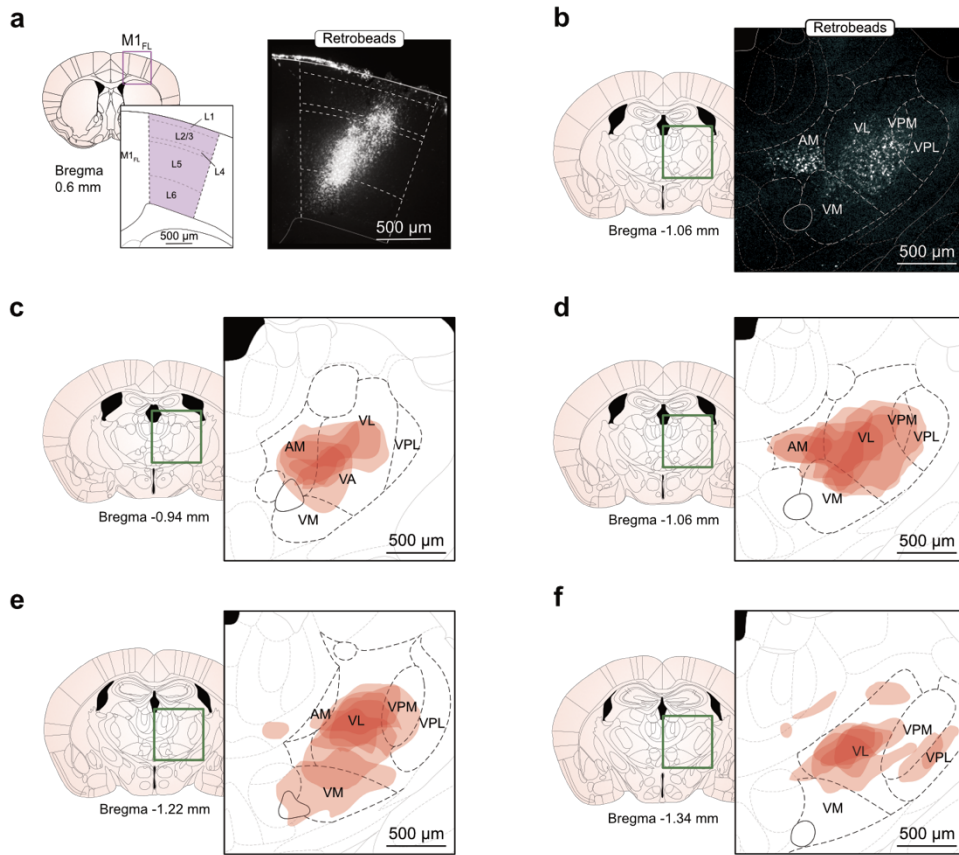


Retrobead tracing



Monosynaptic retrograde rabies tracing

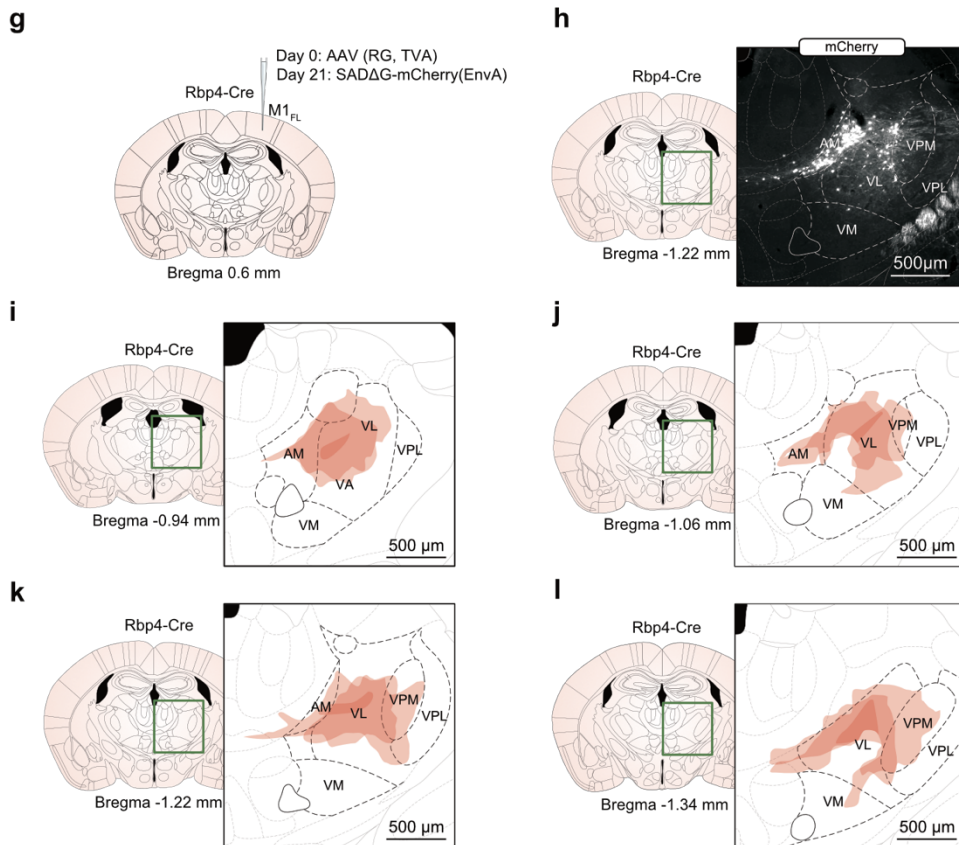


Figure S1. Retrograde mapping of M1_{FL}-projecting motor thalamic neurons.

(a) *Left:* Coronal mouse brain schematic depicting M1_{FL} laminae. *Right:* representative coronal brain slice depicting retrobead injection sites in M1_{FL} demonstrating coverage from L2/3-L6. (b) *Left:* Coronal mouse brain schematic, green square depicts the region of thalamus shown in the expanded view (*right*). *Right:* Representative coronal mouse brain slice displaying M1_{FL}-projecting motor thalamic neurons. (c-f) Distribution of M1_{FL}-projecting motor thalamic neurons labelled by fluorescent retrobeads (red, N = 5) for coronal positions (b) -0.94, (c) -1.06, (d) -1.22, (e) -1.34 mm relative to bregma. Green squares depict the region of thalamus shown in the expanded view (*right*). Motor thalamic nuclei: AM, anteromedial; VL, ventrolateral; VA, ventroanterior; VPM, ventral posteromedial nucleus; VPL, ventral posteromedial; VM, ventromedial. (g) Monosynaptic retrograde rabies tracing strategy: injection of AAV2/1-CAG-FLEX-mTagBFP2-2A-TVA & SADΔG-mCherry(EnvA) into forelimb motor cortex (M1_{FL}) of a layer 5-specific Rbp4-Cre mouse. (h) *Left:* Coronal mouse brain schematic, green square depicts the region of thalamus shown in the expanded view (*right*). *Right:* Representative coronal mouse brain slice displaying M1_{FL}-projecting motor thalamic neurons. (i-j) Distribution of M1_{FL}-projecting motor thalamic neurons labelled by monosynaptic retrograde rabies tracing (red, N = 3) for coronal positions (b) -0.94, (c) -1.06, (d) -1.22, (e) -1.34 mm relative to bregma. Green squares depict the region of thalamus shown in the expanded view (*right*). Motor thalamic nuclei: AM, anteromedial; VL, ventrolateral; VA, ventroanterior; VPM, ventral posteromedial nucleus; VPL, ventral posteromedial; VM, ventromedial.

