Excitatory cholecystokinin neurons of the midbrain integrate diverse temporal responses and drive auditory thalamic subdomains

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The central nucleus of the inferior colliculus (ICC) integrates information about different features of sound and then distributes this information to thalamocortical circuits. However, the lack of clear definitions of circuit elements in the ICC has limited our understanding of the nature of these circuit transformations. Here, we combine virus-based genetic access with electrophysiological and optogenetic approaches to identify a large family of excitatory, cholecystokinin-expressing thalamic projection neurons in the ICC of the Mongolian gerbil. We show that these neurons form a distinct cell type, displaying uniform morphology and intrinsic firing features, and provide powerful, spatially restricted excitation exclusively to the ventral auditory thalamus. In vivo, these neurons consistently exhibit V-shaped receptive field properties but strikingly diverse temporal responses to sound. Our results indicate that temporal response diversity is maintained within this population of otherwise uniform cells in the ICC and then relayed to cortex through spatially restricted thalamic subdomains.

Results

Excitatory Cholecystokinin-Containing (CCK+) Neurons Are the Majority Subpopulation of Excitatory ICC Neurons. We found that a significant population of excitatory neurons in the ICC expresses mRNA for CCK (CCK+). To quantify the fraction of CCK+ neurons vs. the total neuron population and the more restricted glutamatergic neuron population, we used in situ probes to endogenous CCK, VGlut2, and GAD2. We found that CCK+ neurons make up patterns in vitro (12–16) or with in vivo receptive fields (15–17), and it has remained unclear how much intrinsic membrane properties interact with synaptic inputs to shape auditory information.

Here we identify an ICC neuron population that forms the major lemniscal excitatory projection to the MGB. These cholecystokinin (CCK) expressing neurons exhibit homogeneous intrinsic firing patterns and synaptic properties in vitro and V-shaped frequency receptive fields in vivo. However, even within this seemingly uniform population, we observed diverse temporal responses to tones in vivo, likely reflecting complexity in both intrinsic and extrinsic connectivity. Our results are consistent with a view of midbrain processing where individual cell types perform diverse processing tasks but mediate the convergence of this information onto specific target areas.

Significance

Our ability to identify sounds and understand communication signals depends upon our brains’ capacity to combine information about diverse sound features, including temporal patterns. The central nucleus of the inferior colliculus (ICC) performs an initial stage of this integration, but a circuit-based understanding of these processes has been hampered by difficulties in separating clearly defined functional cell types. Here we identify and characterize a major excitatory projection neuron of the ICC. These neurons show uniform intrinsic firing patterns and tuning to frequency, but strikingly diverse temporal responses to sound. Our results suggest that diversity in temporal coding is represented even within a single cell class and is likely primarily driven by differences in circuit connectivity.
53.4 ± 6.6% of the total gerbil ICC population (CCK\textsubscript{E}/VGluT2) and 62.9 ± 7.8% of the glutamatergic ICC population [CCK\textsubscript{E}/(VGluT2+GAD2)] (n = 1,220 cells, n = 5 gerbils; Fig. 1C).

To target CCK\textsubscript{E} neurons specifically, we chose a set intersectional approach (18) using two interdependent adeno-associated virus (AAV) vectors (Fig. 1D and E, see Methods). Targeted neuron characterization was performed via in situ hybridization and the RNAscope system to evaluate the specificity and coverage of virus-mediated reporter expression in CCK\textsubscript{E} neurons (details in SI Appendix). Viral-mediated targeting of CCK\textsubscript{E} neurons was highly specific, assessed through colocalization of tdTomato mRNA with CCK, VGluT2, and lack of colocalization with GAD2 mRNA (Fig. 1D). A total of 98.1 ± 2.1% of neurons positive for tdTomato mRNA were also positive for CCK mRNA (n = 1,216 cells, n = 5 gerbils) and 98.7 ± 0.9% were positive for VGluT2 mRNA (n = 950 cells, n = 4 gerbils). A total of 2.5 ± 1.5% of neurons positive for tdTomato mRNA were also positive for GAD2 mRNA (n = 432 cells, n = 3 gerbils). There was no overlap between GAD2 and VGluT2 mRNA labeling (n = 432 cells, n = 3 gerbils). Viral-mediated expression of tdTomato overlapped a large fraction of CCK\textsubscript{E} neurons (Fig. 1E). A total of 77.4 ± 5.5% of neurons positive for both CCK and VGluT2 had viral-mediated expression of tdTomato (n = 735 cells, n = 3 gerbils). Furthermore, 45.8 ± 6.3% of VGluT2-containing neurons had virally-mediated expression of tdTomato (n = 950 cells, n = 4 gerbils).

