Supplementary Information

Supplementary Methods:

Cutaneous conditioning of the MSR in rats (for Supplementary Fig. 2).

A similar examination of how PAD affected the MSR in mice was performed in rats with percutaneous tail EMG recording. However, in this case PAD was evoked by a cutaneous conditioning stimulation of the tip of the tail (0.2 ms pulses, 3xT, 40 – 120 ms prior to MSR testing) using an additional pair of fine Cooner wires implanted at the tip of the tail (separated by 8 mm). In rats the MSR latency is later than in mice due to the larger peripheral conduction time, ~12 ms (as again confirmed by a similar latency to the F wave). This MSR was thus quantified by averaging rectified EMG over a 12 – 20 ms window. Also, to confirm the $GABA_A$ receptor involvement in regulating the MSR, the antagonist L655708 was injected systemically (1 mg/kg i.p., dissolved in 50 µl DMSO and diluted in 900 µl saline). Again, the MSR was tested at matched background EMG levels before and after conditioning (or L655708 application) to rule out changes in postsynaptic inhibition.

Conditioning of the MSRs in humans (for Supplementary Fig. 3).

H-reflex as an estimate of the MSR. Participants were seated in a reclined, supine position on a padded table. The right leg was bent slightly to access the popliteal fossa and padded supports were added to facilitate complete relaxation of all leg muscles. A pair of Ag-AgCl electrodes (Kendall; Chicopee, MA, USA, 3.2 cm by 2.2 cm) was used to record surface EMG from the soleus muscle. The EMG signals were amplified by 1000 and band-pass filtered from 10 to 1000 Hz (Octopus, Bortec Technologies; Calgary, AB, Canada) and then digitized at a rate of 5000 Hz using Axoscope 10 hardware and software (Digidata 1400 Series, Axon Instruments, Union City, CA)[1]. The tibial nerve was stimulated with an Ag-AgCl electrode (Kendall; Chicopee, MA, USA, 2.2 cm by 2.2 cm) in the popliteal fossa using a constant current stimulator (1 ms rectangular pulse, Digitimer DS7A, Hertfordshire, UK) to evoke an H-reflex in the soleus muscle, an estimate of the MSR[2]. Stimulation intensity was set to evoke a test (unconditioned) MSR below half maximum. MSRs recorded at rest were evoked every 5 seconds to minimize RDD [3] and at least 20 test MSRs were evoked before conditioning to establish a steady baseline because the tibial nerve stimulation itself can presumably also activate spinal GABAergic networks, as in rats. All MSR were recorded at rest, except when the motor unit firing probabilities were measured (see below).

Conditioning of the MSR. To condition the soleus MSR by cutaneous stimulation, the cutaneous medial branch of the superficial peroneal nerve (cDP) was stimulated on the dorsal surface of the ankle using a bipolar arrangement (Ag-AgCl electrodes, Kendall; Chicopee, MA, USA, 2.2 cm by 2.2 cm), set at 1.0xT, where T is the threshold for cutaneous sensation. A brief burst (3 pulses, 200 Hz for 10 ms) of cDP stimuli was applied before evoking a MSR at various inter-stimulus intervals (ISIs; interval between tibial and cDP nerve stimuli) within the window expected for phasic PAD evoked by cutaneous stimuli, presented in random order at 0, 30, 60, 80, 100, 150 and 200 ms ISIs. Seven conditioned MSR at each ISI were measured consecutively and the average of these MSR (peak-to-peak) was used as an estimate of the conditioned MSR. This was compared to the average MSR without conditioning, computed from the 7 trials just prior to conditioning.

The cDP nerve was also stimulated with a 500 ms long train at 200 Hz to condition the MSR, and examine the effect of tonic PAD evoked by such long trains, as in rats. Following the application of at least 20 test MSRs (every 5 s), a single cDP train was applied 700 ms before the next MSR and following this the MSR continued to be evoked for another 90 to 120 s (time frame of tonic PAD). We also conditioned the soleus MSR with tibialis anterior (TA; antagonist muscle, flexor) tendon vibration (brief burst of 3 cycles of vibration at 200Hz) to preferentially activate Ia afferents[2].

Motor unit recording to examine postsynaptic actions of conditioning. Surface electrodes were used to record single motor units in the soleus muscle during low level contractions by placing electrodes on or near the tendon or laterally on the border of the muscle[4]. Alternatively, single motor unit activity from the soleus muscle was also recorded using a high density surface EMG electrode (OT Bioelettronica, Torino, Italy, Semi-disposable adhesive matrix, 64 electrodes, 5x13, 8 mm inter-electrode distance) with 3 ground straps wrapped around the ankle, above and below the knee. Signals were amplified (150 times), filtered (10 to 900 Hz) and digitized (16 bit at 5120 Hz) using the Quattrocento Bioelectrical signal amplifier and OTBioLab+ v.1.2.3.0 software (OT Bioelettronica, Torino, Italy). The EMG signal was decomposed into single motor units using custom MatLab software as per[5]. Intramuscular EMG was used to record MUs in one participant[6] to verify single motor unit identification from surface EMG.

To determine if there were any postsynaptic effects from the conditioning stimulation on the motoneurons activated during the MSR, we examined whether the cDP nerve stimulation produced any changes in the tonic firing rate of single motor units, which gives a more accurate estimate of membrane potential changes in motoneurons compared to compound EMG. Single motor units were activated in the soleus muscle by the participant holding a small voluntary contraction of around 5% of maximum. Both auditory and visual feedback were used to keep the firing rates of the units steady while the conditioning cutaneous was applied every 3 to 5 seconds. The instantaneous firing frequency profiles from many stimulation trials were superimposed and time-locked to the onset of the conditioning stimulation to produce a peri-stimulus frequencygram (PSF, dots in Supplementary Fig. 3b*iii*)[6,7]. A mean firing profile resulting from the conditioning stimulation (PSF) was produced by averaging the frequency values in 20 ms bins across time post conditioning (thick lines in Supplementary Fig. 3b*iii* and c*iii*). To quantify if the conditioning stimulation changed the mean firing rate of the tonically firing motor units, the % change in the mean PSF rate was computed at the time when the H reflex was tested (vertical line in Supplementary Fig. 13b*ii-iii*).

