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Effects of photoinhibition on licking

Photoinhibition of ALM or thalamus caused only small changes in 'lick early' rates, 'no response' rates, and licking latency. ALM photoinhibition caused a small reduction in 'lick early' rates (from 12.8% ± 7.9% to 9.7% ± 8.9%; mean ± sd, $P < 0.001$; $n = 84$ sessions) and did not change 'no response' rates (from 0.5% ± 0.8% to 1.6% ± 0.7%; $P = 0.09$; $n = 84$ sessions). Thalamus photoinhibition also caused at most small changes in 'lick early' rates (from 12.5% ± 11.3% to 29.1% ± 26.4%; $P = 0.07$; $n = 13$ sessions) and 'no response' rates (from 2.9 % ± 3.4% to 1.6% ± 2.8%; $P = 0.22$; $n = 13$ sessions). An increase in 'lick early' rates was apparent in some behavioral sessions, possibly due to rebound activity after transient inhibition of thalamus (the early licks occurred 200 ms after light onset, right after rebound activity; Extended Data Fig. 2).

We also compared licking latency during ALM photoinhibition with or without photoinhibition. The licking latency is defined as the time between the go cue onset and the detected tongue contact with the lickport. Photoinhibition of ALM increased licking latency in contra trials (from 152 ± 43 ms to 166 ± 52 ms; $P = 0.002$; $n = 79$ sessions; in five sessions, there are no correct contra trials during photoinhibition) but not in ipsi trials (from 175 ± 43 ms to 175 ± 48 ms; $P = 0.93$; $n = 84$ sessions). For thalamus photoinhibition, we did not detect significant changes in licking latency in both contra (from 220 ± 24 ms to 209 ± 124 ms; mean ± sd; $P = 0.17$; $n = 13$ sessions) and ipsi trials (from 240 ± 75 ms to 327.3 ± 198 ms; $P = 0.17$; $n = 13$ sessions). All statistics used here are paired *t*-test.

In vivo whole-cell recording methods related to Extended Data Fig. 5

We manipulated the membrane potential during whole-cell recordings in ALM to determine if hyperpolarization with photoinhibition in the thalamus is due to increases in inhibition or decreases in excitation. To enhance either excitatory or inhibitory synaptic potentials, we injected negative or positive currents into the neurons to hyperpolarize or depolarize them. Neglecting spatial components, the membrane potential of ALM neurons is governed by [1]:

$$C_m \frac{dV(t)}{dt} = -g_L(V)\,(V(t) - E_L) - g_E(t)\,(V(t) - E_E) - g_I(t)\,(V(t) - E_I) + I_{inj}(t)$$

g_L, g_E, and g_I are conductances related to leak, excitatory, and inhibitory currents, respectively. E_L, E_E, and E_I are the corresponding reversal potentials. g_L is a function of membrane potential because of intrinsic voltage-dependent currents.

Here, we assume E_L = -50 mV, E_E = 0 mV and E_I = -70 mV. For positive current injection experiments, we depolarized V near 0 mV. Under this condition, contribution of g_E on membrane potential is reduced because $V(t) - E_E$ is near 0. On the other hand, contributions of g_I on membrane potential become stronger, since $V(t) - E_I$ = 70 mV is larger (approximately 3.5 fold) compared to resting conditions ($V(t) - E_I$ = 20mV). Increases or decreases in g_I result in hyperpolarization or depolarization, respectively. Similarly, for negative current injection experiments, we hyperpolarized V near -70 mV. Under this condition, contributions of g_E to membrane potential is increased. Increases or decreases in g_E result in depolarization or hyperpolarization, respectively.

The input resistance, R_{in},

$$R_{in} = \frac{1}{g_L(V) + g_E(t) + g_I(t)}$$

affects the amplitude of membrane potential changes, but not their direction (depolarize or

hyperpolarize).