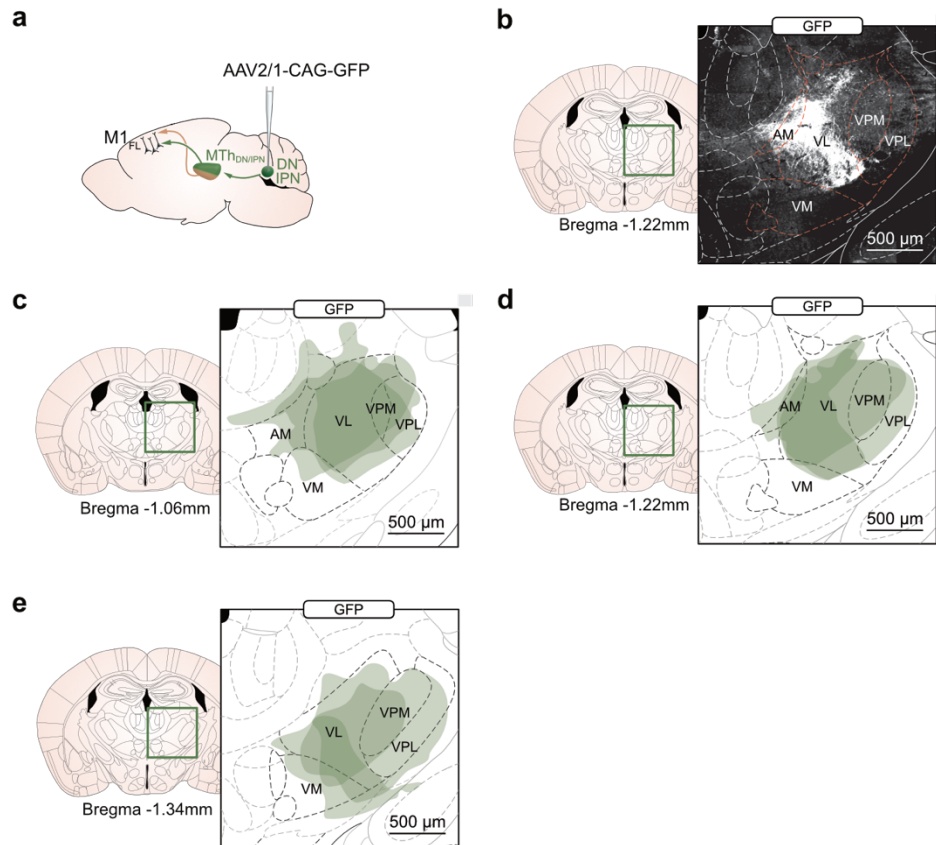


Figure S2. Axon terminal fields of dentate / interpositus nucleus neurons in motor thalamus.

(a) Sagittal mouse brain schematic illustrating viral-based anterograde mapping of dentate nucleus (DN) and interpositus nucleus (IPN) projections to motor thalamus. (b) *Left*: Coronal mouse brain schematic, green square depicts the region of thalamus shown in the expanded view (*right*). *Right*: Representative coronal mouse brain slice displaying anterograde mapping of dentate nucleus (DN) and interpositus nucleus (IPN) axonal arborisation in motor thalamic nuclei. (c-e) Distribution of labelled dentate nucleus and interpositus nucleus axon terminals in motor thalamus (N = 3 mice) for coronal positions (b) -1.06, (c) -1.22, (d) -1.34 mm relative to bregma. Green squares depict the region of thalamus shown in the expanded view (*right*). Motor thalamic nuclei: AM, anteromedial; VL, ventrolateral; VA, ventroanterior; VPM, ventral posteromedial nucleus; VPL, ventral posteromedial; VM, ventromedial.

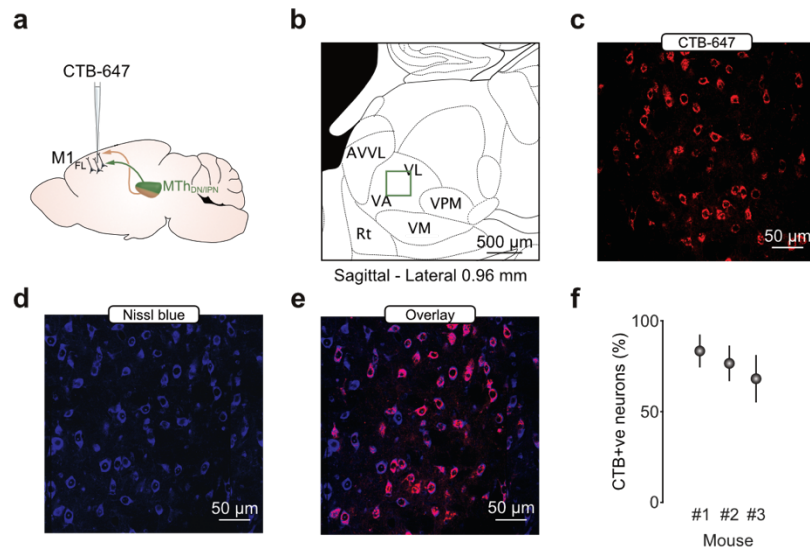


Figure S3. Quantification of M1_{FL}-projecting neurons in ventroanterior / ventrolateral (VA/VL) region of motor thalamus.