Unitary EPSP estimates from PSF. To more directly examine if the facilitation in MSR resulted from changes in transmission in Ia afferents after cutaneous afferent conditioning, we measured changes in the firing probability of single motor units (MUs) during the brief MSR time-course (typically 30 to 45 ms post tibial nerve stimulation) with and without cDP nerve conditioning. Soleus MSRs were as usual evoked by stimulating the tibial nerve, but while the participant held a small voluntary plantarflexion to activate tonic firing of a few single motor units. The size of the MSR was set to just above reflex threshold (when the M-wave was < 5% of maximum) so that single motor units at the time of the MSR could be distinguished from the compound potential from many units that make up the MSR [8]. For a given trial run, test MSRs were evoked every 3-5 s for the first 100 s and then MSR testing continued for a further 100s, but with a cDP-conditioning train (50 ms, 200 Hz) applied 500 ms prior to each MSR testing stimulation. These repeated high frequency trains evoke a tonic PAD in rats that facilitates sensory conduction. A 500 ms ISI was used to ensure the firing rate of the motor unit returned to baseline before the MSR was evoked, and this is also outside of the range of phasic PAD. Approximately 40-50 usable test and conditioned firing rate profiles were produced for a single session where the motor units had a steady discharge rate before the cDP nerve stimulation. Sessions were repeated 3-6 times to obtain a sufficient number of frequency points to construct the PSF (~ 200 trials).

To estimate the EPSP profile and prior background motoneuron activity, motor unit (MU) firing was again used to construct a PSF, as detailed above, but this time locked to the tibial nerve stimulation used to evoke the MSR, so that we could estimate the motoneuron behaviour during the MSR (EPSP). When more than one MU was visible in the recordings firing from these units (usually 2 – 3) were combined into a single PSF. Overall this gave about of 100 – 600 MU MSR test sweeps to generate each PSF. Firing frequency values were initially averaged in consecutive 20 ms bins to produce a mean PSF profile over time before the tibial nerve stimulation, for both unconditioned and conditioned MSR reflex trials. From this, the mean background firing rate within the 100 ms window immediately preceding the tibial stimulation was compared between the test and conditioned MSR trials to determine if the conditioning cDP nerve stimulation produced a change in firing rate, and thus

post-synaptic effect, just before the conditioned MSR was evoked. Next, as an estimate of EPSP size, the mean firing rate during the MSR window was also measured, but computed with smaller PSF bins of 0.5 ms during the MSR. Finally, for each PSF generated with or without conditioning, the probability that a motor unit discharged during the MSR window (30 to 45 ms after the TN stimulation) was measured as the number of discharges during the time of the MSR window divided by the total number of tibial nerve test stimuli.

Temperature, latency and PAD considerations for rats and mice.

In vivo. Large proprioceptive group Ia sensory afferents conduct in the peripheral tail nerve with a velocity of ~33 m/s (33 mm/ms) in mice[9]. Motor axons are similar, though slightly slower (30 m/s)[10]. Thus, in the awake mouse stimulation of Ia afferents in the mouse tail evokes spikes that take ~2 ms to conduct to the motoneurons in the spinal cord ~70 mm away. Following ~1 ms synaptic and spike initiation delay in motoneurons, spikes in the motor axons take a further ~2 ms to reach the muscles, after which the EMG is generated with a further 1 ms synaptic and spike initiation delay at the motor endplate to produce EMG. All told this gives a monosynaptic reflex latency of ~6 ms. The motor unit potentials within the EMG signal have a duration of about 3 – 5 ms, and thus we averaged rectified EMG over 6 – 11 ms to quantify the MSR. We have shown that similar considerations hold for the rat where tail nerve conduction velocities are similar, except the distance from the tail stimulation to the spinal cord is larger (150 mm), yielding a peripheral nerve conduction delay of ~10 ms and total MSR delay of ~12 ms[11]. In humans the MSR latency is dominated by the nerve conduction latency (50 – 60 m/s) over a large distance (~800 mm), yielding MSR latencies of ~30 ms.