During experiments, we partially compensated for series resistance and injected a ramping current until action potentials disappeared[2 3]. Actual membrane potential was calculated post hoc based on injected current and series resistance. Mean membrane potential without photoinhibition was -52 ± 5 mV (mean ± s.d., n = 14). For depolarizations we injected 983 ± 245 pA, resulting in Vm = -7 ± 4.5 mV (mean ± s.d., n = 6). For hyperpolarizations we injected -191± 64 pA, resulting in Vm = -66 ± 4 mV (mean ± s.d., n = 9).

Series resistance did not change before and after current injections (p = 0.058 and 0.55 for positive and negative current injection compared to the baseline, respectively. ranksum test with Bonferroni correction). In 11/21 recordings we were able to release current injections at the end of experiments to confirm that 1) membrane potential returned to spontaneous level, and 2) neurons still produced action potentials, which together confirmed that recordings did not become leaky. This was indeed the case for all 11 cases.

Model related to Extended Data Fig. 6d

We used a leaky integrate-and-fire model [1] to estimate the time required to hyperpolarize/depolarize ALM neurons after photoinhibition/photoactivation of thalamus and other input structures (Extended Data Fig. 6d).

First, we estimated the membrane time constant based on experimental data. We injected a negative current pulse at the end of each behavioral trial (100 ms, -100 pA). Membrane potential during this negative current injection was averaged and fitted using a double-exponential function:

$$V(t) = V(0) - R_p I_{inj}(1 - exp\frac{-t}{R_p C_p}) - R_{in} I_{inj}(1 - exp\frac{-t}{R_{in} C_m})$$

Here, $V(t)$ is the membrane potential, R_p is the pipette resistance, C_p is the pipette capacitance, R_{in} is the membrane input resistance, C_m is membrane capacitance, and I_{inj} is the amplitude of the injected current. The membrane time constant (τ_m) was estimated as:

$$\boldsymbol{\tau_m} = R_{in} C_m$$

We obtained $\tau_m = 20.0 \pm 1.1$ ms. Standard error of the mean was determined by bootstrapping.

Next, we modeled the network. The network consisted of 200 input thalamic neurons projecting to a single ALM neuron. All the input neurons were modeled to generate independent Poisson spikes. The probability of each neuron to fire an action potential during dt is $FR \times dt$. FR is the mean spike rate of the input neurons. We used FR =10 Hz (Fig. 3f).

To model thalamic photoinhibition, mean spike rate of a fraction of thalamic neurons (10-100%, see Extended Data Fig. 6d, Right panel) decayed instantly after photostimulus onset with time constant of τ =1.1 ms, in accordance with our recordings (Fig. 3f). To model thalamic photoactivation, a fraction of thalamic neurons were modeled to fire at the onset of the photostimulus with jitter following normal distribution with mean μ = 1 ms, standard deviation σ = 0.2 ms (spikes before photostimulus onset were excluded).

Membrane potential of ALM neurons is governed by:

$$\tau_m \frac{dV(t)}{dt} = V_r - V(t) + R_{in} \times I_{syn}$$

$V(t)$ is the membrane potential at each time point (time bin: 0.1 ms), τ_m is the membrane time constant (20 ms, following our recording data), V_r is the resting membrane potential (-65 mV), R_{in} is the input

resistance of ALM neuron (20 MΩ), and I_{syn} is a synaptic current driven by the thalamic neurons. The neuron fires at the spike threshold of -40 mV, and recovered back to the reset potential of -43mV after 2 ms of refractory period. The synaptic current follows kick-and-decay dynamics as below:

$$\tau_{syn}\frac{dI(t)}{dt} = -I(t) + I_{max}$$

I(t) is the synaptic current at each time point (time bin: 0.1 ms), τ_{syn} is the synaptic time constant (1.5 ms, assuming only AMPA current), I_{max} is the max synaptic current at the onset (25 pA). For each condition the model was run 3000 times. To estimate the onset of membrane potential change after thalamus photoinhibition or photoactivation, we applied the same criteria we used for actual recording data: we find the time of passing 3 times standard deviation from the baseline. Here, baseline and standard deviation were calculated between -20~0 ms from the light onset.

