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TITLE:

Electrophysiological Investigations of Retinogeniculate and Corticogeniculate Synapse Function

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SUMMARY:

Here, we present protocols for the preparation of acute brain slices containing the lateral geniculate nucleus and the electrophysiological investigation of retinogeniculate and corticogeniculate synapses function. This protocol provides an efficient way to study synapses with the high-release and low-release probability in the same acute brain slices.

ABSTRACT:

The lateral geniculate nucleus is the first relay station for the visual information. Relay neurons of this thalamic nucleus integrate input from retinal ganglion cells and project it to the visual cortex. In addition, relay neurons receive top-down excitation from the cortex. The two main excitatory inputs to the relay neurons differ in several aspects. Each relay neuron receives input from only a few retinogeniculate synapses, which are large terminals with many release sites. This is reflected by the comparably strong excitation, the relay neurons receive, from retinal ganglion cells. Corticogeniculate synapses, in contrast, are simpler with few release sites and weaker synaptic strength. The two synapses also differ in their synaptic short-term plasticity. Retinogeniculate synapses have a high release probability and consequently display a short-term depression. In contrast, corticogeniculate synapses have a low release probability. Corticogeniculate fibers traverse the reticular thalamic nuclei before entering the lateral geniculate nucleus. The different locations of the reticular thalamic nucleus (rostrally from the lateral geniculate nucleus) and optic tract (ventro-laterally from the lateral geniculate nucleus) allow stimulating corticogeniculate or retinogeniculate synapses separately with extracellular stimulation electrodes. This makes the lateral geniculate nucleus an ideal brain area where two excitatory synapses with very different properties impinging onto the same cell type, can be studied simultaneously. Here, we describe a method to investigate the recording from relay

neurons and to perform detailed analysis of the retinogeniculate and corticogeniculate synapse function in acute brain slices. The article contains a step-by-step protocol for the generation of acute brain slices of the lateral geniculate nucleus and steps for recording activity from relay neurons by stimulating the optic tract and corticogeniculate fibers separately.

INTRODUCTION:

Relay neurons of the lateral geniculate nucleus integrate and relay visual information to the visual cortex. These neurons receive excitatory input from ganglion cells via retinogeniculate synapses, which provide the main excitatory drive for relay neurons. In addition, relay neurons receive excitatory inputs from cortical neurons via corticogeniculate synapses. Moreover, relay neurons receive inhibitory inputs from local interneurons and GABAergic neurons of the nucleus reticularis thalami[1]. The nucleus reticularis thalami is present like a shield between thalamus and cortex such that fibers projecting from cortex to thalamus and in the opposite direction must go through the nucleus reticularis thalami[2].

Retinogeniculate inputs and corticogeniculate inputs display distinct synaptic properties[3-8]. Retinogeniculate inputs form large terminals with multiple release sites[9,10]. In contrast, corticogeniculate inputs display small terminals with single release sites[7]. In addition, retinogeniculate synapses efficiently drive action potentials of relay neurons despite constituting only 5−10% of all synapses on relay neurons[3,8,11]. Corticogeniculate synapses, on the other hand, serve as a modulator of retinogeniculate transmissions by controlling the membrane potential of relay neurons[12,13].

These two main excitatory inputs to relay neurons are also functionally different. One prominent difference is the short-term depression of retinogeniculate synapses and the short-term facilitation of corticogeniculate synapses[3,5,8]. Short-term plasticity refers to a phenomenon in which synaptic strength changes when the synapse is repeatedly active within a time period of few milliseconds to several seconds. Synaptic release probability is an important factor underlying short-term plasticity. Synapses, with a low initial release probability, display short-term facilitation due to the buildup of Ca^{2+} in the presynapse and consequently an increase in the release probability is observed upon repeated activity. In contrast, synapses with high release probability usually display short-term depression due to the depletion of ready-releasable vesicles[14]. In addition, desensitization of postsynaptic receptors contributes to the short-term plasticity in some high-release probability synapses[8,15]. High release probability and desensitization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors contribute to the prominent short-term depression of retinogeniculate synapses. In contrast, low-release probability underlies the short-term facilitation of corticogeniculate synapses.

In mice, the optic tract enters the dorsal lateral geniculate nucleus (dLGN) from the caudolateral site, whereas corticogeniculate fibers enter the dLGN rostroventrally. The distance between the two inputs allows for the investigation of the individual properties of two very different excitatory inputs impinging onto the same cell. Here, we build on and improve a previously described dissection method in which retinogeniculate and corticogeniculate fibers are preserved in acute brain slices[3]. We, then, describe the electrophysiological investigation of relay neurons and

stimulation of retinogeniculate and corticogeniculate fibers with extracellular stimulation electrodes. Finally, we provide a protocol for the filling of relay neurons with biocytin and subsequent anatomical analysis.

PROTOCOL:

All the experiments were approved by the Governmental Supervisory Panel on Animal Experiments of Rhineland-Palatinate.

1. Solutions

1.1. Dissection solution

1.1.1. To reduce excitotoxicity, prepare a choline-based solution to be used during the dissection as presented here (in mM): 87 NaCl, 2.5 KCl, 37.5 choline chloride, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, and 25 glucose. Prepare the dissection solution less than 1 week before the experiment.

1.2. Recording solution

1.2.1. Prepare an artificial cerebral spinal fluid (ACSF) solution containing (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO4, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 25 glucose. Add 50 µM D-APV and 10 µM SR 95531 hydrobromide to block N-methyl-D-aspartate (NMDA) and $GABA_A$ receptors, respectively.

1.3. Intracellular solution

1.3.1. Prepare an intracellular solution containing (in mM): 35 Cs-gluconate, 100 CsCl, 10 HEPES, 10 EGTA, and 0.1 D-600 (methoxyverapamil hydrochloride). Adjust the pH to 7.3 with CsOH. Filter, aliquot, and store the stock solution at -20 °C until use.

2. Dissection

2.1. Prepare two slice chambers, of which one is filled with 100 mL of the dissection solution and the other with 100 mL of recording solution. Place the two beakers into a water bath (37 °C) and bubble the solutions with carbogen for at least 15 min before the dissection.

2.2. Fill a plastic beaker with 250 mL of near ice-cold (but not frozen) dissection solution. Bubble with carbogen at least 15 min before use.

2.3. Fill a plastic Petri dish (100 mm x 20 mm), in which the brain dissection will be performed, with the cold dissection solution. Place a piece of filter paper into the Petri dish.

2.4. Cool down the dissection chamber of the vibratome.

2.5. Anesthetize a mouse with 2.5% isoflurane, e.g., in the mouse cage. As soon as the mouse does not respond to a tail pinch, decapitate the mouse near the cervical medulla and immerse the head into the ice-cold dissection solution in the base of the Petri dish.

2.6. Cut the skin of the head from caudal to nasal and keep it laterally with fingers to expose the skull. To take out the forebrain, first cut the skull along with the posterior-anterior midline, and then cut two more times through the coronal suture and lambdoid suture (**Figure 1A**).