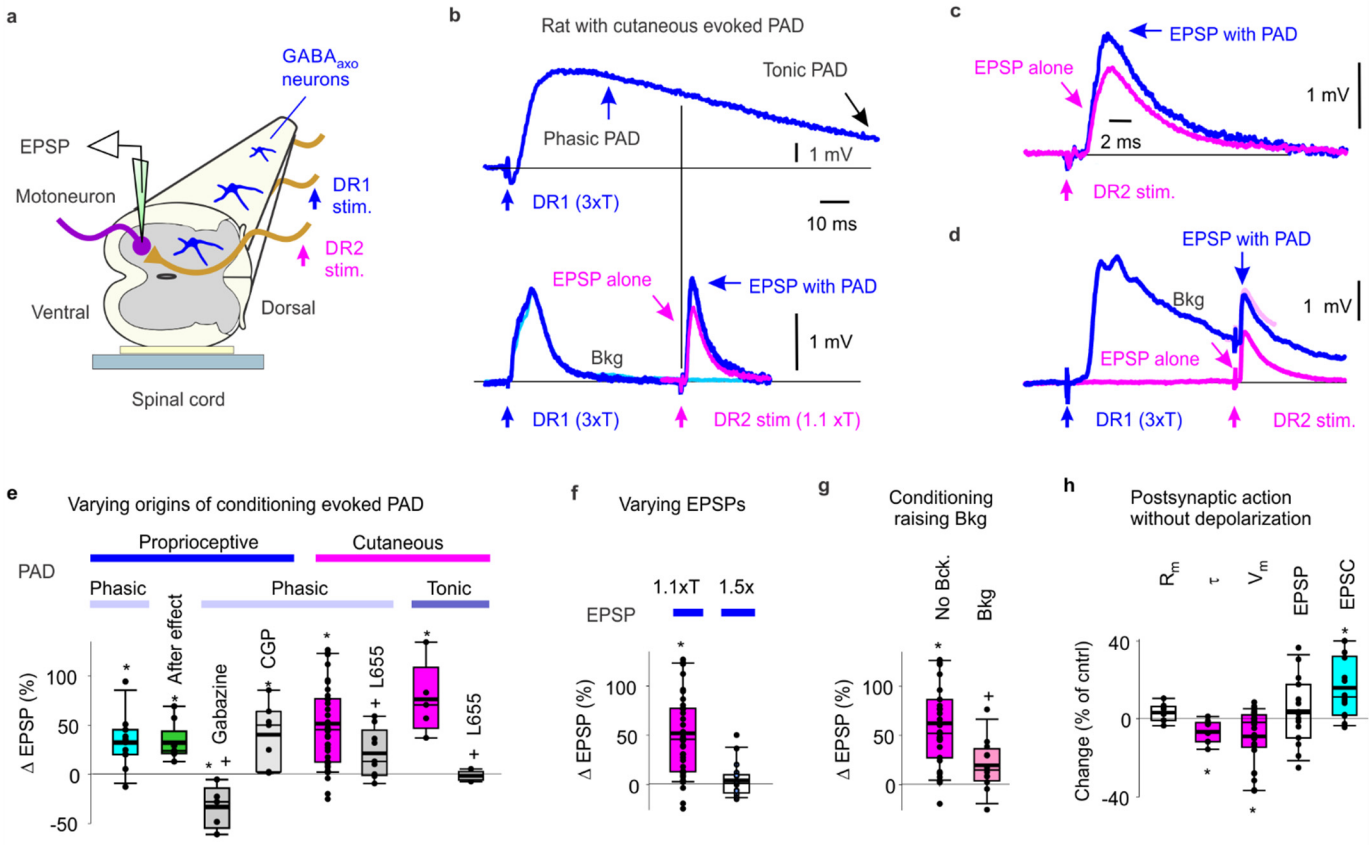
Ex vivo. In our ex vivo whole adult spinal cord preparation the bath temperature was varied between 23 and 32ºC. All data displayed is from 23 – 24ºC, though we confirmed the main results (facilitation of sensory axon transmission to motoneuron by PAD) at 32ºC. The Q10 for peripheral nerve conduction (ratio of conduction velocities with a 10 ºC temperature rise) is about 1.3[12], yielding a Ia afferent conduction in dorsal roots of about 20 m/s at 23 – 24 ºC, as we directly confirmed. Thus, when the DR is stimulated 20 mm from the cord the latency of spike arrival at the cord should be about 1 ms, which is consistent with the time of arrival of afferent volleys that were seen in the intracellular and extracellular recordings from sensory axons (e.g. Figs. 2b and 4e).

PAD and DRR changes with temperature. We did not consistently use high temperature ex vivo baths (32ºC) because the VR and DR responses to activation of DRs or PAD neurons are irreversibly reduced by prolonged periods at these temperatures, suggesting that the increased metabolic load and insufficient oxygen penetration deep in the tissue damages the cord at these temperatures. Importantly, others have reported that in sensory axons PAD-evoked spikes (DRRs) are eliminated in a warm bath and argued that this means they are not present in vivo, and not able to evoke a motoneuron response[13], despite evidence to the contrary[14,15]. However, we find that PAD itself is reduced in a warm bath by the above irreversible damage, and it is thus not big enough to evoke spikes in sensory axons; thus, this does not tell us whether these spikes should be present or not in vivo. Actually, in vivo we sometimes observed that with optogenetic activation of $GABA_{axo}$ neurons and associated PAD there was a direct excitation of the motoneurons (seen in the EMG) at the latency expected for PAD evoked spikes. However, this was also at the latency of the postsynaptic inhibition produced by this same optogenetic stimulation, which often masked the excitation (Fig. 6). In retrospect, examining the $GABA_{axo}$ evoked motoneuron responses during optogenetic-evoked PAD (Fink et al.)[13,16], or sensory-evoked PAD[16,17], there is either outright excitation or an excitation riding on the postsynaptic IPSPs resulting from the activation of there $GABA_{axo}$ neurons. This is consistent with the PAD-evoked spike activating the monosynaptic pathway, which inhibits subsequently tested monosynaptic responses by post activation depression (see Discussion).