**CCK\textsubscript{E} Neurons Contribute to the Lemniscal Tectothalamic Pathway.** We next investigated the extrinsic targets of CCK\textsubscript{E} neurons. Injection of the AAV:CaMKII\textsubscript{−}α tdTomato from a second vector was restricted to excitatory neurons and Flp-recombinase in excitatory and inhibitory ICC neurons. The expression of tdTomato from a second vector was restricted to excitatory neurons and Flp-dependent (AAV:caMKII\textsubscript{−}α–tdTomato\textsuperscript{19}), tdTomato was therefore expressed only in CCK\textsubscript{E} neurons. (A) Brain sections containing viral-mediated expression of tdTomato were analyzed using in situ mRNA hybridization using probes to tdTomato (red), endogenous VGluT2 (magenta), and endogenous CCK (green) with a DAPI counterstain (blue). Arrows mark CCK\textsubscript{E} neurons labeled by virus (CCK\textsubscript{E}/VGluT2+tdTomato+). (B) Brain sections containing viral-mediated expression of tdTomato were analyzed using in situ mRNA hybridization using probes to tdTomato (red), endogenous VGluT2 (magenta), and endogenous GAD2 (green) with a DAPI counterstain (blue). Arrows mark CCK\textsubscript{E} neurons labeled by virus (VGluT2+tdTomato+GAD2–). (C) CCK\textsubscript{E} neurons in the ICC express VGluT2 and CCK and comprise a large fraction of the total ICC neuronal population. A total of 53.4 ± 6.6% of ICC neurons are both CCK\textsubscript{E} and VGluT2+. A total of 62.9 ± 7.8% of VGluT2+ neurons are CCK\textsubscript{E}. Open circles indicate measurements from individual gerbils. Bars: mean ± SEM n = 1,220 cells, n = 5 gerbils. (D) Nearly all neurons targeted by the viruses were CCK\textsubscript{E} neurons. CCK\textsubscript{E}/tdTomato+/tdTomato+, 98.1 ± 2.1%, n = 1,216 cells, n = 5 gerbils; VGluT2+/tdTomato+/tdTomato+, 98.7 ± 0.9%, n = 950 cells, n = 4 gerbils; GAD2+/tdTomato+/tdTomato+, 2.5 ± 1.5%, n = 432 cells, n = 3 gerbils. (E) Targeted neurons represented ~75% of CCK\textsubscript{E} neurons and ~50% of VGluT2+ neurons within the 1-mm IC injected site. CCK\textsubscript{E}/tdTomato+/VGluT2+/CCK\textsubscript{E}/VGluT2+, 77.4 ± 5.5%, n = 735 cells, n = 3 gerbils; tdTomato+/VGluT2+/VGluT2+, 45.8 ± 6.3%, n = 950 cells, n = 4 gerbils.
potentials (EPSPs) from CCKE axons. Neurons were filled with press EGFP in CCKE neurons. EGFP intensity was enhanced with a bio-

**Fig. 3** CCKE synapses onto vMGB neurons, we expressed ChR2 in their targets (21–23). To characterize the functional impact of CCKE inputs, we analyzed EPSPs mediated by both AMPA and NMDA receptors. EPSPs from CCKE inputs had large AMPA- and NMDA-mediated components, but not metabotropic components. (Fig. 3f, n = 10 cells, n = 9 gerbils). The relative amplitudes of AMPA and NMDA contributions were computed from the subtraction of control and drug conditions (Fig. 3j and k). The peak voltage change in the AMPA component (7.00 ± 1.59 mV) was measured from the subtraction of the control and D-AP5 condition. The peak voltage change in the NMDA component (2.49 ± 0.59 mV) was measured from the subtraction of the D-AP5 and D-AP5 + NBOX condition. Taken together, the electrophysiological properties of CCKE synapses onto thalamic neurons fit the profile of a thalamic driver circuit: they comprise large glutamatergic endings or clusters of boutons and provide a powerful but depressing excitatory drive to their thalamic targets mediated by both AMPA and NMDA ionotropic receptors.

