Diversity of interneurons in the lateral and basal amygdala

Jai S. Polepalli1,2,4, Helen Gooch1,4 and Pankaj Sah1,3✉

The basolateral amygdala (BLA) is a temporal lobe structure that contributes to a host of behaviors. In particular, it is a central player in learning about aversive events and thus assigning emotional valence to sensory events. It is a cortical-like structure and contains glutamatergic pyramidal neurons and GABAergic interneurons. It is divided into the lateral (LA) and basal (BA) nuclei that have distinct cell types and connections. Interneurons in the BLA are a heterogeneous population, some of which have been implicated in specific functional roles. Here we use optogenetics and slice electrophysiology to investigate the innervation, postsynaptic receptor stoichiometry, and plasticity of excitatory inputs onto interneurons within the BLA. Interneurons were divided into six groups based on their discharge properties, each of which received input from the auditory thalamus (AT) and auditory cortex (AC). Auditory innervation was concentrated in the LA, and optogenetic stimulation evoked robust synaptic responses in nearly all interneurons, drove many cells to threshold, and evoked disynaptic inhibition in most interneurons. Auditory input to the BA was sparse, innervated fewer interneurons, and evoked smaller synaptic responses. Biophysically, the subunit composition and distribution of AMPAR and NMDAR also differed between the two nuclei, with fewer BA IN expressing calcium permeable AMPAR, and a higher proportion expressing GluN2B-containing NMDAR. Finally, unlike LA interneurons, LTP could not be induced in the BA. These findings show that interneurons in the LA and BA are physiologically distinct populations and suggest they may have differing roles during associative learning.

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RESULTS

Six electrophysiological classes of GABAergic neurons in the BLA Whole-cell current-clamp recordings were obtained from GFP-positive neurons in the BA and LA of GAD67-GFP transgenic mice. No differences were found in the passive membrane properties of interneurons in the two nuclei, and the overall resting membrane potential was $-62.3 \pm 0.6$ mV ($n = 63$) with an input resistance of $252 \pm 17$ MΩ ($n = 63$). As previously described, based on the pattern of action potential discharge evoked by somatic current injection, cells could be grouped into 6 subtypes: accommodating cells (ACC; Fig. 1a, g), regular-spiking.
The BLA receives two streams of auditory input: one directly from the AT and a more processed stream from the AC. The AT is comprised of three nuclei: the medial geniculate body (MGN), comprised of the primary AT (Te1) and neighboring temporal association cortex (TeA), has no direct projections from the inferior colliculus, but receives auditory innervation indirectly via the AT. Te1 is predominantly innervated by MGV30-32, but has sparse projections to the BLA29,33. In contrast, TeA which gets direct input from MGN, PIN, SG, and MGd also receives processed auditory input from Te128,29 and sends long-range projections via the external capsule to densely innervate the BLA26,29,33. Thus, the BLA receives rapid auditory input directly from the thalamus, and a more processed but delayed input from the temporal association cortex, TeA28,29.

To determine the nature of auditory input to BLA interneurons, light-gated channelrhodopsin-2 (ChR2-eYFP) was expressed in the AT (Fig. 2a) or TeA (Fig. 2b) to label thalamic and cortical inputs respectively. Consistent with previous anatomical tracer studies, visualization of transduced YFP-tagged ChR2 terminals from either AT (Fig. 2c) or AC (Fig. 2d) revealed strong labeling within the LA (Fig. 2e), with comparatively sparse innervation in the BA (Fig. 2f), across the entire rostro-caudal extent of the BLA (Fig. 2g). Whole-cell voltage-clamp recordings were then obtained from interneurons in the BLA. Light-activation of AT afferents revealed direct input in ~91% (61/67) of LA interneurons, and the evoked excitatory postsynaptic current (EPSC; Vh = −70 mV) had a peak amplitude of 191 ± 18 pA. For AC afferents, 85% (50/59) of LA interneurons received input (Fig. 3a), and the evoked EPSC had a peak amplitude of 167 ± 25 pA. Although each input was tested independently, these high innervation rates suggest most interneurons receive both AT and AC input.