(a) Sagittal brain slice schematic illustrating retrograde cholera toxin b-Alexa647 (CTB-Alexa647) mapping of M1_{FL}-projecting neurons in ventroanterior / ventrolateral (VA/VL) region of motor thalamus. (b) Sagittal mouse brain atlas overlay of motor thalamic nuclei. Green square depicts the region of thalamus shown in the expanded view in c-e. (c-e) Motor thalamic neurons labelled with CTB-Alexa647 (c), Nissl blue counter stain (d) and overlay (e). (f) Proportion of M1_{FL}-projecting motor thalamic neurons in VA/VL (N = 3 mice, 4-6 slices per mouse, mean \pm bootstrapped 95% CI).

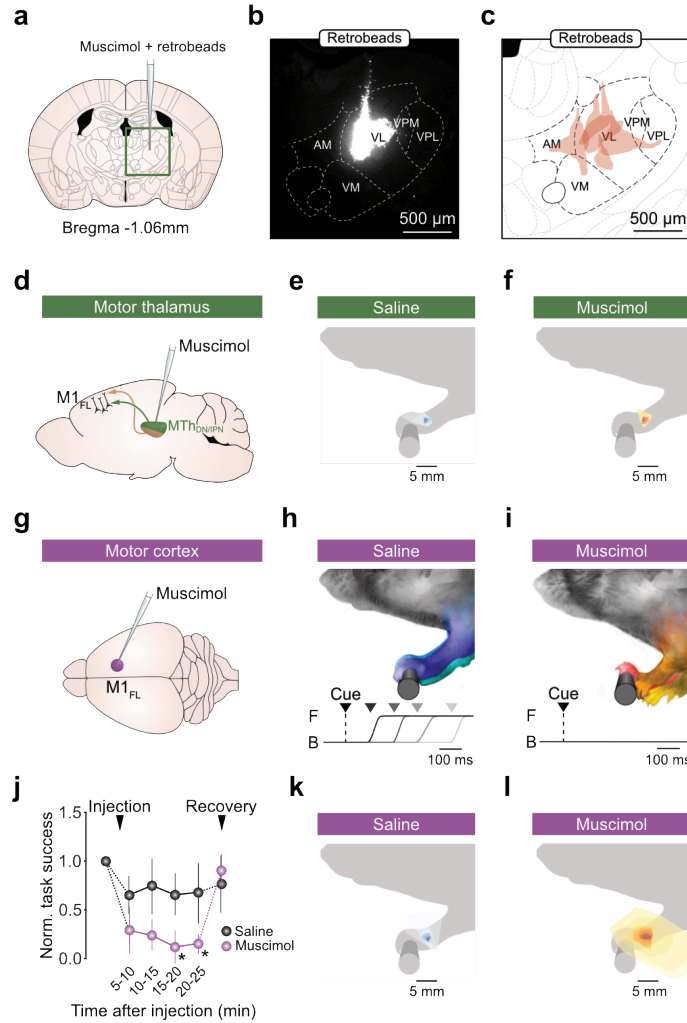


Figure S4. Muscimol inactivation of primary motor cortex and dentate / interpositus nucleus-recipient area of motor thalamus.

(a) Coronal brain schematic illustrating motor thalamus inactivation strategy. Green box identifies expanded view shown in (b-c). (b) Coronal brain slice displaying representative muscimol injection site within motor thalamus. (c) Distribution of muscimol injection sites (N = 5 out of 10 sites recovered). (d) Dentate / interpositus nucleus-recipient motor thalamus inactivation strategy. (e-f) Contour plot of baseline paw positions after (e) saline or (f) muscimol injection into MTh_{DNI/IPN}. Each colored region of the contour plot contains 5% of the data. (g) Top-down view of a mouse brain illustrating M1_{FL} muscimol inactivation strategy. (h-i) Top: Superimposed images of forelimb position at cue onset after (h) saline or (i) muscimol injection into M1_{FL}. Bottom: Example lever trajectories from 4 different trials (black to light grey) following injection of (h) saline or (i) muscimol into M1_{FL}. B, back lever position; F, front lever position; black triangle and dashed line represent cue presentation; grey triangles indicate reaction times. (j) Normalised task success as a function of time after muscimol injection. Colored symbols represent changes in population means \pm 95% CI after saline (black, N = 5) or muscimol (purple, N = 5) injection (* $P < 0.05$, 2-way repeated measures ANOVA, Bonferroni-Holm correction for multiple comparisons). (k-l) Contour plot of baseline paw positions after (k) saline or (l) muscimol injection into M1_{FL}. Each colored region of the contour plot contains 5% of the data.