2.7. Remove the skull between the coronal suture and lambdoid suture such that the cerebrum is exposed. Cut the olfactory bulb and separate the forebrain from the midbrain with a fine blade (**Figure 1B**). Remove the brain from the skull with a thin spatula and place it onto the filter paper in the lid of the Petri dish.

NOTE: Steps 2.6 and 2.7 should be performed as fast as possible to reduce cell death.

2.8. To preserve the integrity of the sensory and cortical inputs to the dLGN in the brain slice, separate the two hemispheres with a parasagittal cut with an angle of 3–5° (**Figure 1C,D**). Dry the mediolateral planes of the two hemispheres by placing them on the filter paper and then glue them onto the cutting stage with an angle of 10–25° from the horizontal plane (**Figure 1E**).

2.9. Place the stage in the center of the metal buffer tray and gently pour the rest of the ice-cold dissection solution into the buffer tray. Perform this step carefully since a strong pour might remove the pasted brain (**Figure 1F**).

2.10. Place the buffer tray into the ice-filled tray, which helps to maintain the low temperature during dissection. Cut 250 µm slices with a razor blade at a speed of 0.1 mm/s and an amplitude of 1 mm.

2.11. Keep slices in the holding chamber filled with oxygenated dissection solution at 34 °C for around 30 min and then allow the slices to recover in the recording solution at 34 °C for another 30 min.

2.12. After recovery, remove the slice holding chamber from the water bath and keep slices at room temperature (RT) until used in experiments.

3. Electrophysiology

3.1. Pull pipettes using borosilicate glass capillaries and a filament puller. Maintain the resistance of recording pipettes at 3–4 MΩ. Pull stimulating pipettes using the same protocol but break the tip slightly after pulling to increase the diameter. Fill recording pipettes with the intracellular solution and biocytin, while fill stimulating pipettes with the recording solution.

3.2. Place the slices into the recording chamber and continuously perfuse the slices with oxygenated recording solution at RT. Check all the slices and select the ones that display an intact optic tract.

3.3. Visualize the slice and cells with an upright microscope equipped with infrared differential interference contrast (IR-DIC) video microscopy.

NOTE: Relay neurons are differentiated from interneurons by their larger soma size and more primary dendrites (branches emerging from the soma). Interneurons display bipolar morphology with a smaller soma.

3.4. Place the stimulating pipette onto the slice before patching the cell with the recording pipette. To investigate retinogeniculate synapses, place the stimulating pipette directly onto the optic tract, where the axon fibers from retinal ganglion cells are bundled (**Figure 2A**). To analyze corticogeniculate synapses, place the stimulating electrode on the nucleus reticularis thalami, which is rostroventrally adjacent to the dLGN (**Figure 2B**).

3.5. Once the recording pipette is immersed into the recording solution, apply a 5 mV voltage step to monitor the pipette resistance. Set the holding potential to 0 mV and cancel the offset potential so that the holding current is 0 pA.

3.6. Approach the cell with the recording pipette while applying positive pressure. When the pipette is in direct contact to the cell membrane, release the positive pressure, and set the holding potential to -70 mV. Apply a slight negative pressure to allow the cell membrane to attach to the glass pipette such that a gigaohm seal forms (resistance >1 GΩ). Compensate the pipette capacitance and open the cell by application of negative pressure pulses.

3.7. To investigate the synaptic function, apply 0.1 ms current pulses (ca. 30 µA) via the stimulation pipette. If no synaptic responses are observed, the stimulating pipettes may have to be replaced. Be careful during placing the stimulating pipettes to a different position such that the recorded cell is not lost.

NOTE: In the example recordings shown in **Figure 2**, synaptic short-term plasticity was investigated by stimulating the optic tract or corticogeniculate fibers twice with 30 ms inter-stimulus intervals. The paired-pulse ratio (PPR) was calculated by dividing the amplitude of the second excitatory postsynaptic current (EPSC) by that of the first EPSC ($EPSC_2/EPSC_1$). Each stimulation protocol was repeated 20 times. The EPSC amplitude was measured from average currents of the 20 sweeps. The time interval between each repetition was least 5 s to avoid short-term plasticity induced by the repetitions.

3.8. Monitor the series resistance continuously by applying a 5-mV voltage step. Only use the cells with the series resistance smaller than 20 MΩ for analysis.

4. Biocytin labeling

4.1. Maintain the whole-cell configuration for at least 5 min to allow the diffusion of biocytin into the distal dendrites.

4.2. After recording, gently remove the pipette from the cell so that the soma is not destroyed.

NOTE: If more than one cell is recorded on one slice, their somas should be sufficiently away from their neighboring cells. Ensure that the dendrites from different cells do not interfere with each other during imaging.

4.3. Prepare a 24-well cell culture plate. Fill each well with 300 µL of 4% paraformaldehyde (PFA). Take care to not contaminate anything with PFA that will be in contact with the slices to be recorded.

4.4. Transfer the slices from the recording chamber to the PFA-containing plate with a rubber pipette and fix the slices overnight. Ensure that the pipette is always prevented from PFA contamination.

CAUTION: Always wear gloves and use a chemical hood when manipulating PFA.

4.5. After fixing slices overnight in PFA, replace PFA with phosphate-buffered saline (PBS). Store the slices in PBS at 4 °C for future processing (less than 2 weeks).

4.6. Wash the slices with fresh PBS 3 times (10 min each wash).

4.7. Block nonspecific binding sites by incubating the slices in the blocking solution (0.2% non-ionic surfactant and 5% bovine serum albumin in PBS) for 2 h at RT on an orbital shaker.

4.8. Discard the blocking solution. Incubate the slices with streptavidin-Alexa 568 diluted into the blocking solution (1:1000) at 4 °C overnight on an orbital shaker.

4.9. Discard the antibody solution and wash slices 3 times with PBS for 10 min at RT on an orbital shaker. Wash the slices with tap water before mounting.

4.10. Put the slices from one mouse on one glass slide. Ensure that the stained cells are present on the top surface of the slices. Absorb the water around the slices with tissues and then leave the slices to dry for approximately 15 min.

4.11. Apply two drops of mounting medium on each slice. Mount a coverslip on the slide without producing air bubbles.

4.12. Store the labeled slices at 4 °C after visualization with a confocal microscope.

5. Cellular imaging and reconstruction

5.1. Scan slices with a confocal microscope and use the associated software for analysis.

5.2. Excite the fluorescence at 561 nm.

5.3. Image the slices with 0.7 numerical aperture (NA), oil-immersion objective.

5.4. Adjust the targeted cell in the middle of the view and apply a 2.5-times magnification.

5.5. Render the Z-stack datasets to scan the whole neuron with the following parameters: format = 2550 x 2550 pixels, scan frequency = 400 Hz, z number = 40. Voxel size was around 0.1211 x 0.1211 x 1.8463 µm3.