In the BA, 67% (12/18) of interneurons received AT input (Fig. 3a) and it was significantly smaller (peak amplitude: 53 ± 14 pA; p < 0.0001) as compared to interneurons in the LA. AC input to BA interneurons was also sparse with 38% of cells (6/16; Fig. 3b) being innervated, but input to individual cells (127 ± 50 pA) was not significantly different to that in the LA (p = 0.5888). For all interneurons, light-evoked EPSCs were time locked to the onset of light stimulation (Fig. 3b) with small synaptic jitter (Fig. 3c), consistent with direct, monosynaptic connections.

These results show that there are clear differences in auditory input to interneurons in the LA and BA. Input from both AT and AC is larger to interneurons in the LA with more cells receiving input, and the overall amplitude of thalamic input to LA interneurons is significantly larger than to those in the BA (Fig. 3d). For cortical input, while fewer BA interneurons were innervated, the absolute size of the input is similar in the LA and BA. It should be noted though that BA interneurons with larger AC inputs were close to the LA/BA boundary, which can be difficult to define clearly. When comparing LA interneurons based on their discharge properties (Fig. 1), all six subtypes received input from both the AT and AC (Supplementary Table 2; Fig. 3e). However, while the total numbers of some subtypes were small, AT input to ACC neurons was significantly larger than AC input (p = 0.0137; Fig. 3e).

Interestingly, when we compared AT and AC inputs to individual interneurons, the rise time of AT EPSCs was significantly shorter (peak amplitude: 191 ± 18 pA; p < 0.0001) compared to AC EPSCs (peak amplitude: 167 ± 25 pA). This difference in rise time suggests that AT EPSCs may have a faster onset, consistent with the more rapid auditory input from the thalamus.
slower than that evoked by AC inputs (AT-LA 10–90% rise time = 1.6 ± 0.09 ms, n = 59; AC-LA rise time = 1.3 ± 0.08 ms, n = 46; p = 0.0482; Fig. 3f), suggesting that cortical inputs to these interneurons may be distributed electronically closer to the soma. However, there was no difference in the ability of inputs to drive cells to threshold. In the LA, thalamic input drove suprathreshold responses in 21% of cells (9/44), and cortical input could drive 19% (7/38) to threshold (Fig. 3h), whereas suprathreshold responses in 21% of cells (9/44), and cortical input could drive 19% (7/38) to threshold. In the LA, thalamic input drove cells to threshold. In the LA, thalamic input drove

Interneurons in the BLA make synaptic connections between cells of the same51,34 and different10,34 families. As most interneurons in the BLA receive auditory input and can drive these cells to threshold, we next tested if auditory inputs drove feed-forward inhibition on interneurons (Fig. 4a). With interneurons voltage clamped at a depolarized membrane potential (~40 mV), stimulation of either AT or AC afferents elicited biphasic synaptic responses (Fig. 4b) in approximately half the interneurons in the LA. The outward component of this biphasic response was blocked by GABA_A receptor antagonist picrotoxin (20 μM; Fig. 4c). To compare the level of feed-forward inhibition for the two inputs onto these cells, we calculated the excitation to inhibition (E/I) ratio. We found that AC input drove feed-forward inhibition with a smaller E/I ratio as compared to AT input (AT-LA E/I ratio = 10.6 ± 1.9; AC-LA E/I ratio = 5.9 ± 1.1; p = 0.0453), consistent with the stronger AT input. Though not significant, a similar difference was observed within the biphasic E/I ratio of AT and AC inputs on ACC interneurons (AT-ACC E/I ratio = 11.4 ± 2.8; AC-ACC E/I ratio = 5.2 ± 1.1; p = 0.0637; Fig. 4f). While we have not directly tested optically driven inhibition to principal cells, paired recordings revealed a high unidirectional inhibitory connection probability between interneurons and pyramidal cells in the LA (Fig. 4g, h), suggesting that auditory stimulation would likely drive feed-forward inhibition onto LA excitatory pyramidal neurons.

