottom: corresponding GABA<sub>A</sub>R currents elicited (left to right) at 23 μm, 62 μm, and 81 μm from the soma center. Gray traces are individual trials, black average overlaid. White trace is single-exponential fit to rising phase used to calculate peak amplitude and τ<sub>rise</sub>. B: summary of peak amplitude (top) and τ<sub>rise</sub> (bottom) of GABA<sub>A</sub>R currents plotted vs. distance from the soma center of the uncaging position. Individual cells in gray, average in 20-μm bins overlaid in black. C, top: MSO neuron filled with Alexa 568; arrows indicate position of pressure application of 40 mM K<sup>+</sup>. Bottom: corresponding currents elicited (top to bottom) at 105 μm, 0 μm, and 85 μm from the soma center. There was no response to pressure application of 40 mM K<sup>+</sup>, indicating a lack of excitable inputs at these locations. Scale bars, 20 μm. D: summary showing locations where we attempted to stimulate synaptic inputs to GABA<sub>A</sub>Rs. Black circles represent a failure to stimulate synaptic input at all positions tested. E: example traces of mIPSCs recorded before (top) and after (bottom) addition of STR. No GABA<sub>A</sub>R miniature event was recorded. DNQX, D-APV, STR, ZD, TTX, 4-AP, and TEA were present in the extracellular solution in A, B, and E. In C and D, DNQX, D-APV, STR, and ZD were present in the extracellular solution.
highly abundant and diffuse in the MSO membrane as part of a nonsynaptic pool of functional GlyRs. Compared with the extrasynaptic site, however, the receptor density in synapses is much higher. Thus receptors need to be efficiently trapped at the postsynapse to allow for this steep gradient of receptor density. The presence of an extrasynaptic pool of receptors could therefore be a side-product of the maintenance of the large numbers of receptors required at the synapse. Alternatively, these extrasynaptic receptors could serve a specific function as sensors for ambient glycine or taurine released by either synaptic or paracrine signaling.

Despite the similarities with the results of anatomical studies (Kapfer et al. 2002; Kuwabara and Zook 1992; Stotler 1953; Werth et al. 2008), it is important to note that the putative segregation of excitatory and inhibitory inputs to different cellular compartments of MSO neurons does not strictly hold. Our results indicate that both input types converge on the soma and proximal dendrites of MSO neurons, and it is only on distal dendritic segments that excitatory inputs are isolated. This overlap at the MSO soma provides a locus for the interaction of excitatory and inhibitory neurotransmitter systems, in which NMDARs are implicated.

NMDARs are functionally expressed on mature neurons of the MSO, but no reliable synaptic input to these receptors could be evoked. Interestingly, NMDARs are biased to the soma, resulting in a distribution profile more similar to GlyRs than AMPARs. This mismatch between the expression pattern of NMDARs and glutamatergic synaptic inputs led us to search for an extrasynaptic role for NMDARs in the MSO. These receptors have a high affinity for glutamate, making them ideal for sensing low levels such as might be encountered extrasynaptically. Using a combination of uncaging and fiber stimulation, we show that glycine liberated from synapses under physiological conditions potentiates NMDARs located at the soma. Although the purpose of this interaction remains speculative, we favor a role in activity-dependent signaling. Since inhibitory and excitatory inputs derive from similar neuronal populations in the anteroventral cochlear nucleus, NMDARs are most likely activated during strong excitatory and inhibitory drive. Ca\(^{2+}\) influx through NMDARs alters the state of synaptic receptors (Bliss and Collingridge 1993; Malenka and Nicoll 1993), thereby providing a mechanism by which NMDARs might adjust synaptic strength in an activity-dependent manner. In addition, the activation of NMDARs provides a slow, summating excitatory drive to MSO neurons, thus slowing the overall speed of excitatory signaling. This might partially compensate for the relatively faster kinetics of AMPA-mediated excitatory responses compared with glycineric inhibitory responses in the adult MSO (Couchman et al. 2010). In any case, our results provide the first evidence for an interaction between NMDARs and glycineric inputs in the mature auditory brain stem.

Surprisingly, the mapping of GABA\(_A\)Rs revealed that they were consistently present along the entire extent of MSO neurons. This is especially interesting given that no fast GABA\(_A\)R current could be synaptically stimulated and no spontaneous GABA\(_A\)-ergic events were observed. GABA signaling in the MSO clearly differs in structure and function from that in the avian analog, the nucleus laminaris (NL). In the NL, GABA is a primary synaptic neurotransmitter, providing a strong depolarizing shunt to sharpen coincidence detection (Monsivais et al. 2000; Yang et al. 1999). In contrast, in the mature superior olivary complex, chloride signaling is inhibitory (Ehrlich et al. 1999; Lohrke et al. 2005; Magnusson et al. 2005) and GABARs are not activated by direct synaptic input (Couchman et al. 2011; Helfert et al. 1989; Smith et al. 2000). This indicates that, as in other brain regions (Farrant and Nusser 2005; Semyanov et al. 2004), background levels of GABA might be present in the superior olivary complex, influencing the MSO circuit through a volume transmission mechanism.

The unique precision of MSO neurons is achieved via the integration of large excitatory and inhibitory fiber inputs mediated by fast AMPARs and GlyRs (Couchman et al. 2010). These inputs are further shaped by postsynaptic membrane properties including a membrane time constant on the order of hundreds of microseconds and an input resistance in mature neurons below 7 M\(\Omega\) (Chirila et al. 2007; Couchman et al. 2010; Magnusson et al. 2005; Scott et al. 2005). It is thought that this circuit relies on the modulation of leak conductances (Scott et al. 2005) to adjust coincidence detection windows. Therefore, the modulation of synaptic input through cross talk or the presence of activity-dependent background NMDA or GABA conductances could dynamically modulate timing, and in turn coincidence detection windows for ITD coding.

Although the role of AMPARs and GlyRs on MSO function is clear, the effects of GABA\(_A\)Rs and NMDARs are still obscure. However, there is mounting evidence that NMDARs and GABARs play more direct functional roles in nuclei of the mature auditory brain stem than previously assumed. In the LSO, the retrograde release of GABA can modulate the input-output function of this circuit in vivo (Magnusson et al. 2008). In the MSO, at least in vitro, presynaptic GABA\(_A\)Rs modulate inhibitory neurotransmission (Hassfurth et al. 2010). The presence of postsynaptic, likely extrasynaptic GABA\(_A\)Rs reported here therefore raises the possibility of functional consequences for in vivo processing. In the cochlear nucleus, where a similar configuration of GABARs has been reported, the activation of GABARs in vitro modulates cellular computation, ultimately converting bushy cells from relays to coincidence detectors (Chanda and Xu-Friedman 2010) in the medial nucleus of the trapezoid body (MNTB), synaptic NMDARs together with nitric oxide activation may be involved in calcium signaling, altering synaptic transmission and postsynaptic excitability (Steinert et al. 2008, 2010). Nevertheless, any computational effects caused by the modulatory actions of GABA\(_A\)Rs and NMDARs on MSO function are still speculative. We find in the MSO that both receptor types are largely extrasynaptic, and are therefore most likely to be activated during periods of high activity. This activation could increase the leak conductance of MSO neurons and, by reducing the membrane time constant, shorten coincidence detection windows. Thus these receptors might be able to dynamically adjust the ITD functions of these neurons, perhaps enabling gain modulation during the transition between auditory environments with different levels of background sound. In any case, the widespread presence of NMDARs and GABARs in the auditory brain stem makes an in vivo investigation into the role of these transmitters important for our understanding of ITD coding.


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