**CCKE Neurons Are Disk-Shaped Laminar Neurons.** Having established the specificity and functional nature of CCKE projections, we next asked if CCKE neurons comprise a single morphological group within the ICC. ICC neurons have been previously separated into two well-defined classes based on dendritic orientation relative to the tonotopic lamina (4). Disk-shaped neurons have elongated dendrites oriented parallel to the tonotopic lamina while stellate neurons have radiate dendrites that cross the lamina (8, 10). These two morphologically defined neuron classes include subclasses with distinct physiological properties and neurochemical markers (14, 24–26).

To classify the CCKE neurons within this diverse framework, we sliced the ICC in the horizontal plane. This plane allowed for the most reliable and reproducible visualization and reconstruction of dendrites within large sections of isofrequency lamina (Fig. 4a–c). Polar histograms with 5° bins around the soma center were used to describe the dendritic orientation of CCKE neurons. The average polar histogram of all reconstructed neurons (Fig. 4d) revealed that dendrites of CCKE neurons are oriented along the rostromedial to caudolateral axis. The shorter average vector radiating caudolaterally from the cell soma center indicates some truncation of the dendritic tree due to the steeper curvature of the ICC isofrequency lamina as it runs toward the lateral edge of the nucleus. Together, qualitative categorization and quantitation of dendritic orientation suggests CCKE neurons can be classified as disk-shaped neurons, consistent with past descriptions of this morphological cell type (8, 10).

**CCKE Neurons Exhibit Uniform Intrinsic Firing Patterns.** To understand whether CCKE neurons exhibit distinctive in vitro electrophysiological features relative to the total ICC population, we made whole-cell patch-clamp recordings in slices prepared in either the coronal or ICC laminar planes (Fig. 5a). To compare intrinsic firing patterns across multiple ICC neuron populations, we recorded from nonvirally targeted ICC neurons representing the total ICC population, as well as the AAV-labeled excitatory,
CCK and CCKE populations. The data contributing to the total ICC population were not combined with those from animals with virally labeled neurons. However, excitatory, CCK, and CCKE neurons will have been represented in the population data due to random sampling of unlabeled cells. In both the total ICC and the excitatory populations, we observed the full extent of firing property diversity in the gerbil ICC that has been previously described for other rodents—adapting, sustained, and onset firing patterns (unlabeled ICC: \( n = 68, 79 \), and 5/152 neurons, respectively; CaMKII\(\alpha \): \( n = 16, 6 \), and 4/26 neurons, respectively; Fig. 5B). CCK neurons exhibited adapting and sustained firing patterns (34 and 5/39 neurons, respectively), but not onset, reflecting a more restricted population of neurons. However, when recordings targeted the CCKE population, only the adapting firing pattern was observed (\( n = 35/35 \) neurons). The rate of adaptation was not a distinguishing property across CCKE neurons and the general ICC or excitatory cell populations when compared across trains of different rates (Fig. 5C). Thus, while firing rate adaptation was a consistent feature of CCKE neurons, it was not unique to this population.

Next we explored if action potential or subthreshold membrane properties would distinguish CCKE neurons from the less restricted ICC populations. Measures of action potential height and half-width were not distinguishing characteristics between the total, excitatory, and CCKE populations (Fig. 5D). Subthreshold membrane properties were also not distinguishing features of CCKE neurons (Fig. 5E). Taken together, our results show that CCKE neurons form a uniform neuron population only when intrinsic firing pattern and reporter expression profile are considered together (Fig. 5B).