LA and BA interneurons have divergent synaptic properties

Our results show clear asymmetries in auditory input to interneurons in the LA and BA. We have previously shown that in a population of interneurons in the LA, glutamatergic synapses express strongly rectifying AMPA receptors, and at these synapses there are few if any postsynaptic NMDA receptors35,37. We therefore compared the biophysical properties of glutamatergic synapses on interneurons in the LA and BA. Synaptic inputs were evoked using electrical stimulation of the internal or external capsules to evoke thalamic and cortical inputs, respectively36,37. It should be noted that while stimulation of the internal and external capsule has generally been accepted to recruit thalamic and cortical inputs respectively, other afferents such as those arising from the hippocampus, are also likely to be present. Electrical stimulation is indiscriminate as to the source of afferents stimulated, however, in these cells synaptic currents evoked by stimulation in the external or internal capsule, as well as those occurring spontaneously have identical kinetics, indicating that all glutamatergic synapses on individual interneurons express similar ionotropic receptors36. Using a cesium based internal solution, and with GABA_A receptors blocked, glutamatergic inputs were evoked at holding potentials of −60 mV and −40 mV, and the NMDA/AMPA receptor ratio was used as a measure of the relative synaptic weights of the stimulated input (see Methods). As reported previously35, a population of cells in the LA had synapses that lacked NMDA receptors (Fig. 5h). At synapses where NMDA receptors were present, the NMDA/AMPA ratio of inputs to interneurons was comparable between the LA and BA (BA, 0.90 ± 0.07, n = 45; LA, 0.73 ± 0.10, n = 54; p > 0.05; Fig. 5a, b; Supplementary Fig. 2a).

**Fig. 2 Auditory input targets the lateral amygdala (LA).** a, b Virus encoding ChR2-eYFP was stereotactically targeted to the auditory thalamus (AT) a or the auditory cortex (AC) b. ChR2-eYFP expressing afferents from the AT (c) and AC (d) were detected throughout the rostro-caudal extent of the BLA (right). Schematics (left) show corresponding locations of interneurons that received AT input (green), AC input (blue) or no input (red). e, f Shown are representative cells in the LA (e) and BA (f) recovered following immunohistochemistry for biocytin. AT afferents (ChR2-eYFP) are also shown (green) highlighting the higher density of auditory projections within the LA (e) compared to the BA (f). Insets show respective somatic GAD67-eGFP fluorescence for GABAergic identification. g Within-slice ratio of BA:LA fluorescence across the rostro-caudal range (rostral BLA, AT = 0.42 ± 0.1 (n = 7), AC = 0.47 ± 0.07 (n = 4), p = 0.6695; middle BLA, AT = 0.37 ± 0.07 (n = 10), AC = 0.48 ± 0.08 (n = 9), p = 0.3219; caudal BLA, AT = 0.34 ± 0.05 (n = 6), AC = 0.43 ± 0.7 (n = 9), p = 0.3403). Mean ± SEM (unpaired two-tailed t test with Welch’s correction).
The AMPAR rectification index was measured as the ratio of peak AMPAR EPSC amplitude at −40 mV and −60 mV in the presence of NMDAR blocker d-AP5 (30 μM), and was significantly higher in BA interneurons (BA: 0.35 ± 0.04, n = 45; LA: 0.25 ± 0.02, n = 54; p < 0.05; Fig. 5a, c; Supplementary Fig. 2b), suggesting that glutamatergic synapses on BA interneurons contained fewer GluR2-lacking, calcium permeable (CP)-AMPAR receptors.

We next assessed the subunit composition of NMDA receptor subunits by measuring the weighted decay time constant (τ) of the synaptic current at +40 mV in the presence of AMPAR blocker NBQX (10 μM)35. The distribution of time constants showed differences between interneurons in the LA and BA (Fig. 5d), which was also reflected in the cumulative probability plots (Fig. 5e). Overall, the NMDAR EPSC on interneurons in the LA had a faster decay time constant (59 ± 3 ms, n = 54) as compared to that in the BA (91 ± 7 ms, n = 45) (Fig. 5e inset; p = 0.001; K-S test), suggesting the presence of a higher percentage of GluN2A-containing heterodimeric NMDA receptors in LA interneurons38–40.