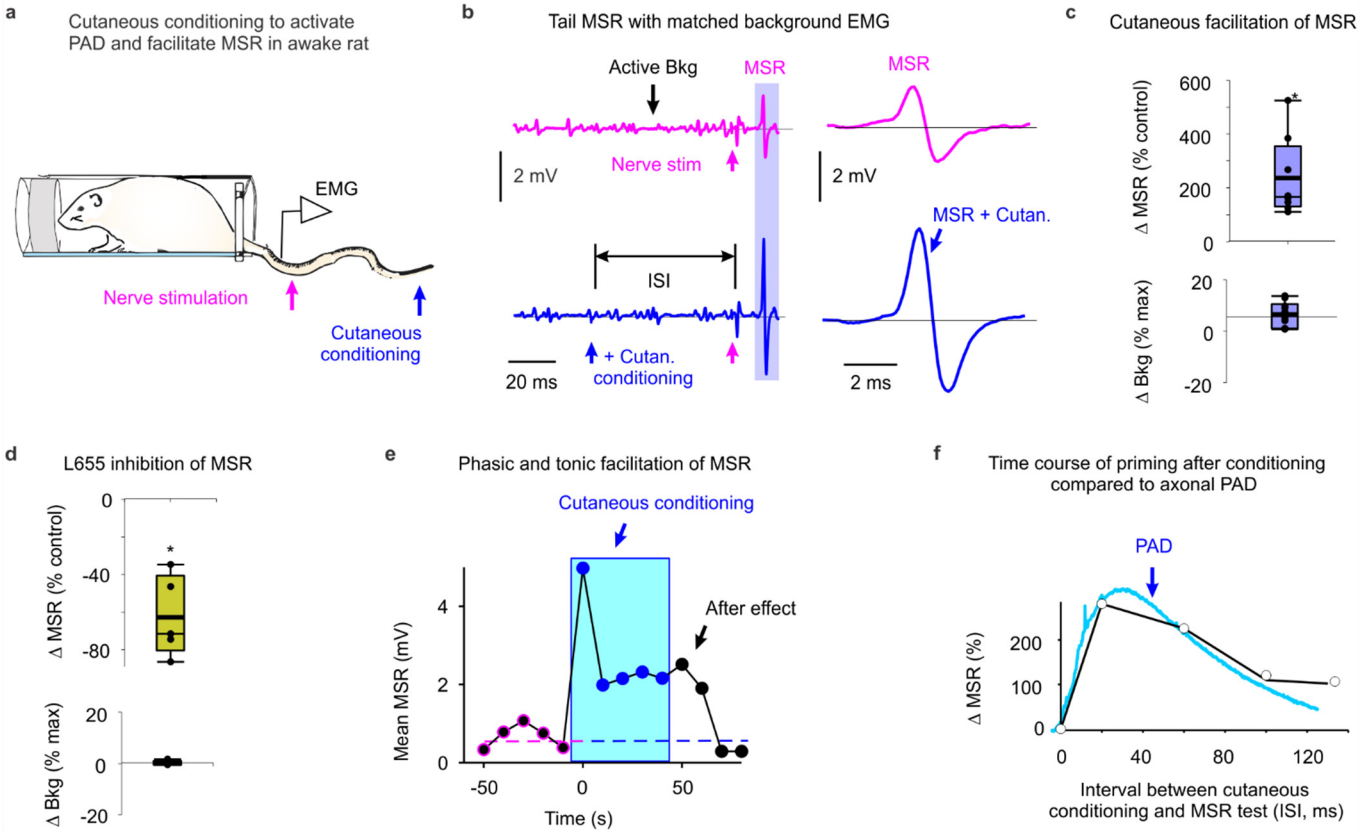
Synaptic latency. The latency of a single synapse in our ex vivo preparation at 23 – 24ºC was estimated from the difference between the time arrival of the sensory afferent volley at the motoneurons (terminal potential seen in intracellular and extracellular recordings) and the onset of the monosynaptic EPSP in motoneurons. This was consistently 1 – 1.2 ms (Fig. 5b and e). This is consistent with a Q10 of about 1.8 – 2.4 for synaptic transmission latency [18,19], and 0.4 ms monsynaptic latency at body temperature [20,21]. Based on these considerations we confirm that the PAD evoked in sensory axons is monosynaptically produced by optogenetic

activation of $GABA_{axo}$ neurons with light, since it follows ~1 ms after the first spike evoked in GABAaxo neurons by light (Fig. 3a). This first spike in $GABA_{axo}$ neurons itself takes 1 – 2 ms to arise and so the overall latency from light activation to PAD production can be 2 - 3 ms (Fig. 3f), as seen for IPSCs at this temperature in other preparations [22]. With DRs stimulation PAD arises with a minimally 4 – 5 ms latency, which is consistent with a trisynaptic activation of the sensory axon, after taking into account time for spikes to arise in the interneurons involved (Fig. 4a,e).

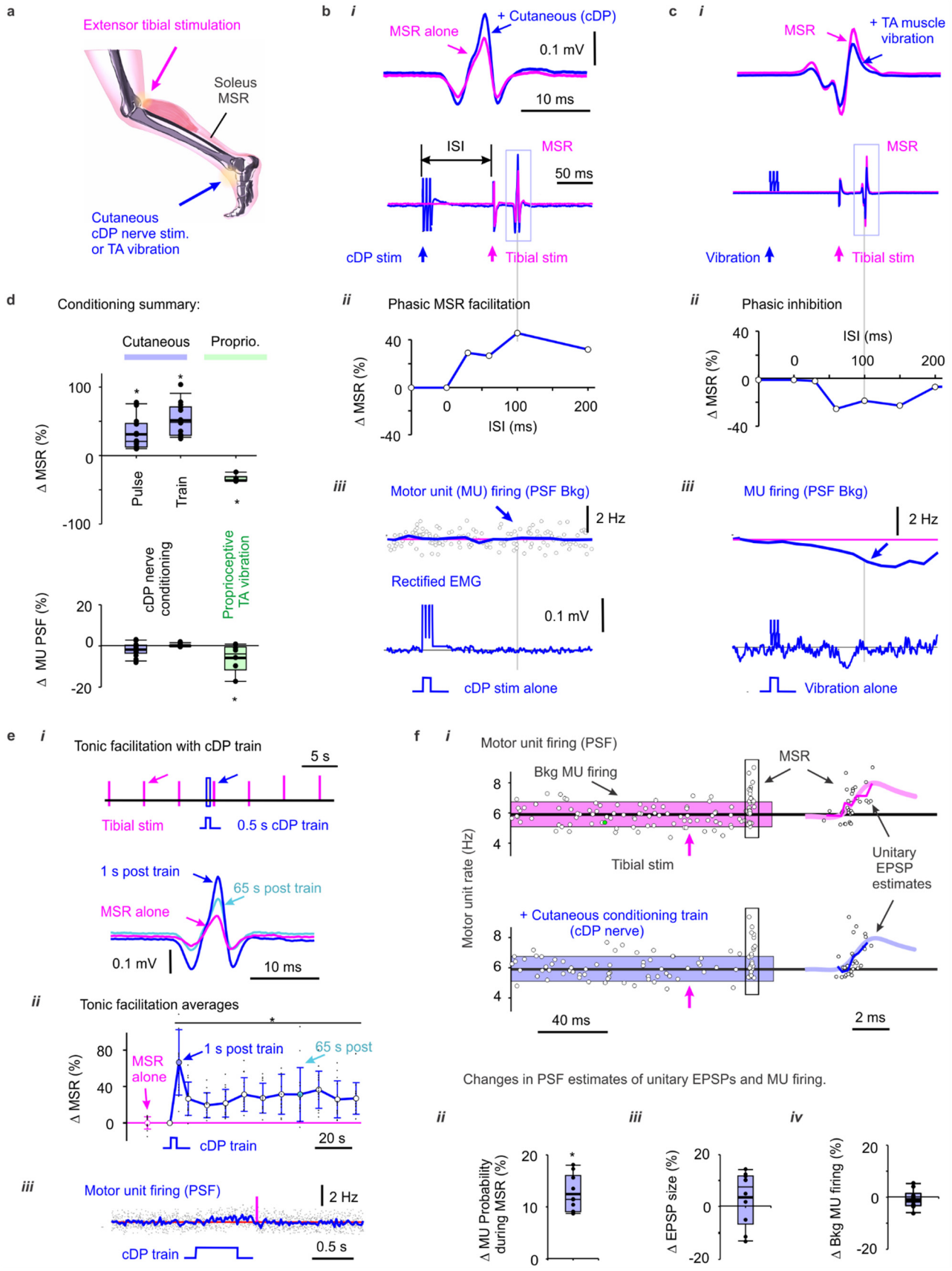
Supplementary Figures and Tables:



Supplementary Fig. 1 | Sensory evoked facilitation of monosynaptic EPSPs by $GABA_A$ receptors.
a, Whole spinal cord ex vivo preparation for intracellular recording of EPSPs from motoneurons while stimulating dorsal roots (DRs). **b**, Monosynaptic EPSP in an S4 motoneuron evoked by a proprioceptive group I stimulation of the S4 DR (1.1xT, denoted DR2, lower traces; resting potential -75 mV: black line; T: EPSP threshold, similar current to afferent volley threshold), alone (pink) and 60 ms after (blue) a conditioning stimulation of cutaneous afferents in rat to evoked PAD (stimulation of the largely cutaneous Ca1 DR, 2.5xT; denoted DR1). Averages of 10 trials each at 10 s intervals. PAD evoked by the same cutaneous conditioning stimulation in a proprioceptive S4 DR afferent is shown for reference (top, recorded separately, as in Fig. 4b). **c**, EPSPs from (**b**) on expanded time scale. **d**, Similar to (**b**), but stronger conditioning stimulation (DR1, 3xT) evoking background postsynaptic activity (blue, Bkg) that lasted longer than 60 ms, and slightly inhibited the EPSP, likely from increased postsynaptic conductances shunting the EPSP (postsynaptic inhibition; light pink: overlay of EPSP alone) and masking nodal facilitation. **e**, Summary box plots of facilitation of EPSPs during phasic PAD evoked by either proprioceptive conditioning (S3 or contralateral S4 DR stimulation, 1.1xT, *n* = 11 motoneurons EPSPs in 5 mice, blue) or cutaneous conditioning (Ca1 DR stimulation, 2-3xT, in rats, *n* = 42 motoneurons/EPSPs in 10 rats, pink), and action of $GABA_A$ and $GABA_B$ antagonists (gabazine 50 μM, CGP55845 0.3 μM and L655708 0.3 μM grey, *n* = 5, 7, 9 EPSPs respectively in same animals, with again mice proprioceptive conditioning and rats cutaneous). EPSPs evoked in S3 and S4 motoneurons by DR2 (S3 or S4) stimulation at 1.1T, as in (**b**). Facilitation measured 60 ms post conditioning during phasic PAD (phasic condition indicated) and when postsynaptic actions of conditioning (Bkg) were minimal (as in **b**). After conditioning was completed EPSP testing continued and revealed a residual facilitation that lasted for 10 - 100 s (After effect, green, *n* = 9 EPSPs in 5 mice), due to a build up of tonic PAD, after which the EPSP returned to baseline, similar to post-tetanic potentiation[23]. Also, a brief high frequency cutaneous stimulation train (200 Hz, 0.5 s, 2.5xT) that led to a very long lasting depolarization of proprioceptive axons (Tonic PAD, example in Fig. 5g) caused a facilitation of the monosynaptic EPSP that lasted for minutes (average shown, tonic cutaneous condition), and this was blocked by L655708 (in rats, *n* =5 EPSPs in 4 rats). * significant change with conditioning, two-sided paired two-sided paired t-test, $P < 0.05$. + significant change with antagonist, two-sided paired t-test, $P < 0.05$. Raw data points show occasional inhibition of the MSR by conditioning, but overall facilitation. ChR2 activation of GABAaxo neurons lacked these long tonic PAD-mediated after effects on the EPSP facilitation (Fig. 5e-f, Post), suggesting an additional source of GABA mediating after effects. **f**, Summary box plots of change in EPSP induced by cutaneous DR (DR1) conditioning (and associated phasic PAD) 60 ms prior to evoking the EPSP, with varying EPSP stimulation intensity. When the DR that evoked the test EPSP (DR2) was stimulated at an intensity that produced less than half the maximal EPSP height (1.1xT, ~ 30% max EPSP, *n* =42, same data as in **e**) the facilitation of EPSP by conditioning was larger than when this DR2 stimulation was increased to produce a test EPSP near maximal (1.5xT, prior to conditioning, *n* = 18 EPSPs from same rats as in **e**). * significant change with conditioning, two-sided paired t-test, $P < 0.05$. This is likely because the stronger test stimulation reduced the headroom for increasing EPSPs by recruiting more proprioceptive axons, and increased self-facilitation prior to conditioning, the latter during repeated testing used to obtain EPSP averages. **g**, Summary of cutaneous facilitation of EPSPs from (**f**) (evoked by DR2 at 1.1xT), but separated into trials without (as in (**b**), *n* = 31 EPSPs, in 10 rats) and with (as in (**d**), *n* = 11 EPSPs in 10 rats) large background postsynaptic changes induced by conditioning that lasted up to and during the EPSP testing (at 60 ms post conditioning, Bkg). * significant change with conditioning evoked PAD, two-sided paired t-test, $P < 0.05$. +significant reduction facilitation with increased background activity (Bkg), two-sided paired t-test, $P < 0.05$. **h**, Remote postsynaptic inhibition from conditioning. Long lasting changes in intrinsic proprieties of motoneurons (S4 and S3) following a mixed proprioceptive and cutaneous conditioning DR stimulation (on S3 or contralateral S4 DR, 2.5xT, DR1) that only produced a transient postsynaptic depolarization that ended prior to EPSP testing (as in B), including a reduction in time constant (τ) and slight hyperpolarization of potential (V_m), both measured at the time of EPSP testing (measured at 60 ms post conditioning, but in trials without EPSP testing; *n* = 15 motoneurons in 5 rats). At this time, there was little change in somatic membrane resistance (Rm) with conditioning, suggesting that conditioning induced postsynaptic activity at a remote location in distal dendrites of the motoneuron. Indeed, when we voltage clamped the membrane potential during monosynaptic testing (DR2 at 1.1-1.5xT) to directly measure the synaptic current (EPSC) and minimize that inhibitory action of postsynaptic conductance increases, we found that the conditioning stimulation (DR1) produced a larger facilitation of the monosynaptic EPSC than the EPSP measured in current clamp in the same motoneurons (same n = 15 motoneurons). These results are consistent with the facilitation of the EPSP being masked by postsynaptic inhibition from increases in remote dendritic postsynaptic conductances triggered by the conditioning stimulation. *significant change with conditioning, two-sided unpaired t-test, $P < 0.05$. Box plots show the interquartile range (box), median (thin line), mean (thick line), 10 and 90 percentiles (whiskers) and extremes (dots).