Network model related to Extended Data Fig. 7c-e

The goal of the models was to explore possible architectures of multiregional models that may be consistent with our key experimental results: 1) selective firing in ALM and thalamus, 2) loss of ALM firing following strong photoinhibition of thalamus, 3) Strong reduction in thalamic firing after photoinhibition of ALM (not shown), 4) Loss of selectivity in ALM after weak, non-selective inactivation of thalamus.

Models are based on standard methods [4,5]. The models consist of two neurons (with selectivity for 'left' (ipsi) or 'right' (contra) trial types) each in thalamus and ALM. Dynamics of the network are governed by the equation

$$\tau\frac{dx_i}{dt} = -x_i + \sum_j W_{i,j} f(x_j) + T_i + s_i I(t)$$

where τ is the synaptic time constant (10 ms) and x_i is the activity of neuron i. Neuron indices 1, 2, 3 and 4 correspond to right ALM, left ALM, right thalamus and left thalamus neurons, respectively. $W_{i,j}$ is the connection strength from neuron j onto neuron i, $f(x)$ is a synaptic non-linearity, T_i is the tonic (non-selective) input. $I(t)$ is the temporal profile of the sensory input which is constant during the sample period and zero for all other times and s_i is the strength of this input to each neuron.

We first tried to identify a network architecture (i.e. connection matrix) capable of reproducing the key results with linear synapses, $f(x) = x$ (Extended Data Fig. 7c). A multitude of networks with selective firing in both ALM and thalamus were consistent with the strong photoinactivation experiments. However, no connection matrix was able to explain the loss of ALM selectivity produced by weak thalamic photoinhibition. This effect requires that non-selective perturbations to thalamus cause changes in ALM activity that for a given neuron can be either excitatory or inhibitory, depending on its firing rate. Such an effect cannot be explained with linear interactions.

A reduction in selective firing following a reduction of non-selective excitatory drive can be achieved with a non-linear current-firing relationship. In this model, the same small-amplitude change in input to a neuron would produce different changes in firing rate during preferred vs. non-preferred trial types resulting in a change in selectivity. However, changes in selectivity are always accompanied by reduction in firing rate, inconsistent with our data.

We next constructed networks with non-linear synapses (Extended Data Fig. 7d, e). After weak, non-selective inhibition of thalamus these were capable of producing a reduction in selective firing in ALM without altering average firing rates. We built a network with non-selective inputs from thalamus to ALM (Extended Data Fig. 7d, top), threshold linear excitation and non-linear inhibition

given by

$$f_{inh.}(x) = \tan\left(10(x - x0)\right) + 1$$

Because of the sharp onset of inhibition created by this non-linearity, a slight downward shift in activity created by weak and non-selective inhibition of non-selective thalamic inputs to ALM could effectively turn of the inhibitory connections responsible for maintaining persistent firing (Extended Data Fig. 7d, bottom).

We then removed the crossing thalamo-cortical connections, increased the strength of the sensory input to ALM (Extended Data Fig. 7e, top) and found that a network with selective thalamo-cortical connections could also produce a loss of selectivity in response to weak thalamic inhibition.

The parameters for the non-selective and selective networks were:

$$W_{non\_selective} = \begin{pmatrix} 0 & -0.2 & 0.2 & 0.2 \\ -0.2 & 0 & 0.2 & 0.2 \\ 0.25 & 0 & 0 & 0 \\ 0 & 0.25 & 0 & 0 \end{pmatrix}$$

$$W_{selective} = \begin{pmatrix} 0 & -0.2 & 0.35 & 0 \\ -0.2 & 0 & 0 & 0.35 \\ 0.25 & 0 & 0 & 0 \\ 0 & 0.25 & 0 & 0 \end{pmatrix}$$

$\mathbf{s}$ = (1/15,0,0,0),

$\mathbf{T}$ = (0, 0, 0.35, 0 .35)/100.