5.6. Semi-automatically trace the neurons using a neuronal reconstruction software (e.g., NeuronStudio). An example of a 3D traced relay neuron is shown in **Figure 3B**.

REPRESENTATIVE RESULTS:

The slice preparation of dLGN containing the retinogeniculate and corticogeniculate pathways is shown under a 4x objective (**Figure 2**). Axons of retinal ganglion cells bundle together in the optic tract (**Figure 2**). The stimulating pipette was placed on the optic tract to induce retinogeniculate synapse-mediated current (**Figure 2A**) and on nucleus reticularis thalami to induce corticogeniculate synapses-mediated current (**Figure 2B**), respectively. Of note, axons from cortical neurons to LGN neurons projects from the cortex to thalamus traverse the nucleus reticularis thalami. Corticogenicluate synapses can, therefore, be activated by stimulation in the nucleus reticularis thalami.

The paired-pulse ratio of AMPA receptor-mediated EPSCs is below 1 when stimulating the optic tract with 30 ms inter-stimulus interval, but above 1 when stimulating corticogeniculate fibers with the same inter-stimulus interval (**Figure 2C,D**). The paired-pulse depression of retinogeniculate synapses is due to a high vesicle release probability and desensitization of postsynaptic AMPA receptors. The paired-pulse facilitation of corticogeniculate synapses is consistent with a low vesicle release probability[16].

Figure 3A shows IR-DIC image showing the soma of a dLGN relay neuron along with the tip of the patch pipette. Biocytin staining enables us to gain a view of the recorded neuron. Different from interneurons, which have bipolar morphology, relay neurons have multipolar dendritic arbors (**Figure 3B**) containing more than 3 primary dendrites[8,17]. Three-dimensional (3D) reconstruction of a biocytin-labeled relay neuron was then generated which shows a radially symmetric dendritic architecture (**Figure 3C**).

FIGURE LEGENDS:

Figure 1: Schemes representing the dissection protocol. (**A**) Horizontal view of the mouse skull, showing the position of the initial cuts. The first cut was performed along with the sagittal suture,

followed by another two cuts along with the coronal suture and lambdoid suture, respectively. Dashed lines represent the incisions. (**B**) Sagittal view of the whole brain. Dashed lines indicate that the cerebrum is isolated from the olfactory bulb and midbrain. (**C**-**D**) These panels show the first cutting angle to separate the two hemispheres from horizontal (**C**) and coronal (**D**) view, respectively. (**E**) Coronal view of one hemisphere on the cutting stage. Dashed lines indicate the slicing direction. l, m, d, and v represent the lateral, medial, dorsal and ventral aspects, respectively, for the hemisphere in panels D and E. (**F**) Diagram of the dissection chamber with two hemispheres on the cutting stage. The dashed arrow indicates the moving direction of the cutting blade. a, p, and d represent the anterior, posterior, and dorsal aspects of the left hemisphere, respectively. (**G**) Scheme representing a properly cut slice with a relatively large part of the dLGN and intact preservation of the optic tract. dLGN, dorsal lateral geniculate nucleus; vLGN, ventral lateral nucleus; OT, olfactory bulb; NRT, nucleus reticularis thalami. Panels C, D and G have been modified from **Figure 1** of Turner and Salt[3].

Figure 2: Slice preparation of dLGN containing the retinogeniculate and corticogeniculate pathways and example recordings. (**A**-**B**) These panels show a dLGN slice with preservation of retinogeniculate and corticogeniculate inputs. The stimulation electrode was placed on the optic tract to activate retinogeniculate synapses (**A**) and on the nucleus reticularis thalami to activate corticogeniculate synapses (**B**). (**C**) AMPA receptor-mediated EPSCs in response to stimulation of the optic tract twice with 30 ms inter-stimulus interval. (**D**) Corticogeniculate synapse-mediated currents with 30 ms inter-stimulus interval. The stimulation artifacts were removed for clarity.

Figure 3: Morphological analysis of a relay neuron. (**A**) IR-DIC image showing the soma of a dLGN relay neuron with the tip of the patch pipette. (**B**) Confocal image of a relay neuron labeled with biocytin and visualized with streptavidin-Alexa 568. (**C**) Three-dimensional (3D) reconstruction of the same neuron, showing a radially symmetric dendritic architecture.

DISCUSSION:

We describe an improved protocol based on a previously published method[3], which allows for the investigation of the high probability of release retinogeniculate synapses and low probability of release corticogeniculate synapses from the same slice. This is of great importance since these two inputs interact with each other to modulate the visual signal transmission: retinogeniculate inputs are the main excitatory drive of relay neurons, whereas corticothalamic inputs function as a modulator, which influences the gain of retinogeniculate transmission by affecting the state of relay neurons. As expected, we observed that retinogeniculate and corticogeniculate synapses display different short-term plasticity due to the difference in their release probabilities. Additionally, AMPA receptor desensitization also contributes to the strong depression in retinogeniculate synapses[9,15,16]. We have recently shown that short-term depression is modulated by CKAMP44[16], an AMPA receptor auxiliary protein of the CKAMP family that slows the recovery from desensitization of AMPA receptors[18-20]. Furthermore, retinogeniculate synapse-mediated current amplitudes are higher than those of corticogeniculate synapse-mediated currents under the same stimulating strength, consistent with the large retinogeniculate synapses with multiple releasing sites, and relatively small corticogeniculate synapses[7,21].

The slicing protocol is essentially the same as the one developed by Turner and Salt[3]. Some modifications that were based on our experience improved slice quality. Similar to Hauser et al.[22], we used a choline-based instead of a sucrose-based dissection solution. Recovery after dissection was done at 34 °C in the cutting solution for 30 min and then in the recording solution for another 30 min and not at room temperature. Slices were slightly thinner (250 µm instead of 400 µm). We dissected brains using a wedge with an angle of 10−25°, which facilitates and increases the reliability of the slicing procedure.

Recording and stimulation conditions for the investigation of relay neuron synapse function are essentially the same as previously described by Chen and Regehr[8]. Activation of optic tract fibers usually elicits large currents and can be in the range of several nA when all retinogeniculate synapses on one relay neurons are activated[23]. To prevent series resistance errors, especially when investigating the maximal current by activating all retinogeniculate synapses on one relay neurons, Chen and Regehr used recording low resistance pipettes (<2 MΩ). However, to investigate synaptic short-term plasticity, it suffices to activate one or few retinal ganglion cell axons, which elicits currents that are in the range of 34−579 pA (unpublished results, Chen et al.). Smaller pipettes with a resistance between 3−4 MΩ can, therefore, be used when investigating retinogeniculate synapse function using weak stimulation of the optic tract. Activation of corticogeniculate synapses elicits relatively small currents[16]. Series resistance errors are, therefore, a smaller problem when investigating corticogeniculate synapse function.