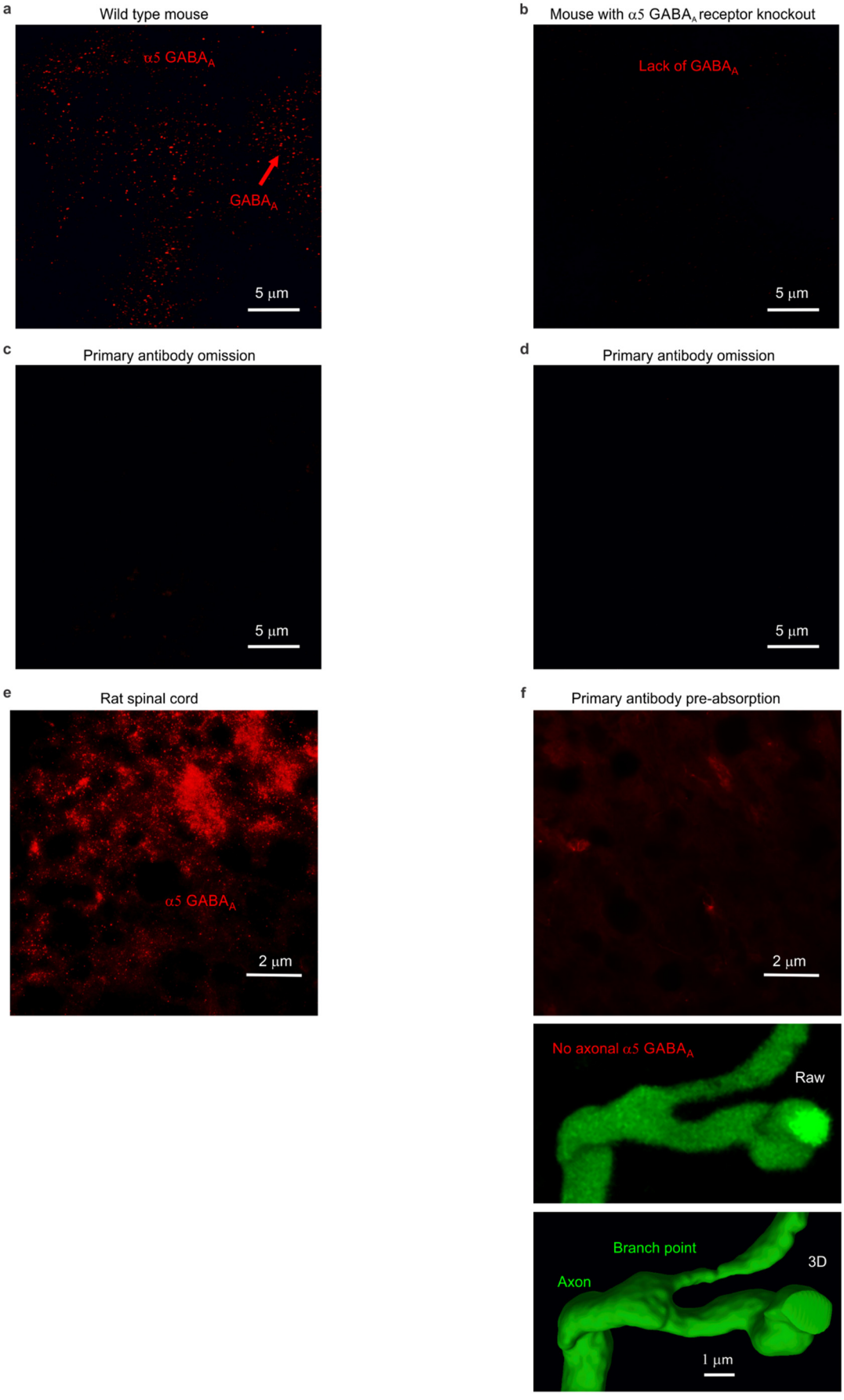


Supplementary Fig. 2 | Facilitation of reflexes in awake rats | a-c, MSR recorded as in Fig 6, but in rat and with PAD instead activated with cutaneous conditioning (tip of tail, 0.2 ms, 2xT, 60 ms prior, 0.1 Hz repetition), at matched active Bkg EMG. * significant change with conditioning, two-sided paired t-test, $P < 0.05$, $n = 8$ rats (**c**). **d**, Decrease in MSR with L655708 (1 mg/kg i.p.) at matched Bkg EMG. Box plot. * significant change, two-sided paired t-test, $P < 0.05$, $n = 5$ rats. **e**, Typical MSR amplitude before, during and after conditioning as in (**a-c**) with after effect. **f**, Typical change in MSR with cutaneous conditioning as in (**a-c**) when the ISI is increased, compared to PAD (from Fig. 4). (**e,f**) similar results in $n = 5/5$ rats. **Summary of findings in awake rats**: Increasing $GABA_{axo}$ neuron activity with a brief cutaneous stimulation (**a**) increased the MSR (**b-c**) during a period consistent with nodal facilitation by PAD (30 – 200 ms post stimulation; **f**). We again kept the conditioning stimulation small enough to not change the background (**b**) to rule out postsynaptic actions. Blocking $GABA_A$ receptor tone (with L655708) decreased the MSR, at matched levels of background EMG (**d**), suggesting a spontaneous tonic PAD facilitating the MSR. Repeated cutaneous conditioning stimulation (trains) to induce a buildup in this tonic PAD caused an associated buildup of the MSR that outlasted the conditioning and its postsynaptic actions by many seconds (after effect; **e**). Box plots show the interquartile range (box), median (thin line), mean (thick line), 10 and 90 percentiles (whiskers) and extremes (dots).