$x_0 = 0.2$

Networks with non-linear synapses, and either selective or non-selective thalamocortical connectivity, are capable of reproducing the key experimental results.

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Figure panel	Recording method (location)	Mouse genotype or virus (injection parameters, number of mice)	Manipulation conditions (location)
Fig. 1c	N.A.	PV-ires-Cre x Ai32 (Rosa-CAG-LSL-ChR2(H134R)-EYFP-WPRE) (11 mice)	Photoinhibition 0.75 or1.5mW (ALM)
Fig. 1d	N.A	Gad2-ires-cre + AAV2/10 CAG-flex-ChR2(H134R)-tdTomato (Virus injection: bregma AP -0.7, ML 1.6, DV 3.7 and 3.3 mm, 150 nl each, 4 mice)	Photoinhibition 10 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
Fig. 2e	Silicon probe recordings (ALM)	PV-ires-Cre x Ai32 (7 animals, including 3 mice used in Fig. 1c)	N.A.
		VGAT-ChR2-EYFP (3 mice)	N.A.
Fig. 2f	Silicon probe recordings (VM/VAL)	Gad2-IRES-Cre4 x Ai35D (Rosa-CAG-LSL-Arch-GFP-WPRE, JAX 012735, 2 mice)	N.A.
		C57Bl/6J (2 mice)	N.A.
		PV-ires-Cre x Ai32 (7 mice)	N.A.
Fig. 3a-e	Silicon probe recordings (ALM)	VGAT-ChR2-EYFP (3 mice, also used in Fig. 2c)	Photoinhibition 10 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
Fig. 3f	Silicon probe recordings (VM/VAL)	VGAT-ChR2-EYFP (2 mice)	Photoinhibition 10 mW (Thalamus; optrode: bregma AP -1.5, ML 0.85, DV 4.0 mm)
Fig. 3g, 4a-c, EDF. 5a, b & EDF. 6b	Whole cell recordings (ALM)	VGAT-ChR2-EYFP (4 mice)	Photoinhibition 10 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
Fig. 4d-i	Whole cell recordings (ALM)	PV-ires-cre x Ai32 (5 mice)	Photoinhibition 1mW (M1: bregma AP 0, L 1.8 mm; contra ALM: bregma AP 2.5, L -1.5mm)
Fig.5 & EDF. 7	Silicon probe recordings (ALM)	VGAT-ChR2-EYFP (2 mice)	Photostimulation 0.5 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
Fig 6 a-d, e top	Silicon probe recordings (VM/VAL)	PV-ires-Cre x Ai32 (7 animals, also used in Fig. 2f)	Photoinhibition 0.75 or 1.5mW (ALM)
Fig. 7 a-e top	Silicon probe recordings (VM/VAL)	PV-ires-Cre x Ai32 (7 mice, also used in Fig. 1d,f, 2f)	Photoinhibition 1.5mW (ALM)
Fig 6 e bottom	Silicon probe recordings (ALM)	PV-ires-Cre x Ai32 (3 mice, same as in Fig. 1c)	Photoinhibition 0.75-1.5mW (ALM)
EDF. 2c	Silicon probe recordings (thalamus)	Gad2-ires-cre + AAV2/10 CAG-flex-ChR2(H134R)-tdTomato (2 mice)	Photoinhibition 10 mW (Thalamus; optrode: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 2d	Silicon probe recordings (Thalamus)	VGAT-ChR2-EYFP (2 mice, same data as in Fig. 3f)	Photoinhibition 10 mW (Thalamus; optrode: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 2e	Silicon probe recordings (ALM)	VGAT-ChR2-EYFP (3 mice, same data as in Fig. 3e)	Photoinhibition 10 mW (Thalamus; Fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 3a-f	N.A.	C57Bl6/J (3 mice)	Muscimol infusion (Thalamus; cannula: bregma AP -1.5, ML 0.75, DV 4.2 mm, left and right hemispheres)
		C57Bl6/J (2 mice)	Muscimol infusion (Thalamus; cannula; bregma AP -0.3, ML 0.75, DV -4.0 mm, left and right hemispheres)
		C57Bl6/J (2 mice)	Muscimol infusion (Thalamus; cannula: bregma AP -1.5, ML 0.75, DV -3.2 mm, left and right hemispheres)
EDF. 5c-h	Whole cell recordings (ALM)	Gad2-ires-cre + AAV2/10 CAG-flex-ChR2(H134R)-tdTomato (Virus injection: bregma AP -0.7, ML 1.6, DV 3.7 and 3.3 mm, 150 nl each, 3 mice)	Photostimulation 10mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 5i-n	Whole cell recordings (ALM)	PV-ires-cre x Ai32 (2 mice)	Photostimulation 1 mW (ALM)
EDF. 6c	Whole cell recordings (ALM)	Olig3-ires-cre x Ai32 (2 mice)	Photostimulation 10 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 6e	Whole cell recordings (M1)	VGAT-ChR2-EYFP (2 mice: 2 of the animals also used in Fig. 4, 5a)	Photostimulation 10 mW (Thalamus; fiber: bregma AP -1.5, ML 0.85, DV 4.0 mm)
EDF. 6f	Whole cell recordings (ALM)	VGAT-ChR2-EYFP (2 mice)	Photostimulation 1 mW (M1: bregma AP 0, L 1.8 mm)
EDF. 8g	Silicon probe recordings (thalamus)	PV-ires-Cre X Ai32 (1 mice, also used in Fig. 6 a-c)	Photostimulation 1.5 mW (vM1: bregma AP 0.5, L 0.5 mm)
EDF. 10	Silicon probe recordings (SNr)	PV-ires-Cre x Ai32 (3 mice, same as in Fig. 1c)	Photoinhibition 1.5mW (ALM)