We here show that stimulation of retinogeniculate and corticogeniculate synapses can be used to investigate short-term plasticity of AMPA receptor-mediated currents. The same protocol can be applied to record NMDA receptor-mediated current[24]. In fact, one of the first indications that AMPA receptor desensitization contributes to the short-term plasticity in retinogeniculate synapses came from experiments in which paired-pulse ratios of AMPA receptor-mediated currents were compared with paired-pulse ratios of NMDA receptor-mediated currents. Corticogeniculate synapses were stimulated by positioning the stimulation pipette in the nucleus reticularis thalami because axons of cortical neurons that excite dLGN relay neurons travel across this thalamic nucleus. The same stimulation position can be used to study inhibitory synaptic currents in the dLGN as shown for example by Govindaiah and Cox[25].

Relay neurons express $GABA_A$ and $GABA_B$ receptors. The release of GABA activates synaptic $GABA_A$ receptors. $GABA_B$ receptors, which are extrasynaptically localized, are activated when nucleus reticularis thalami are strongly activated for example during burst firing[26-28]. Spillover of GABA, which is usually restricted due to GABA uptake by astrocytes, can activate during intense synapse activation the extrasynaptic $GABA_B$ receptors[27,28]. We did not block $GABA_B$ receptors because stimulation intensity and frequency were comparably low. However, when investigating corticogeniculate synapse function using intense stimulation protocols it might be preferable to add $GABA_B$ receptors.

Recordings of dLGN relay neurons can readily be performed when using slices that are approximately 2 mm medial from the lateral surface of the brain (start collecting approximately

the 8th of the 250 µm slices during the cutting procedure). In some slices, not both inputs to a given relay neuron can be detected because one of the two pathways are severed close to the relay neuron. In this case, it is usually still possible to investigate retinogeniculate and corticogeniculate synapses in the same slice by selecting different relay neurons for each input. To record retinogeniculate synapse mediated current, ventrally located relay neurons are selected since the optic tract fibers to these cells are more likely preserved than in the dorsally located relay neurons. In contrast, corticogeniculate inputs are more likely preserved onto relay neurons that are located dorsally in the dLGN.

Slice quality determines the reliability of the data. Based on our experience, the following parameters are essential to ensure good slice quality. Cooling and oxygenation are important factors during the dissection procedure. The head of the mouse must be kept in cold solution immediately after the decapitation of the mouse. When taking the brain out of the skull, it should be immersed in ice-cold solution every 3 to 4 s in order to reduce cell death. Similarly, the slicing procedure should be performed at around 4 °C. Moreover, dissection solution and recording solution must be oxygenated at least 15 min before use to maintain sufficient oxygen concentration. In addition, blocking sodium channels can efficiently reduce the neuron activity. Therefore, a dissection solution containing 37.5 mmol choline was used.

Relay neurons display low-threshold spikes, which are generated by the transient Ca^{2+} current[29]. To block voltage gated Ca^{2+}-channels, we added D-600 (methoxyverapamil) to the intracellular solution. D-600 should not be used if the purpose of the study is to investigate low-threshold spikes in relay neurons. The precision of whole-cell voltage clamp recordings is affected by space clamp errors. This technical problem is more relevant for inputs that are far away from the recording electrode (thus for distal synapses)[30,31]. To minimize the impact of the space clamp error, we used a Cs^+-based instead of a K^+-based intracellular solution. Channels that are K^+ permeable (e.g., leak channels) are usually Cs^+ impermeable[32]. Thus, the use of a Cs^+-based intracellular solution increases the impedance of the cell.

Stable patch clamp recording depends on the selection of healthy cells. Neurons with round soma shape, dark color or swollen soma should be avoided. Furthermore, relay neurons are distinguished from the local interneurons by their larger soma size and more elaborate primary dendritic arbors. Therefore, we selected cells that have bigger soma size (bigger than 15 µm in diameter) and non-bipolar morphology. The example cell shown in **Figure 3B** has a symmetrical dendritic architecture and can, therefore, be classified as relay neuron resembling Y-neurons that are for example found in the cat LGN[17].

Series resistance should be as small as possible and maintained constant. Moreover, since the inputs from two directions are preserved in one slice, it is important to get rid of the interference between the two inputs. To avoid activating corticogeniculate fibers when recording retinogeniculate synapse-mediated currents or vice versa, the stimulating pipette should not be placed into the dLGN and the distance between stimulating and recording pipette should be as far as possible. In addition, the long distance between stimulating and recording pipettes requires relatively intact axon preservation. Therefore, a proper cutting angle must be selected for the

dissection. In general, only one or two slices with preservation of two inputs can be obtained from each hemisphere.

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1 Guido, W. Development, form, and function of the mouse visual thalamus. *Journal of Neurophysiology.* **120**, 211-225, doi:10.1152/jn.00651.2017 (2018).

2 Guillery, R. W., Feig, S. L., Lozsadi, D. A. Paying attention to the thalamic reticular nucleus. *Trends in Neurosciences.* **21**, 28-32 (1998).

3 Turner, J. P., Salt, T. E. Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones in vitro. *The Journal of Physiology.* **510 (3)**, 829-843 (1998).

4 Lindstrom, S., Wrobel, A. Frequency dependent corticofugal excitation of principal cells in the cat's dorsal lateral geniculate nucleus. *Experimental Brain Research.* **79**, 313-318 (1990).

5 Granseth, B., Ahlstrand, E., Lindstrom, S. Paired pulse facilitation of corticogeniculate EPSCs in the dorsal lateral geniculate nucleus of the rat investigated in vitro. *The Journal of Physiology.* **544**, 477-486 (2002).

6 Hamos, J. E., Van Horn, S. C., Raczkowski, D., Uhlrich, D. J., Sherman, S. M. Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat. *Nature.* **317**, 618-621 (1985).

7 Kielland, A. et al. Activity patterns govern synapse-specific AMPA receptor trafficking between deliverable and synaptic pools. *Neuron.* **62**, 84-101, doi:10.1016/j.neuron.2009.03.001 (2009).

8 Chen, C., Regehr, W. G. Developmental remodeling of the retinogeniculate synapse. *Neuron.* **28**, 955-966 (2000).

9 Budisantoso, T., Matsui, K., Kamasawa, N., Fukazawa, Y., Shigemoto, R. Mechanisms underlying signal filtering at a multisynapse contact. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience.* **32**, 2357-2376, doi:10.1523/JNEUROSCI.5243-11.2012 (2012).

10 Morgan, J. L., Berger, D. R., Wetzel, A. W., Lichtman, J. W. The Fuzzy Logic of Network Connectivity in Mouse Visual Thalamus. *Cell.* **165**, 192-206, doi:10.1016/j.cell.2016.02.033 (2016).

11 Usrey, W. M., Reppas, J. B., Reid, R. C. Paired-spike interactions and synaptic efficacy of retinal inputs to the thalamus. *Nature.* **395**, 384-387, doi:10.1038/26487 (1998).