a
Extensor tibial stimulation
Soleus MSR
Cutaneous cDP nerve stim. or TA vibration
b i
MSR alone
+ Cutaneous (cDP)
0.1 mV
10 ms
ISI
MSR
50 ms
cDP stim
Tibial stim
c i
MSR
+ TA muscle vibration
MSR
Vibration
Tibial stim
d
Conditioning summary:
Cutaneous
Proprio.
Δ MSR (%)
Pulse
Train
cDP nerve conditioning
Proprioceptive TA vibration
Δ MU PSF (%)
ii
Phasic MSR facilitation
Δ MSR (%)
ISI (ms)
Phasic inhibition
ISI (ms)
iii
Motor unit (MU) firing (PSF Bkg)
2 Hz
Rectified EMG
0.1 mV
cDP stim alone
MU firing (PSF Bkg)
Vibration alone
e i
Tonic facilitation with cDP train
5 s
Tibial stim
0.5 s cDP train
1 s post train
65 s post train
MSR alone
0.1 mV
10 ms
ii
Tonic facilitation averages
Δ MSR (%)
MSR alone
1 s post train
65 s post
cDP train
20 s
iii
Motor unit firing (PSF)
2 Hz
cDP train
0.5 s
f i
Motor unit firing (PSF)
Bkg MU firing
MSR
Motor unit rate (Hz)
Tibial stim
Unitary EPSP estimates
+ Cutaneous conditioning train (cDP nerve)
40 ms
2 ms
Changes in PSF estimates of unitary EPSPs and MU firing.
ii
iii
iv
Δ MU Probability during MSR (%)
Δ EPSP size (%)
Δ Bkg MU firing (%)

Supplementary Fig. 3 | Facilitation of reflexes in humans.
a, To estimate the role of $GABA_{axo}$ neurons in humans we employed the sensory-evoked depolarization of proprioceptive axons by $GABA_{axo}$ neurons (sensory-evoked PAD; Fig. 4), which is known to occur in humans[24]. For this we recorded the MSR in the soleus muscle in response to tibial nerve stimulation. **b,** MSR in soleus EMG evoked by a tibial nerve pulse (1.1xT, 0.2 Hz, **b***i*), and phasic facilitation of the MSR following a brief conditioning of the cutaneous medial branch of the superficial peroneal nerve (cDP nerve) at varying intervals (ISIs, **b***ii*, 1.0xT, perception threshold T, at rest), and lack of changes in background (Bkg) motor unit (MU) activity or EMG evoked by conditioning alone (**b***iii*, peri-stimulus frequencygram, PSF; with weak contraction). **c**, Same as (**b**), but with proprioceptive conditioning evoked by a brief tibial anterior (TA) muscle tendon vibration, which alone inhibited MU activity (postsynaptic inhibition, PSF Bkg, **c***iii*). **d**, Summary box plots of changes in MSR and postsynaptic (MU) activity with brief conditioning (cDP, *n* = 14 subjects; or TA vibration, n = 6 subjects; as in **b**-**c**) and long cutaneous conditioning trains (**e**, *n* = 14 subjects). * significant change with conditioning, two-sided paired t-test, $P < 0.05$. **e**, Tonic increase in MSR (tonic facilitation) after 0.5 s cutaneous conditioning train (cDP, 1.1xT, 200 Hz) at rest (**e***i*-*ii*), without prolonged changes in MU activity induced by conditioning alone (**e***iii*, PSF in weak contraction). MSR evoked by tibial stimulation every 5 s, with averages from repeated conditioning shown in (**e***ii*). * significant change in MSR, one way ANOVA with Tukey correction for multiple comparisons, $P < 0.05$, n = 5 subjects. **f,** Overlay of all MU firing rates (PSF) with repeated MSR testing (at 5 s intervals) during ongoing weak contraction, and effect of the 0.5 s cutaneous conditioning train (**f***i*). Summary box plots of increased probability of MU firing during MSR (**f***ii*), without changing estimated EPSP size (**f***iii*, PSF thin line; thick line unitary EPSP shape from Fig. 5j) or background MU firing (Bkg, **f***iv*). * significant change with conditioning, two-sided paired t-test, $P < 0.05$, *n* = 10 subjects.

Summary of findings in humans: Increasing $GABA_{axo}$ neuron activity with a brief cutaneous stimulation increased the MSR (**a, b***i***, d**) during a period consistent with nodal facilitation by PAD (30 – 200 ms post stimulation; **b***ii*). We again kept the conditioning stimulation small enough to not change the background EMG or single motor unit (MU) firing (**b*iii***) to rule out postsynaptic actions. When we instead increased PAD by a proprioceptive conditioning (via muscle TA vibration) the soleus MSR was inhibited (for up to 200 ms; **c***i*-**c***ii*), as previously reported[2]. However, the vibration alone inhibited the ongoing MU discharge (**c***iii*), implying that this MSR inhibition was caused in part by postsynaptic inhibition, rather than PAD-mediated presynaptic inhibition[2]. Repeated cutaneous conditioning stimulation (trains) to induce a buildup in this tonic PAD caused an associated buildup of the MSR that outlasted the conditioning and its postsynaptic actions by many seconds (after effect; **d,e**). Finally, the probability of a single MU contributing to the MSR was increased by cutaneous conditioning (**f***i*-*ii*), without increasing the estimated EPSP amplitude or rise time (PSF; see Methods; **f***iii*) or changing in the MU firing prior to the MSR testing (**f***iv*; motoneuron not depolarized closer to threshold), consistent with decreased branch point failure (Fig. 5).