**CCKE Neurons Respond to Sound Stimuli with Diverse Temporal Patterns.** To understand the role of CCKE neurons in conveying auditory information to the thalamus, we measured receptive fields and temporal properties of CCKE neuron responses to pure tone sound stimuli in vivo. To identify these neurons in vivo, we activated the expressed ChR2 with blue light delivered through an independently placed optic fiber at or just under the dorsal surface of the inferior colliculus (IC) (Fig. 6A and SI Appendix, Fig. 5; see Methods). We recorded the frequency response areas (FRAs) of ICC units (CCK: \( n = 10 \) units, \( n = 7 \) gerbils; total ICC population: \( n = 61 \) units, \( n = 37 \) gerbils) and divided them into three groups based upon the shape of their excitatory receptive fields in response to contralateral monaural pure tones, as previously described (3). In nontargeted units, we recorded type V units, which had excitatory areas that widened with increasing sound levels; type I units, which had tuning whose shape was insensitive to sound level; and type O units, which displayed an on-center-surround receptive field shape. The inhibitory areas for all FRAs were not prominent and spontaneous activity was minimal due to ketamine anesthesia (CCKE:

![Image](https://doi.org/10.1073/pnas.2007724118)
Neurons in brain circuits are typically categorized based on neurotransmitter phenotype, intrinsic electrical properties, dendritic morphology, and network connectivity (30–34). In the ICC, a major center for integrating ascending and descending auditory information, both inhibitory and excitatory cell types share overlapping anatomical and physiological features, underscoring the need to include other information such as molecular-genetic markers. Here, we have combined anatomical and physiological methods with selective virus-based targeting to isolate and define the major excitatory ascending pathway from the ICC to the auditory thalamus. CCKE neurons of the gerbil ICC have uniform neurochemical, anatomical, and electrophysiological profiles but diverse temporal responses to pure tones. These results support a model of ICC function in which the convergence patterns of inputs, rather than the intrinsic properties of individual cell classes, determines temporal response patterns to sound.

**Discussion**

Neurons in brain circuits are typically categorized based on neurotransmitter phenotype, intrinsic electrical properties, dendritic morphology, and network connectivity (30–34). In the ICC, a major center for integrating ascending and descending auditory information, both inhibitory and excitatory cell types share overlapping anatomical and physiological features, underscoring the need to include other information such as molecular-genetic markers. Here, we have combined anatomical and physiological methods with selective virus-based targeting to isolate and define the major excitatory ascending pathway from the ICC to the auditory thalamus. CCKE neurons of the gerbil ICC have uniform neurochemical, anatomical, and electrophysiological profiles but diverse temporal responses to pure tones. These results support a model of ICC function in which the convergence patterns of inputs, rather than the intrinsic properties of individual cell classes, determines temporal response patterns to sound.

**Intersectional Viruses Provide Access to a Family of Excitatory Tectothalamic Neurons.** Previous studies of ICC circuit function have described excitatory and inhibitory cell types as having overlapping morphological and physiological characteristics (26, 35, 36). However, using interdependent viral vectors, we have been able to isolate a distinct family of excitatory neurons, comprising ~60% of all excitatory neurons (Fig. 1). These CCKE neurons have a laminar dendritic orientation and axonal targets specific to neurons of the vMGB. By contrast, nonspecific anatomical tracer studies as well as our own data have shown that axons exiting the ICC diverge to make extensive connections across the auditory system: commissural projections to the contralateral ICC, ascending tectothalamic projections to the MGB, and descending projections to a variety of lower auditory structures (37–39).

CCKE neurons are distinct from previously identified excitatory neuron subclasses in the ICC. These include regularly firing vasoactive intestinal peptide (VIP)-positive excitatory neurons that display a stellate dendritic morphology and give rise to many ascending and descending projections to both auditory and nonauditory targets (40), and transiently firing excitatory neurons that have low input resistances and express hyperpolarization and cyclic nucleotide-gated ion channels (36). Thus, like inhibitory neurons of the cortex and hippocampus, excitatory neurons of the ICC appear to consist of distinct functional classes whose roles are only beginning to be distinguished. Given the large size of the CCKE population, it will be important to understand whether this family of neurons can be further parsed into finer subcategories that might carry more restricted kinds of auditory information. These finer subcategories might reflect further intersections with other molecular-genetic markers (33, 41).