To evaluate the proportion of interneurons expressing each type of NMDA receptor, LA and BA interneurons were separated into three classes based on the kinetics of NMDAR EPSCs: those expressing receptors largely containing GluN1/2A (τ < 80 ms), those expressing receptors containing GluN1/2B (τ > 80 ms), and those lacking NMDAR receptors (Fig. 5f)38. This analysis shows that in the LA, 18% of interneurons had synapses that did not express synaptic NMDARs (n = 11/64), 72% expressed NMDARs largely composed of GluN2A-heterodimers (n = 47/64), and 10% expressed GluN2B-containing NMDARs (n = 7/64). In contrast, in the BA, 51% of cells had synapses expressing mostly GluN2A-heterodimeric NMDARs (n = 23/45), 49% expressed GluN2B-containing NMDARs (n = 22/45), and no cells were found with synapses that lacked NMDA receptors (Fig. 5h).

Interneuron LTP is restricted to the lateral amygdala We have shown that excitatory inputs to interneurons in the BLA form synapses that largely contain GluR2-lacking AMPA receptors, but there is diversity in the types of NMDA receptors present. In the LA, cortical input to interneurons shows a form of NMDA receptor independent LTP, that is initiated by calcium influx via Ca2+ permeable (CP) GluR2-lacking AMPAR receptors35 but is limited to cells that do not express GluN2B subunits38. Given the differences in auditory innervation and synaptic properties between the LA and the BA, we next asked if inputs to interneurons in the BA undergo LTP. All interneurons in the BA express synaptic NMDA receptors (Fig. 5h), however, tetanic stimulation of cortical input to interneurons in the BA failed to evoke LTP (n = 8; Fig. 6a, b). In contrast, as reported previously38, tetanic stimulation of cortical inputs onto LA interneurons expressing GluN2A-containing NMDA receptors reliably evoked LTP (normalized EPSC amplitude = 1.5 ± 0.1; n = 9; Fig. 6c, d).
DISCUSSION

The BLA is a cortical-like structure that plays a central role in processing, storage, and retrieval of associative fear memories. It contains two main types of neuron: glutamatergic pyramidal-like neurons comprising ~85% of the population, while the remaining ~15% are GABAergic interneurons.4,41 Interneurons in the BLA tightly control the excitability of principal neurons and play a key role in associative learning.6–13 The BLA is anatomically divided into the LA and BA, but functional studies treat interneurons of these nuclei as one population. In this study we have characterized interneurons in the LA and BA, along with their innervation by auditory inputs, and show that while sharing some properties, they also have clear differences.

Interneurons are a heterogeneous population that are separated into groups by expression of cytosolic markers, electrical discharge properties, and the synaptic connections they make.16,17,49,50 Of these, the best understood are those expressing PV and those expressing SOM. While these markers define two distinct developmental classes, there are different cell types within each. For example, among PV interneurons, some innervate the soma and axon initial segment, a different type innervates the soma alone, while another type innervates the proximal dendritic tree6,21,22 and they each have different physiological impacts.45,51 Markers that separate these classes are not currently available, and we separated BLA interneurons on their discharge properties. Based on these criteria, cells were divided into six types: accommodating cells (ACC), regular-spiking cells (REG), fast-spiking cells (FS), studding cells (ST), irregular-spiking cells (IS), and burst-spiking cells (BS). All six classes have been previously described in the cerebral cortex2, and four types (ACC, FS, ST, IS) have been previously described in the BA.21,22