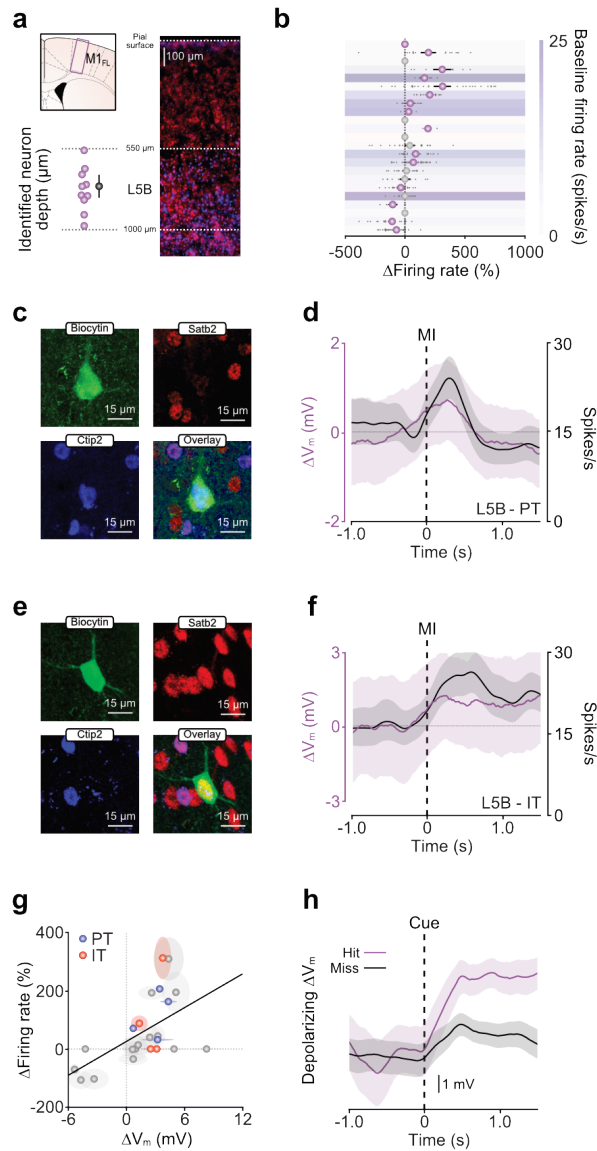


Figure S5. Membrane potential dynamics and projection class identity of individually recorded L5B neurons.

(a) *Top left:* Coronal brain slice schematic showing M1_{FL}. The purple rectangle depicts the expanded view (*right*). *Right:* Distribution of PT-type (blue, CtIP2 staining) and IT-type (red, Satb2 staining) projection neurons in L5B of M1_{FL}. *Bottom left:* depth of recovered L5B neurons as measured perpendicularly from the pial surface ($n = 11/23$ neurons identified, black symbol represents mean \pm 95% CI). (b) Average change in L5B projection neuron peri-movement firing rate \pm 95% CI. Grey dots represent individual trials, purple symbols represent significant changes in firing rate, white symbols represent non-significant changes, defined by comparing 95% bootstrapped confidence intervals. (c) Biocytin staining (green) and post-hoc immunohistochemical staining for Satb2 (red) and CtIP2 (blue) confirmed the PT-type projection class identity of an individually recorded L5B pyramidal neuron. (d) Mean subthreshold V_m and firing rate of the L5B PT-type projection neuron depicted in (c). (e) Biocytin staining (green) and post-hoc immunohistochemical staining for Satb2 (red) and CtIP2 (blue) confirmed the IT-type projection class identity of an individually recorded L5B pyramidal neuron depicted in (e). (g) Correlation between movement-related subthreshold ΔV_m and firing rate changes. Blue/red symbols represent means \pm 95% CI from individual PT-/IT-type neurons respectively, black line is a linear fit to the data. Grey symbols represent means \pm 95% CI from neurons where projection class identity was not determined. (h) Example cue aligned hit-trial mean ΔV_m (purple) vs miss-trial mean ΔV_m (black) trajectories from a representative M1_{FL} layer 5B neuron.