**CCKE Neurons Comprise a Powerful Thalamic Driving Circuit in the Auditory Pathway.** Using slice optogenetics, we directly demonstrated that activation of CCKE axons and terminals consistently triggered strong excitatory depolarizations in intracellularly recorded thalamic neurons of the vMGB (Fig. 3). The terminal fields of these afferents did not span the length of a thalamic tonotopic lamina but were instead highly restricted to subdomains (SI Appendix, Fig. S2) with tightly clustered large to medium terminal boutons (Fig. 2). Our data are consistent with the driver hypothesis (19), whereby primary ascending lemniscal inputs provide sparse but powerful excitatory drive on the proximal dendrites of excitatory neurons (42, 43). Our observations of small terminal bouton chains that sometimes appeared to target stretches of individual dendrites (e.g., Fig. 3F) suggest that large terminal size may not be a prerequisite for strong excitatory drive. Consistent with previous studies of ICC cell types identifying consistent features of ascending driver synapses, CCKE-mediated synaptic potentials are generated by ionotropic receptors, including a significant NMDA receptor component exhibiting strong short-term depression (21, 23, 44). The magnitude and time course of recovery of short-term depression of optically evoked EPSPs from CCKE inputs were similar to measurements in studies employing nonselective electrical stimulation of ascending axons (44), indicating that synaptic dynamics of excitatory inputs are conserved across cell classes. The properties of inhibitory synapses also do not distinguish different input classes to ICC neurons (45), further highlighting the fact that ascending pathways in the ICC must be defined jointly by synaptic physiology, intrinsic electrical properties as well as anatomical features.

Tectothalamic afferents of the auditory system and retinotthalamic afferents of the visual system share a general anatomical and physiological framework in which parallel inputs carrying sets of highly processed signals provide powerful “driving” input to the thalamus (19). In the visual system, retinal ganglion.
cells (RGCs) send highly processed information to the visual thalamus (lateral geniculate nucleus) in parallel pathways. Our data for CCKE terminal morphologies closely match previously reported RGC Y-axon size and termination patterns (20). In mice, RGCs have been classified into at least 25 functionally distinct populations based on molecular, morphological, and physiological criteria, carrying diverse information such as color, temporal pattern, or spatial movement (41, 46–48). In the auditory system, pathways carrying similar spectral bandwidth filters to incoming frequency information revealed V-shaped FRAs, indicating these neurons ascribe similar temporal responses found in the full ICC population (Fig. 6). Such diversity likely reflects differences in how excitatory and CCKE neurons are driven, both monaural and binaural envelopes that are a hallmark of ICC processing and the salient features of speech and communication signals (55–57).

The striking diversity of subthreshold membrane properties observed in vitro, even within the CCKE population, likely contributes to the differential filtering properties of ICC neurons in vivo and is consistent with the view that cell types in the ICC convey convergent information from multiple sources. Within each tonotopic ICC lamina, ascending inputs are segregated in

**Functional Implications.** In all mammalian species examined, ICC neurons exhibit high diversity in their in vivo spectral tuning with units generally showing V-shaped (most common), I-shaped, and O-shaped FRAs, often in adjacent topographic locations (3, 53). Diversity in the in vivo temporal patterns is also apparent, where ICC units can be described as pauser/build, sustained, regular/sustained, and onset firing groups (16, 27–29). Analyses restricted to neurons with laminar dendritic arbors have also failed to correlate subsets of in vivo responses with dendritic/axonal morphology (17). In vivo recordings of CCKE neurons revealed V-shaped FRAs, indicating these neurons ascribe similar spectral bandwidth filters to incoming frequency information. By contrast, CKE neurons exhibited the full diversity of temporal responses found in the full ICC population (Fig. 6). Such diversity likely reflects differences in how excitatory and inhibitory activity is balanced during acoustic responses (7, 54). Diversity in CKE temporal responses may also improve the encoding of both monaural and binaural envelopes that are a hallmark of ICC processing and the salient features of speech and communication signals (55–57).
overlapping functional zones, called synaptic domains, that are targeted by a specific subset of lower auditory nuclei (49–52). CCKE neurons appear to be distributed evenly across and within tonotopic lamina, and thus neurons within the same and adjacent fibroendritic laminae likely receive synaptic inputs from different sources, including synapses from ICC collaterals themselves. Given that CCKE neurons are similar, the unique pattern of inputs each CCKE neuron receives will result in neurons with different temporal responses to pure tones.