Our data show that the distribution of inhibitory interneurons in the BLA is different from other cortical and hippocampal regions. Whereas FS cells form ~30–50% of the interneuron population in the cortex and hippocampus, using a pan interneuron GFP-expressing mouse line (GAD67-EGFP), we find these neurons form a relatively minor population in the BLA. Interneurons in the BLA were initially distinguished by expression of calcium binding proteins, aspiny dendritic trees,6,41,48,51 and electrophysiologically
as cells with fast-spiking discharge properties. However, with the introduction of mice with genetically labeled interneurons, it is clear that not all interneurons in the BLA are FS cells. Indeed, as shown here, the most common BLA interneuron subtype is accommodating, a firing pattern characteristic of BLA excitatory principal neurons. Moreover, while fast-spiking is widely used to identify parvalbumin interneurons in the hippocampus and the cortex, we have shown that FS cells are only a proportion of the parvalbumin cell population in the BLA. These electrophysiological data are complemented by our immunohistochemistry (Supplementary Fig. 1) finding that LA but not BA PV+ interneurons receive excitatory input from the AT, and that PV interneurons drove feed-forward inhibition in the LA but not the BA. Together, these data show that auditory processing in the LA is tightly controlled by inhibitory networks that operate by a combination of feed-forward inhibition and disinhibitory control. The clear differences in innervation patterns in the BLA also suggest differing roles for LA and BA interneurons during auditory fear conditioning.

As expected by the low expression of GluR2 subunits in interneurons, AMPA receptors at glutamatergic inputs were inwardly rectifying. This rectification was more prominent for interneurons in the LA as compared to the BA, suggesting a higher fraction of synaptic AMPA receptors lacking GluN2 subunits in LA interneurons. There were also clear differences in the distribution of synaptic NMDA receptors. Firstly, a small population of interneurons in the LA do not express synaptic NMDA receptors. These interneurons were initially described in the LA as FS interneurons and, consistently with the lower proportion of parvalbumin interneurons, they do not appear to be present in the BA. As FS interneurons are a proportionally small population, it is possible that we missed some of these cells in the BA. Of cells that express NMDA receptors, one population had receptors containing GluN2B subunits, while a different population expressed receptors that appear to be GluN2A only heterodimers. This second population is proportionally larger in the LA, where it marks cells at which cortical inputs undergo long-term potentiation. In the BA, however, expression of these two types of NMDA receptors was evenly distributed, and inputs to these cells do not appear to undergo LTP. The reason for this lack of LTP at inputs to interneurons in the BA is not clear. In the LA, LTP is seen at inputs that have steep inward rectification and is evoked by calcium influx via calcium permeable AMPA receptors. As described above, synaptic AMPA receptors on interneurons in the BA show significantly less rectification, showing that the complement of GluR2-lacking receptors is lower. Thus, one possibility is that the calcium rise at synapses on BA interneurons is not sufficient to trigger LTP. We initially mapped auditory input to interneurons in the LA and BA using channelrhodopsin expressed in thalamic and cortical auditory nuclei. However, the biophysical analysis of glutamatergic input to these cells and whether they could undergo plasticity was done using extracellular stimulation in the internal and external capsules. Unlike optogenetic stimulation, this technique will activate a range of inputs, raising the question of how these data can be reconciled with those obtained in the first part of our study. However, we have shown in the BLA that all glutamatergic synapses on particular interneurons show identical biophysical properties, and thus thalamic and cortical auditory input to interneurons are not expected to be different, and consistent with this the only differences we detected were between interneurons in the LA and BA, rather than differences in input from the external and internal capsule. With regard to synaptic plasticity, as channelrhodopsin increases release probability, tetanic stimulation as a means of evoking plasticity is problematic. Our results again show that there are differences in plasticity between cells in the LA and BA rather than in the input. Thus, while the method of stimulation is different both optical stimulation and extracellular stimulation show that interneurons in the LA and BA should be treated as different populations.