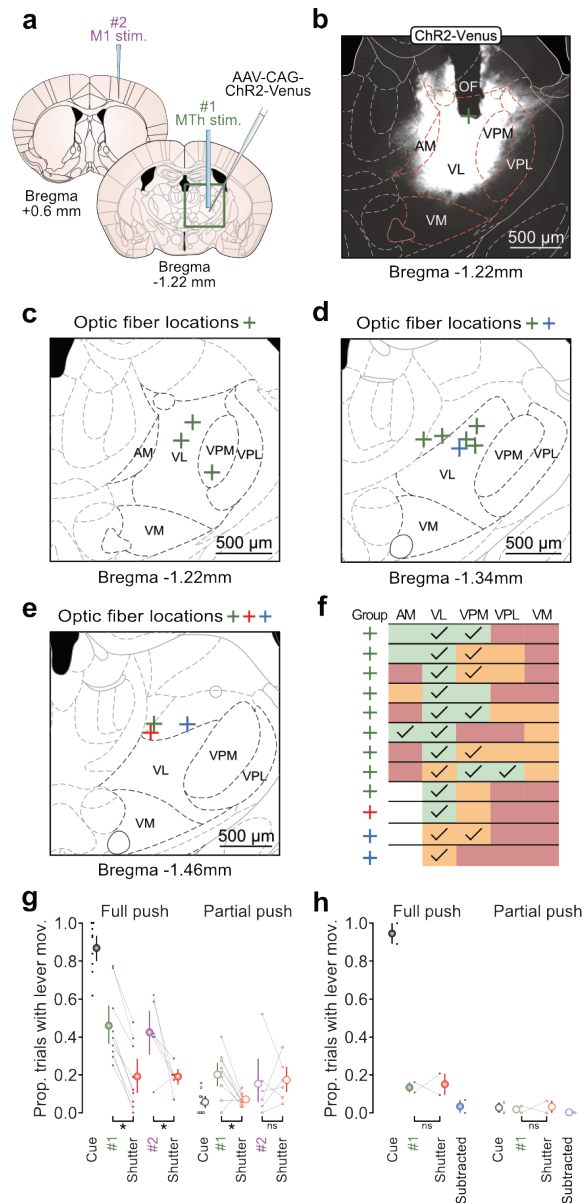


Figure S6. Viral expression and selective optogenetic stimulation of dentate / interpositus nucleus-recipient region of motor thalamus.

(a) Coronal brain slice schematics illustrating dual MTH optogenetic stimulation strategy: channelrhodopsin 2 (ChR2) expression was targeted to neurons in MTH_{DNI/PIPN} and stimulated via an optic fiber placed directly above MTH_{DNI/PIPN} (#1) or via a tapered optic fiber implanted directly into forelimb motor cortex (M1_{FL}) (#2). Green square overlay identifies the expanded view shown in (b). **(b)** Representative coronal section showing expression of ChR2-Venus and position of the optic fiber (OF), centre marked by green cross. **(c-e)** Anatomical locations of optic fiber placements in MTH_{DNI/PIPN} (N = 12 mice, green, red and blue crosses indicate positive behavioral effects, no behavioral effects and control mice, respectively). **(f)** Quantification of viral expression in motor thalamic nuclei (green, nuclei with 50-100% coverage; orange, nuclei with 0-50% coverage; red, no expression; ticks represent nuclei that are within the cone of 470nm light emitted from the optic fiber during optogenetic stimulation). **(g)** Comparison of cue-evoked and ChR2-evoked task engagement represented as the proportion of trials that evoked either full (left) or partial (right) lever push movements. Black, cue + shutter trials; green, direct MTH_{DNI/PIPN} stimulation + shutter trials; purple, direct stimulation of MTH_{DNI/PIPN} axon terminals in M1_{FL} + shutter trials; orange, shutter only trials. Colored dots represent data from individual mice, colored symbols represent mean \pm 95% CI. (Cue, N = 9 mice; #1, N = 9 mice, #2, N = 6 mice. * $P < 0.05$, ns = non-significant, two-sample t -test) **(h)** Comparison of cue-evoked and ChR2-evoked task engagement represented as the proportion of trials that evoked either full (left) or partial (right) lever push movements in control mice. Black, cue + shutter trials; green, direct MTH_{DNI/PIPN} stimulation + shutter trials; orange, shutter only trials; blue, (#1 - shutter) trials. Colored symbols represent mean \pm 95% CI. (N = 2 mice, ns = non-significant, two-sample t -test).