12 Steriade, M., Jones, E. G., McCormick, D. A. *Thalamus*. (Elsevier, 1997).

13 Wang, W., Jones, H. E., Andolina, I. M., Salt, T. E., Sillito, A. M. Functional alignment of feedback effects from visual cortex to thalamus. *Nature Neuroscience.* **9**, 1330-1336, doi:10.1038/nn1768 (2006).

14 Zucker, R. S., Regehr, W. G. Short-term synaptic plasticity. *Annual Review of Physiology.* **64**, 355-405, doi:10.1146/annurev.physiol.64.092501.114547 (2002).
15 Chen, C., Blitz, D. M., Regehr, W. G. Contributions of receptor desensitization and saturation to plasticity at the retinogeniculate synapse. *Neuron.* **33**, 779-788 (2002).
16 Chen, X., Aslam, M., Gollisch, T., Allen, K., von Engelhardt, J. CKAMP44 modulates integration of visual inputs in the lateral geniculate nucleus. *Nature Communications.* **9**, 261, doi:10.1038/s41467-017-02415-1 (2018).
17 Krahe, T. E., El-Danaf, R. N., Dilger, E. K., Henderson, S. C., Guido, W. Morphologically distinct classes of relay cells exhibit regional preferences in the dorsal lateral geniculate nucleus of the mouse. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience.* **31**, 17437-17448, doi:10.1523/JNEUROSCI.4370-11.2011 (2011).
18 von Engelhardt, J. et al. CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. *Science.* **327**, 1518-1522, doi:10.1126/science.1184178 (2010).
19 Khodosevich, K. et al. Coexpressed auxiliary subunits exhibit distinct modulatory profiles on AMPA receptor function. *Neuron.* **83**, 601-615, doi:10.1016/j.neuron.2014.07.004 (2014).
20 Farrow, P. et al. Auxiliary subunits of the CKAMP family differentially modulate AMPA receptor properties. *eLife.* **4**, e09693, doi:10.7554/eLife.09693 (2015).
21 Rafols, J. A., Valverde, F. The structure of the dorsal lateral geniculate nucleus in the mouse. A Golgi and electron microscopic study. *The Journal of Comparative Neurology* **150**, 303-332, doi:10.1002/cne.901500305 (1973).
22 Hauser, J. L., Liu, X., Litvina, E. Y., Chen, C. Prolonged synaptic currents increase relay neuron firing at the developing retinogeniculate synapse. *Journal of Neurophysiology.* **112**, 1714-1728, doi:10.1152/jn.00451.2014 (2014)
23 Hooks, B. M., Chen, C. Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse. *Neuron.* **52**, 281-291, doi:10.1016/j.neuron.2006.07.007 (2006).
24 Liu, X., Chen, C. Different roles for AMPA and NMDA receptors in transmission at the immature retinogeniculate synapse. *Journal of Neurophysiology.* **99**, 629-643, doi:10.1152/jn.01171.2007 (2008).
25 Govindaiah, G., Cox, C. L. Metabotropic glutamate receptors differentially regulate GABAergic inhibition in thalamus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience.* **26**, 13443-13453, doi:10.1523/JNEUROSCI.3578-06.2006 (2006).
26 Fogerson, P. M., Huguenard, J. R. Tapping the Brakes: Cellular and Synaptic Mechanisms that Regulate Thalamic Oscillations. *Neuron.* **92**, 687-704, doi:10.1016/j.neuron.2016.10.024 (2016).
27 Jacobsen, R. B., Ulrich, D., Huguenard, J. R. GABA(B) and NMDA receptors contribute to spindle-like oscillations in rat thalamus in vitro. *Journal of Neurophysiology.* **86**, 1365-1375, doi:10.1152/jn.2001.86.3.1365 (2001).
28 Kulik, A. et al. Distinct localization of GABA(B) receptors relative to synaptic sites in the rat cerebellum and ventrobasal thalamus. *The European Journal of Neuroscience.* **15**, 291-307 (2002).
29 Gutierrez, C., Cox, C. L., Rinzel, J., Sherman, S. M. Dynamics of low-threshold spike activation in relay neurons of the cat lateral geniculate nucleus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience.* **21**, 1022-1032 (2001).

30 Armstrong, C. M., Gilly, W. F. Access resistance and space clamp problems associated with whole-cell patch clamping. *Methods in Enzymology.* **207**, 100-122 (1992).

31 White, J. A., Sekar, N. S., Kay, A. R. Errors in persistent inward currents generated by space-clamp errors: a modeling study. *Journal of Neurophysiology.* **73**, 2369-2377, doi:10.1152/jn.1995.73.6.2369 (1995).

32 Clay, J. R., Shlesinger, M. F. Analysis of the effects of cesium ions on potassium channel currents in biological membranes. *Journal of Theoretical Biology.* **107**, 189-201 (1984).