What explains differences in the physiological properties of interneurons in the BLA? The BLA contains a population of precursor cells that gives rise to newborn interneurons in the adult that integrate into the local circuitry. Newborn neurons will receive synaptic inputs that undergo a period of maturation, a process during which the biophysical properties and subunits of glutamatergic synapses change. Thus, it is possible that some interneurons sampled in our study are still not fully mature, accounting for the differences in biophysical properties. The

![Fig. 6 Interneurons in the BA are resilient to LTP induction.](npj Science of Learning (2020) 10 Published in partnership with The University of Queensland)
physiological role of NMDA receptors in BLA interneurons is not completely clear, we suggest that like cytosolic markers and transcription factors that are used as markers for developmental fate mapping, expression of ionotropic glutamate receptors may also be developmental markers that delineate different interneuron types and mark specific circuits in the LA and BA.

The BLA plays a key role in auditory fear conditioning, and a host of studies have identified changes in the response of BLA pyramidal cells and interneurons to auditory input following fear learning. However, not all studies disentangle recordings obtained from cells in the LA from those in the neighboring the BA. Moreover, using cytosolic markers such as PV and SOM, interneurons are generally treated as single populations while there is clearly significant diversity even within these populations. In part, this is no doubt due to the difficulty in separating these nuclei, and different cell types, particularly in vivo. Synthetic plasticity of input to the BLA is widely accepted to underpin fear conditioning. This plasticity has been thought to be of input to principal neurons in the BLA. It is clear that interneurons play key roles in fear learning, and following fear conditioning, the response of some interneurons to the CS also changes suggesting that synaptic plasticity of input to interneurons plays a role in fear learning. It will be interesting to identify the cell types that mediate these responses, and our data suggests that they are likely to be located in the LA.

METHODS

Animals

All experimental and animal care procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and approved by the University of Queensland Animal Ethics Committee. Predominantly GAD67-GFP knock-in mice on a C57BL/6 background were used, which allowed for the visual differentiation of GABAergic interneurons from excitatory neurons in acute slice recording conditions.

Virus

Third generation lentiviruses were produced in house for the transgene Channelrhodopsin-2 [pLenti-synapsin-hChR2(H134R)-eYFP-WPRE, kind gift from Karl Deisseroth, Stanford University]. Adeno-associated viruses (AAV) were obtained from Penn Vector Core (AAV2/5-hSyn-hChR2(H134R)-eYFP-WPRE-Igh). Third generation lentiviruses were produced in house for the transgene Channelrhodopsin-2 [pLenti-synapsin-hChR2(H134R)-eYFP-WPRE-Igh].

Surgery

Mice (p21–60) were anesthetized with a ketamine (100 mg/kg)/xylazine (20 mg/kg) mixture intraperitoneally, and the head shaved and secured in a stereotaxic frame. Rectal temperature was monitored and maintained at 37 ± 0.5 °C throughout the procedure by a feedback-controlled heat pad. The scalp was hemisected and secured laterally, and a small craniotomy was transferred to the recording chamber. During recordings, slices were perfused with oxygenated aCSF, heated to 32 ± 2 °C and secured with a platinum harp strung with parallel nylon threads. Recording pipettes were fabricated from borosilicate glass and pulled to a tip resistance between 3 and 5 MΩ (GC150F, 1.5 mm, Harvard Apparatus, UK) when filled with internal solution containing (in mM): 135 KMeSO4, 8 NaCl, 10 HEPES, 2 Mg2ATP, 0.3 Na3GTP, 0.1 spermine, 7 phosphocreatine, 8 biocytin, and 0.3 EGTA. Recordings were performed with a patch clamp amplifier (Axopatch 200B, Axon Instruments, Molecular Devices). Recordings were filtered at 6 kHz, and digitized at 10–20kHz using an ITC-18 board (Instrutech, Port Washington, NY) attached to an iMac. Recordings were acquired and analyzed offline using Axograph software for Windows (Axograph X, version 1.4.4), run through virtual machine software (VMWare Fusion 6).