Taken together, the present data indicate that CCKE neurons are a family of neurons that comprise a large portion of the excitatory tectothalamic driver pathway. These neurons convey temporally diverse auditory information to the vMGB, with temporal response structure determined both by the combination of extrinsic inputs as well as local circuitry. Given its large size, it seems likely that the family of CCKE neurons may well be divided into subsets of neurons, possibly reflected in characteristic molecular-genetic profiles that will result in further restricted functional properties of these neurons. The intersectional viral approach employed here will be critical to addressing these questions.

Methods

Animal Use. All procedures were approved by and conducted in accordance with The University of Texas at Austin Institutional Animal Care and Use Committee. Male and female Mongolian gerbils (Meriones unguiculatus) were bred at the Animal Resource Center at The University of Texas at Austin or obtained from Charles River Laboratories. Gerbils were housed in groups of up to five animals and maintained on a 12 h light/dark cycle.

ICC Neuron Targeting. We restricted targeting to ICC excitatory CCK (CCKE) neurons using a set intersectional strategy involving two viruses (18). AAV:CB3-Flp and AAV:CaMKII-(XFP)Flp ensured that the reporter was expressed only in neurons where both the CB3 and CaMKII promoters were active (details in SI Appendix, Detailed Methods). The two viruses were combined in a 1:2 vector ratio (recombinase:reporter) based on titer. None of our GABAergic promoters (18) were active in the IC likely due to the developmental origin of IC inhibitory cells (39). To express channelrhodopsin-2 (ChR2) in CCK neurons, ChR2(H134R)-sfGFP fusion protein (60) was used in place of fluorescent reporter in the recombinase-dependent vector AAV:CaMKII-(ChR2-sfGFP)X. Typical titers were $1 \times 10^{10}$ genomes/microliter.

Histology. For immunohistochemical labeling, tissue was permeabilized and blocked in a solution of 0.2% Triton X-100 and 5% normal goat serum in phosphate-buffered saline. Tissue sections were then incubated with primary antibodies: guinea pig anti-VGluT2 (AB-2251-I, Millipore) and biotinylated goat anti-GFP (ab6658, Abcam) in blocking solution overnight at 4 °C. For fluorescence labeling, sections were incubated with secondary antibody in blocking solution for 2 h. When using biotinylated antibodies, tissue was incubated in an avidin-biotin solution (Vectastain Elite ABC HRP Kit, Vector Laboratories) and developed in a nickel-diaminobenzidine solution (DAB peroxidase (HRP) Substrate Kit with nickel, Vector Laboratories). Tissue sections were mounted on gelatin-coated slides and coverslipped (Vectashield Hardset Antifade Mounting Medium with DAPI, Vector Laboratories). Histology sections were stained with 3,3-nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (XGal) in CCKE neurons, ChR2(H134R)-sfGFP fusion protein (60) was used in place of fluorescent reporter in the recombinase-dependent vector AAV:CaMKII-(ChR2-sfGFP)X. Typical titers were $1 \times 10^{10}$ genomes/microliter.

In thalamic sections developed with Ni-DAB, individual bouton terminals that fell within a cluster of four or more boutons within at least one axonal termination field were traced with Neurolucida software. En passant

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**Fig. 6.** CCKE neurons recorded in vivo have uniform V-shaped FRAs and diverse temporal responses to tones. (A) Examples of light-evoked (Left, 50 ms blue light) and sound-evoked (Right, 100 ms 1.5 kHz pure tone) voltage traces from one CCKE unit. Action potentials from light or sound presentations from the respective experiment are aligned and overlaid (gray traces, 103 light-evoked and 1,106 sound-evoked action potentials); black traces, average waveform. Inserts: Example spike train from one stimulus presentation. (Scale bars: 0.3 mV, 50 ms.) (B) Example FRAs from four CCKE neurons (B1 through 4) and their associated peristimulus time histograms (PSTHs) are shown below each respective
bouts corresponding to fibers of passage were not included in the analysis. Divisions of the MGB can be defined immunohistochemically with anti-calretinin antibody labeling (1:1,500, CR7077, Swant). Calretinin expresses strongly in the dorsal division of the MGB, but not in the ventral division (61).