Supplementary Table 1. List of mice used for behavioral experiments and recordings

To silence ALM during extracellular recordings, the average laser power was 0.75 mW or 1.5 mW. Laser powers were randomly chosen on a trial-by-trial basis. Photoinhibition with the two powers were pooled as there was no detectable difference in inhibition onset. For M1 silencing AP location was varied slightly for individual animals to avoid the coronal suture, where the skull is not transparent.

Figure panel	Mouse genotype (number of mice)	virus or tracer injections
EDF. 1a-e, & g	C57Bl/6J (2 mice)	AAV2/1-CAG-EGFP WGA-Alexa Fluor® 555 (ALM, bregma AP 2.5, ML 1.5, DV 0.4 and 0.8 mm, 50 nl at each depth for both reagents. 2 weeks after the virus injection, WGA was injected into the same location. A day after animals were perfused)
EDF. 1f	C57Bl/6J (2 mice)	Mixture of FLAG (AAV1-CAG-mRuby2-FLAG) and red RetroBeads at 1:1 ratio (ALM, bregma AP 2.5, ML 1.5, DV 0.4 and 0.6mm, 75 nl at each depth)
EDF. 8h & i	Gad2-ires-cre (1 mice)	AAV2/1-CAG-flex-EGFP (ALM, bregma AP 2.5, ML 1.5, DV 0.4 and 0.6 mm, 75 nl each)
EDF. 9	C57Bl/6J (2 mice)	Red RetroBeads (VM, bregma AP -1.5, ML 0.85, DV 4.1 mm, 50 nl)

Supplementary Table 2. List of mice used for anatomical experiments
All reagents were introduced into the left hemisphere. Unless described, after virus injections expression was allowed for more than two weeks before perfusion.