To estimate the intrinsic membrane properties of interneurons, somatic current injections were applied in 500 ms square pulses at 50 pA incremental steps from −100 to 600 pA. Synaptic inputs were evoked at 0.1 Hz electrically (Digitimer, DS2A) or optically using 5 ms pulses of whole-field illumination at blue excitation wavelengths −470 nm (Cairn OptoLED), and recorded in whole-cell patch-clamp configuration. Light-gated synaptic inputs met monosynaptic inclusion criteria when onset latency was <7 ms, synaptic jitter was <0.5, and average input amplitude was >5 pA (cut-off threshold for a miniature EPSC event). To examine synaptic properties, EPSCs were recorded in voltage-clamp at holding potentials of −60 and +40 mV, to determine: (1) NMDAR-to-AMPA ratio; (2) AMPAR rectification in the presence of the NMDAR blocker AP5 (30 µM); (3) kinetics of the NMDAR mediated EPSC in the presence of the AMPAR blocker 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dion (DNQX, 10 µM). Bipolar stimulating electrodes were placed on the external capsule (EC) to stimulate cortical inputs, and medially within the internal capsule (IC) to stimulate thalamic inputs. The NMDAR/AMPAR ratio was calculated as the NMDA current at 25 ms after the peak of the dual component EPSC at +40 mV, divided by the peak AMPAR-mediated EPSC at −60 mV. The rectification index of AMPAR-mediated EPSCs was calculated as the peak amplitude at +40 mV divided by the peak AMPAR. For comparison of AMPA/ NMDA EPSCs, deactivation kinetics, 5–10 electrically evoked EPSCs were recorded at +40 mV and averaged. The synaptic current decay rates were fitted with a double exponential equation of the form: \( I(t) = I_f e^{-t/I_f} + I_s e^{-t/I_s} \), where \( I_f \) and \( I_s \) are the amplitudes of the fast and slow decay components, respectively.
and $t_1$ and $t_2$ are their respective decay time constants. The weighted time constant was calculated as: $t_{w} = (t_1 / (t_1 + t_2)) t_1 + (t_2 / (t_1 + t_2)) t_2$, and was used for statistical comparisons of the decay times of recorded EPSCs. LTP was evoked with a tetanic stimulus (100 Hz, 300 ms × 3, 10 s interval).

Immunohistochemistry

For biocyton recovery and immunohistochemistry, brain slices were fixed in 4% paraformaldehyde in 0.1 M PBS overnight at 4 °C. Slices were washed three times with 0.1 M PBS, and then blocked and permeabilized with a blocking solution (PBS 0.1 M, bovine serum albumin 3%, saponin 0.1%, and sodium azide 0.05%) for 30 min at room temperature. Slices were then washed three times in 0.1 M PBS and incubated in blocking solution containing anti-GFP primary antibodies that recognize eYFP (anti-GFP-mouse 1:2000, Millipore; or anti-GFP-chicken 1:4000, Aves) for 2 days at room temperature on an orbital shaker. Slices were then washed three times for 15 min each time in 0.1 M PBS, then incubated in blocking solution containing Alexa Fluor-conjugated species-specific secondary antibodies (anti-mouse-AF488 1:1000, Molecular Probes; anti-chicken-AF488 at 1:1000, Molecular Probes) and streptavidin (Alexa Fluor 555 at 1:1000, Invitrogen) for 2 h at room temperature on an orbital shaker. Slices were then washed three times in 0.1 M PBS, briefly rinsed in saline (9 g/L NaCl) containing DAPI, then mounted from 0.1 M PBS into fluorescent mounting media (DakoCyomation) onto glass slides and coverslipped. Slices were imaged using an upright Axio Imager (Zeiss) microscope (5x or 20x objective), equipped with Zen Software (Zeiss), and an ApoTome grid for optical fluorescence sectioning. Images were produced by flattening z-stacks to a maximum projection image using the Z Project function within Fiji (ImageJ, 1.47 g). Within-slide BALA mean fluorescence ratios were quantified using Fiji, with LA and BA ROI boundaries determined using the mouse brain atlas. Ranges across the rostro-caudal axis were dequantified using Fiji, with LA and BA ROI boundaries determined using the brain atlas. Ranges across the rostro-caudal axis were determined using the brain atlas.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY

All data are available in the main text or the Supplementary Materials.

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