Figure 1

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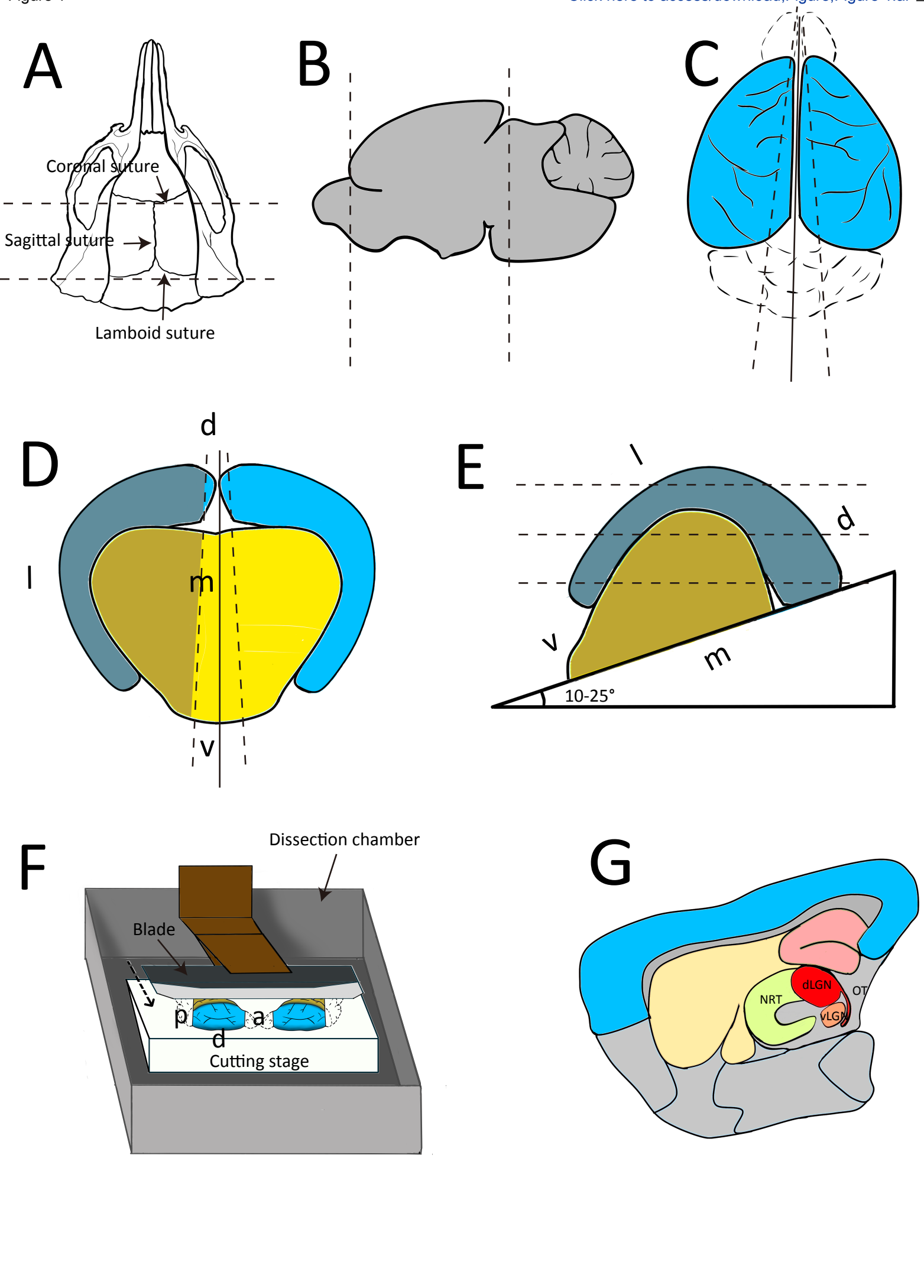
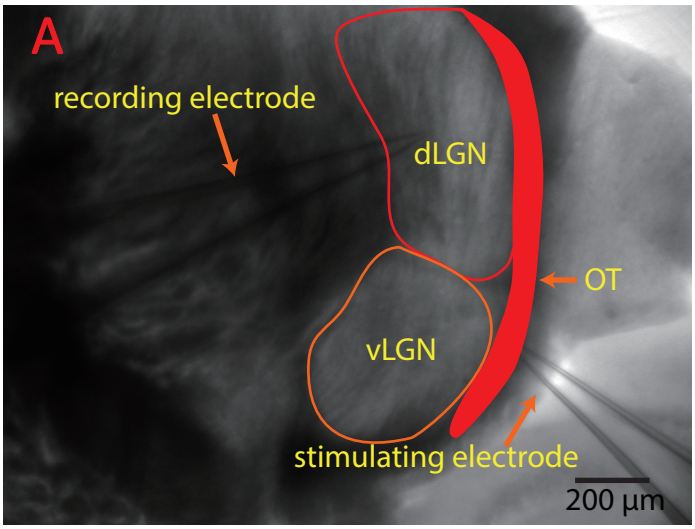
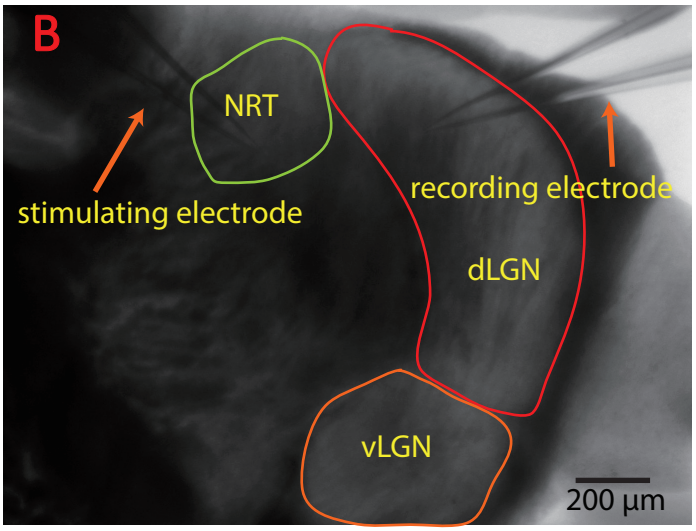
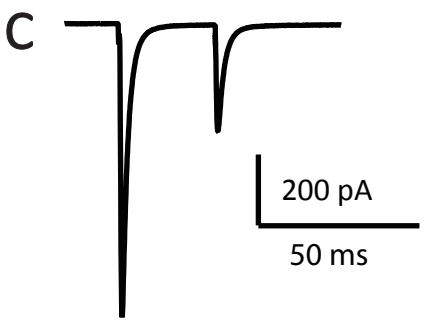


Figure 2







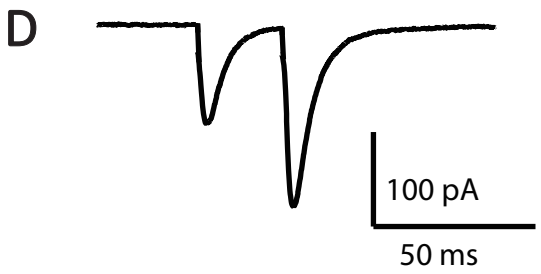
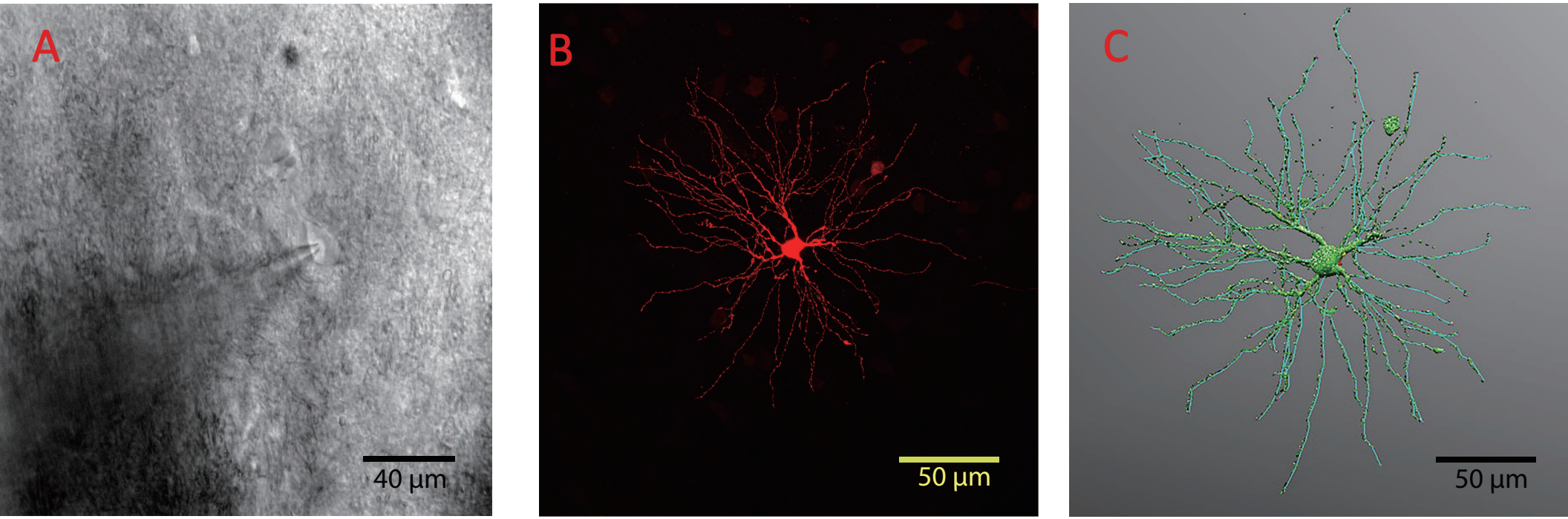