We examined the specificity and coverage of virus-mediated reporter expression in CCKe neurons by multiplexed in situ hybridization using proprietary probes (Advanced Cell Diagnostics) to neuronal marker transcripts: CCK, vesicular glutamate transporter 2 (VGluT2), glutamate decarboxylase 2 (GAD2), and αTomato reporter. ICC sections were DAPI stained to label nuclei and to aid the colocalization of fluorescence signals (Fig. 1D).

Acute Slice Electrophysiology. Data from 230 neurons were obtained from 119 gerbils of both sexes, aged 5 to 8 wk. Gerbils were deeply anesthetized with isoflurane, perfused transcardially with either room temperature or ice-cold artificial cerebral spinal fluid (ACSF; 125 mM NaCl, 25 mM glucose, 25 mM NaHCO3, 2.5 mM KCl, 1.25 mM NaH2PO4, 1.5 mM CaCl2, and 1.5 mM MgSO4, pH adjusted to 7.45 with NaOH). Each animal was decapitated, and the brain rapidly removed and submerged in ACSF. For recordings from inferior colliculus neurons (n = 212 neurons, n = 107 gerbils), 200- to 250-μm slices were prepared in either the coronal or IC laminar plane (45° to horizontal, parallel to IC isofrequency laminae) in room-temperature ACSF. For recordings in the thalamus (n = 12 neurons, n = 12 gerbils), 200- to 250-μm slices were prepared in the horizontal plane in ice-cold ACSF. Slices were cut with a vibrating microtome (Leica VT1200S; Leica Systems). All preparations were incubated for 30 min at 35 °C, then allowed to recover at room temperature for at least 30 min. ACSF was continuously bubbled with 95% O2/5% CO2.

Whole-cell recordings were conducted at 35 °C using a Dagan BVC-700A recorder (Dagan Corporation) in current-clamp mode using standard methods (Optro Detailed Methods). Membrane potentials are corrected for a 10-mV junction potential. For cell-type-specific fluorescence-targeted or photoactivation recordings, AAV-Ca3.2-fFlp and AAV-CaM4Dii-flp (ChR2-αFP)19,20 vectors were injected into the inferior colliculus to sensitize CCKe neurons to light. Full-field 470 nm blue light from a custom LED system (Buckpuck DC driver, Luxeon Star).

Neural signals were amplified and filtered (2400B Extracellular Preamplifier; Dagan), before being digitized (R26) and sent to the custom software. All sound stimuli were of 100-ms duration and presented at 2/s or 4/s with a 5 ms or 10 ms rise/fall (details for stimuli, histological verification of recording sites, and analyses are included in SI Appendix, Detailed Methods). Stimulus delivery and neural recordings were controlled via custom-made MATLAB software. Acoustic stimuli were generated digitally by an R26 Multi-IO Processor (Tucker-Davis Technologies) and delivered by a calibrated closed-field speaker (ER2; Etymotic Research). Speaker calibration was completed using a 1/2-inch pressure field microphone (4192; Brüel & Kjær). Neural signals were amplified and filtered (2400B Extracellular Preamplifier; Dagan), before being digitized (R26) and sent to the custom software. All sound stimuli were of 100 ms duration and presented at 2/s or 4/s with a 5 ms or 10 ms rise/fall (details for stimuli, histological verification of recording sites, and analyses are included in SI Appendix, Detailed Methods). Data Availability. All study data are included in the article and/or supporting information.

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In Vivo Surgery and Recordings. Recordings were performed within a sound-attenuation chamber. Forty gerbils aged 11 to 14 wk were anesthetized with an intraperitoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine (18 mg/kg), being secured to a custom-made head-post holder. Atropine sulfate (0.04 mg/kg) was administered intramuscularly every 30 to 60 min as needed to reduce respiratory mucus secretions. Animals were maintained at 37 °C with a rectal probe feedback low-voltage DC electric heating pad (Harvard Apparatus). Glass-coated tungsten electrodes (2 MΩ) or KCl-filled borosilicate glass electrodes (4 to 10 MΩ) were used for single-unit recordings. For light stimulation, a multimode fiber optic (NA = 0.39, 200 μm core; Thorlabs Inc.) with a polished tip was coupled to an analog modulated blue diode-pumped solid-state laser (i = 473 nm; Lasergrow Technologies) and independently lowered into the craniootomy at 15° off vertical, and ~2.5 mm below the IC surface.

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