Figure 3

Click here to access/download;Figure;Figure 3.eps



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
		EPC 10 USB	
	HEKA	Double patch	
Amplifier	Elektronik	clamp amplifier	
Biocytin	Sigma-Aldrich	B4261-250MG	
$CaCl_2$	EMSURE	1.02382.1000	
choline chloride	Sigma-Aldrich	C1879-1KG	
	Leica		
Confocal Laser Scanning Microscop	Microsystems	TCS SP5	
CsCl	EMSURE	1.02038.0100	
Cs-gluconate	Self-prepared		Since there was no commercial Cs-gluconate, we prepared it by ours
D-600	Sigma-Aldrich	M5644-50MG	methoxyverapamil hydrochloride
D-APV	Biotrend	BN0085-100	NMDA-receptor antagonist
Digital camera for microscope	Olympus	XM10	
EGTA	SERVA	11290.02	
Forene	Abbvie	2594.00.00	isoflurane
Glucose	Sigma-Aldrich	49159-1KG	
HEPES	ROTH	9105.2	
	World Precision		
High Current Stimulus Isolator	Instruments	A385	
KCl	EMSURE	1.04936.1000	
$MgCl_2$	EMSURE	1.05833.0250	
	Luigs &		
Micromanipulators	Neumann	SM7	
Miroscope	Olympus	BX51	
	ThermoFisher		
mounting medium	Scientific	P36930	Prolong Gold Invitrogen
NaCl	ROTH	3957.1	

NaH_2PO_4	EMSURE	1.06346.1000	
$NaHCO_3$	EMSURE	1.06329.1000	
Pipette	Hilgenberg	1807502	
Puller	Sutter	P-1000	
razor blade	Personna	60-0138	
Semiautomatic Vibratome	Leica Biosystems	VT1200S	
SR 95531 hydrobromide	Biotrend	AOB5680-10	GABAA-receptor antagonist

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Author(s): Xufeng Chen, Danni Wang, Jakob von Engelhardt

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Institution:	University Medical Center Mainz
Title:	Professor Dr.
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Dear Dr. Steindel,

In your mail you asked us to address the editorial and peer review comments and to send a revised version of our manuscript. Below you find the point-by-point responses to your and to the reviewer's comments

We thank the reviewers and you for your time and effort, and we hope that the new version will find the reviewers' approval.

Response to editor

1. 2.5: What concentration of isoflurane is used here?

We added the information of isoflurane concentration (2.5%) in line 111.

Figures:

1. Figure 2: Please include a scale bar in A and B. Please also adjust the font of '100 pA' in panel C to match with panel D.

We added the scale bar in Figure 2 A and B. We also adjusted the font of '100 pA' in panel C.

Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

We added the information of all materials and equipment in the file of table of materials.

Response to referees

We would like to thank both Reviewers for their critical comments and thoughtful suggestions, since these comments lead to an essential improvement of the manuscript. We elaborated the introduction and discussion to address both Reviewers' concerns.

Point-by-point response:

Reviewer #1:

Manuscript Summary:

The lateral geniculate nucleus (LGN) with its bottom up and top down inputs is a very interesting structure to study the corticofugal modulation of sensory inputs. This is well described in the abstract of the manuscript.

Major Concerns:

It will not be clear to the readers why stimulation at the location of the NRT is a useful approach to activate corticofugal fibers projecting to the LGN. Many may know about the NRT-LGN circuitry. Therefore (a) the connectivity between VC - NRT - LGN should be more precisely addressed.

We thank Reviewer 1 for the pertinent suggestion about a precise introduction of VC - NRT – LGN circuit. Indeed, many readers might not know that cortiogeniculate fibers also pass NRT, and it is therefore peculiar for them why we positioned the stimulation pipette in the NRT to stimulate corticogeniculate synapses. To avoid this ambiguity, we described the neuronal circuit of VC - NRT – LGN in the introduction of the manuscript (Line 47-54). In the same context, as suggested by Reviewer 2, the same preparation can also be used to study inhibitory input from NRT to thalamus. This point was discussed in line 305-308.

In this context (b) the rationale behind the application of the GABAa-Blocker should be made clear: the main impact of NRT on LGN is mediated via GABAa receptors but there is also a small conribution of GABAb - is it negligible? Useful additional references would be for a) Guido et al., J Neurophysiol 120: 211-225, 2018 and for b) Fogerson and Huguenard, Neuron 92: 687-7054, 2016

Indeed, $GABA_A$ receptors and $GABA_B$ receptors exist in relay neurons. However, $GABA_B$ receptors are mainly located extrasynaptically, which are activated only by strong NRT neuron firing (Jacobsen et al. 2001; Kulik, A. et al. 2002). However, in our protocol, stimulation was applied every 5 seconds at relatively low intensity, which should not be able to activate $GABA_B$ receptors. Therefore, we think that the influence of $GABA_B$ receptors can be negligible. This issue was discussed in line 309-316.

Minor Concerns:

There are no further concerns.

Reviewer #2:

We very much appreciate Reviewer 2's detailed evaluation and criticisms. Reviewer 2 correctly pointed out several factual errors and typos we made. We are grateful and corrected all of them. Moreover, we replenished the citations as advised by him/her. In addition, we also agree with his/her suggestion that the difference between previously published protocol and ours should be addressed.

The authors should clearly state in the introduction or discussion how their modified method differs from the Turner and Salt description of the preparation. Aside from using a wedge platform, is anything else altered in the cutting protocol? They should also

mention in the legend of Figure 1 that panels C,D and F are modified from Figure 1 of Turner and Salt 1998.

Our protocol for the dLGN preparation is indeed a modified version of the one established by Turner and Salt in 1998. In the new manuscript (line 280-286) we discussed modifications such as in cutting solution, mounting of hemispheres for cutting, slice thickness. We now cited Turner and Salt 1998 in Figure 1.

Similarly, they should describe how their method of patch clamp recordings and stimulation of the retinogeniculate synapse differs from the methods described by Chen and Regehr 2001.

We indeed used a similar stimulation protocol as Chen and Regehr. The main difference is the pipette resistance. To reduce series resistance error, they used pipettes with resistances below 2 MΩ. Very low resistance pipettes were not required for an investigation of short-term plasticity of retinogeniculate and corticogeniculate synapses, as it is usually possible to elicit EPSCs with relatively small amplitudes (34-579 pA, our observation) with recruitment of few axons. Very low resistance pipettes would certainly be necessary for an investigation of e.g. the maximal AMPA receptor current amplitude that a relay neuron receives and the quantification of the fiber fraction (as done for example be by Chen and Regehr). This issue was discussed in line 287-298.

Major Concerns:

Factual errors

Line 28 of Abstract: Retinal ganglion cells receive in addition top-down excitation from the cortex.: Should be "relay neurons" instead of "Retinal ganglion cells"

"Retinal ganglion cells" was changed to "Relay neurons" (see line 26).

Line 270: "retinogeniculate synapse-mediated current amplitudes are higher than that of retinogeniculate synapse-mediated currents under same stimulating strength, consistent with the large": The second "retinogeniculate" should be "corticogeniculate" synapse

"retinogeniculate" was changed to "corticogeniculate"(see line 277).

Citations:

Line 50: "the two inputs…" needs citation which should include: Turner and Salt 1998 among others including studies in cat and other species.

We now cited Turner and Salt, 1998; Chen and Regehr, 2000; Lindstrom and Wrobel, 1990; Granseth et al., 2002; Hamos et al.,1985; Kielland et al.,2009 (see line 55).

Line 53: this first sentence needs citations which should include: Budisantoso et al, 2012 and Morgan et al, 2016 Cell 165:192

We now cited Budisantoso et al., 2012 and Morgan et al., 2016 (see line 51).

Line 56: Corticogeniculate synapses… needs citations

We now cited Steriade, Jones, and McCormick, 1997 and Wang et al., 2006 (see line 56).

Line 60: One prominent difference…": needs citations which should include Turner and Salt 1998

We now cited Turner and Salt, 1998; Chen and Regehr, 2000; and Granseth et al., 2002 (see line 64).

Line 68: In addition, desensitization… : citation #2 never touches upon AMPAR desensitization, cite Chen, Blitz and Regehr, 2002.

We now referenced the correct paper: Chen, Blitz and Regehr, 2002 (see line 72).

Line 267: "the strong depression in retinogeniculate synapse": Chen, Blitz and Regehr 2002 should be cited.

We now cited Chen, Blitz and Regehr, 2002 (see line 274).

Clarification needed:

Cutting Methods:

1. Specify what type of cutting blade you use

We used razor blade for dissection. The information has been added in line 132.

2. Are the two hemispheres mounted on the same wedge? If so, it would be helpful to diagram the arrangement of the two hemispheres on the wedge.

We indeed mounted two hemispheres on the same wedge, and we agree that it is helpful to present such procedure. The additional figure diagramming two hemispheres was shown in Figure 2.

Electrophysiology

1. For the retinogeniculate synapse, recording pipette resistances of 3-4 MOhm are too high. Since the synaptic currents can range in the 10's of nA in amplitude, the pipet resistance should be less than 2 MOhms to avoid significant series resistance error.

See above our response to comment 2 of reviewer 2. An explanation of when low resistance pipettes are necessary has been added.

2. Figure 2C, D: where are the stimulus artifacts? The example trace of retinogeniculate AMPAR currents shows a PPR that is significantly higher than previously published studies, including the last author's work. A more appropriate trace should be used.

Stimulus artifacts are blanked for clarity, and we agree that the example trace here is less representative. A better example trace is now shown in Figure 3

3. While the authors record only from AMPAR from this prep, it should be noted that the prep can be used to study NMDAR as well as inhibitory synaptic currents

We discussed this point in line 299-305.

Figure legends:

Figure 1: A. Please label sagittal, coronal and lamboid sutures

sagittal, coronal and lamboid sutures were labeled as shown in Figure 1A

B. this is not a horizontal view, it is a sagittal view.

"horizontal" was changed to "sagittal"

D. Would be helpful to also label lateral, medial, ventral, dorsal to match with E.

"lateral", "medial", "ventral", "dorsal" were labeled in Figure 1D

E. label angle of the wedge with 10-25 degrees

The angle of the wedge (10-25 degrees) was labeled in Figure 1E

Text:

Line 36: "The optic tract enters the lateral geniculate distant from the cortical input.", this sentence is not clear. Perhaps the authors mean "lateral" rather than "distant".

We described the different locations of optic tract and reticular thalamic nuclei more precisely in line 34-40.

Line 259: "This protocol describes a method which allows the investigation of high-released retinogeniculate synapses and low-released corticogeniculate synapses from the same slice." Please change "high-released" and "low-released" to "high probability of release" and "low probability of release" to make it clearer for the readers.

high-released" and "low-released" were changed to "high probability of release" and "low probability of release" respectively (see line 266).

Minor Concerns:

Spelling errors (not a complete list):

General: "angel" should be "angle" in line 122 and many other sentences

"angel" were changed to "angle" (see line 125, 127, 285 and 358).

General: "scull" should be "skull"

"scull" were changed to "skull" (see line 116, 118, 120, 237 and 330).

Line 154: dL'GN

' is removed (see line 157).

Line 264: w

"w" was changed to "we" (see line 271).

Line 308: lager

"lager" is changed to "larger" (see line 150 and Line 347).

Dear Dr. Cao,

We thank you for your careful reading of the manuscript and helpful comments. We have made revisions according to your comments, as described below.

I.

Response to editor

1. Please note that the editor has formatted the manuscript to match the journal's style. Please retain the same. The updated manuscript is attached and please use this version to incorporate the changes that are requested.

We retained the same style as formatted by the editor to match the journal`s style.

2. Please address specific comments marked in the attached manuscript. Please turn on Track Changes to keep track of the changes you make to the manuscript.

We addressed all the comments proposed by the editor. The detailed response is listed as follows:

We rephrased the sentence “In contrast, corticogeniuclate synapses facilitate, consistent with their low release probability” (See line 38).

We replaced Tri~~n~~ton X1000 with a more generic term (non-ionic surfactant) (see Line

245).

We cited Figure 3 in the last paragraph of “representative results” section (see line 300 and 302).

We uploaded the copyright information in the Editorial Management account.

We added the title of Figure 3 (see line 330).

We now cited Turner and Salt, 1998 (see line 335).

We rephrased the sentence “To prevent series resistance errors especially when…” (see line 362-365)

“In some slices, not both inputs to a given relay neuron can be detected because one of the two pathways is severed close to the relay neuron.” This means that we sever axons/inputs/pathways during slicing procedure. This cut is sometimes close to the cell of interest. We could replace severed with cut but that does not increase accuracy.

3. Please add more details to section 5 (particularly for reconstruction). There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how

is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Our idea was to not take a video of this part, because it would be rather boring (use of a confocal, reconstruction using Neurostudio). However, we could of course also film that. We added a more detailed information that should help perform these experiments.

4. References: Please do not abbreviate journal titles.

We now used the full journal name in the reference section.

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