a
Wild type mouse
α5 GABA$_A$
GABA$_A$
5 μm
b
Mouse with α5 GABA$_A$ receptor knockout
Lack of GABA$_A$
5 μm
c
Primary antibody omission
5 μm
d
Primary antibody omission
5 μm
e
Rat spinal cord
α5 GABA$_A$
2 μm
f
Primary antibody pre-absorption
2 μm
No axonal α5 GABA$_A$
Raw
Branch point
Axon
3D
1 μm

Supplementary Fig. 4 | Lack of α5 $GABA_A$ receptor immunolabelling after receptor knockout or antibody pre-absorption. **a**, Immunolabelling of α5 $GABA_A$ receptors with antibody to rabbit anti-α5 $GABA_A$ receptor subunit (1:200; TA338505, OriGene Tech), as used in Fig 1 and Extended Data Fig. 1. Images taken in hippocampal region of wildtype adult mouse brain where neuronal α5 $GABA_A$ receptors are highly enriched. **b**, Lack of α5 $GABA_A$ receptor immunolabelling in α5 $GABA_A$ receptor knockout mouse (Gabra5 KO), from same region. **c-d**, Primary antibody omission controls in wild type and Gabra5 KO mice, respectively, where sections were processed as in (**a**) and (**b**), but no rabbit anti-α5 $GABA_A$ receptor antibody applied. This is used as a control to show that the secondary antibodies do not non-selectively bind to the tissue. Tissue sections in (**a**) and (**b**) were processed for immunolabelled side-by-side on the same slide, and images were obtained with identical confocal microscope settings and displayed at the same brightness as in antibody omission controls of (**c**) and (**d**) where no labelling was observed. **e**, Immunolabelling of α5 $GABA_A$ receptors with the same antibody as in (**a**) but in the spinal cord, where Fig 1 shows these receptors in branch points of sensory axons. **f**, Same immunolabelling as in (**e**), but with the primary antibody to α5 $GABA_A$ receptors preabsorbed with the antigen used to make the antibody, as detailed in the methods, showing a lack of labelling anywhere in the spinal cord, and specifically no labelling at branch points of sensory axons identified by neurobiotin injections, using the methods of Fig. 1. (**a-f**) representative of 3 animals.

Supplementary Table 1. Chronological list of evidence contradicting the classical concept of presynaptic inhibition of transmitter release from proprioceptive sensory axon terminals on motoneurons.

Date	Contradictions in classic view of terminal presynaptic inhibition mediated by terminal $GABA_A$ receptors and PAD	Resolution of contradictions
1938	*Primary afferent depolarization (PAD) directly evokes spikes in sensory axons, producing excitation rather than presynaptic inhibition.* Barron and Matthews (1938)[25] discovered that sensory nerve conditioning evokes a long depolarization in many other sensory afferents (primary afferent depolarization, PAD), which we now know is mostly $GABA_A$ mediated[26]. They and others noted that sometimes this PAD was large enough to directly induce axon spiking, even in vivo[15], including spikes in the sensory axons mediating the MSR itself, raising a contradiction with the notion of GABA mediated presynaptic inhibition[27]. While these PAD-triggered spikes do not fully propagate antidromically out the DRs in many axons (they fail en route), they are actually initiated in most axons and more likely to conduct orthodromically[27], making most axons and their motoneuron synapse refractory to subsequent testing (*post activation depression*). Indeed, numerous groups have shown that these spikes directly activate the MSR pathway[13,14,28,29]. Thus, these PAD-evoked spikes must inhibit afferent transmission in the MSR pathway by making axons refractory and producing post activation depression of their terminal synapse, even in humans where PAD evoked spikes occur[24]. This indirect inhibition is $GABA_A$ mediated and thus readily mistaken for presynaptic inhibition (sensitive to $GABA_A$ antagonists)[30,31], even though the PAD-evoked spike is fundamentally excitatory. Even Eccles noted this issue, and showed that just the refractory period alone in the sensory axon inhibits the MSR[14].	*Post activation depression from PAD evoked spikes inhibits the MSR and masks facilitation of the MSR by nodal facilitation.* We find that facilitation of the MSR by conditioning evoked PAD is always reduced when it is associated with a large enough conditioning stimulation to evoke spikes in sensory afferents, which likely results from post activation depression of axon transmission. This likely explains why Fink et al.[13,16] recently saw inhibition of the MSR with optogenetic or sensory activation of $GABA_{axo}$ neurons (see Fig 4.12c in Fink[16] for PAD evoked EPSC inhibiting the MSR). When looking for MSR facilitation, avoiding these spikes and post activation depression requires using weak conditioning stimuli, unlike previous studies[14,17].
1949	*Post-tetanic potentiation (PTP) of the MSR increases sensory nerve conduction, but its mechanisms have remained elusive.* Lloyd (1949)[32] concluded that increasing conduction along sensory axons (not just terminals) contributed to the minutes of facilitation of the MSR seen after a high frequency nerve stimulation train (PTP). However, he supposed this might be due to hyperpolarization of the sensory axons, even though we now know that such trains depolarize axons via tonic PAD[27]. The tonic PAD from these bursts must overwhelm the hyperpolarization driven by Na-K pump activity[33]. Lloyd also concluded that PTP only occurred when the same nerve is used for the train (tetanus) as for testing the MSR.	*Repetitive nerve stimulation produces a tonic $GABA_A$ mediated depolarization (PAD) of axons that facilitates nodal conduction, and increases the MSR.* This PAD likely contributes to PTP, and is largest when the same nerve is tetanized, compared to other nerves, explaining why Lloyd missed the subtler facilitation from other nerves.
1958	*PAD is associated with a lowering of the threshold for activating spikes.* Early on Wall (1958)[34] noted that a conditioning nerve stimulation that depolarized sensory axons (PAD) was associated with a lower threshold to extracellularly activate these axons. Subsequently this was assumed to be due to the action of terminal $GABA_A$ receptors and presynaptic inhibition, and spike threshold changes were used to estimate the size of PAD[26,35].	*PAD lowers the spike threshold via $GABA_A$ receptors at or near nodes assisting the sodium spike.* This is not related to presynaptic inhibition, but can still be used to estimate PAD, as Rudomin and others have done.
1957 - 1994	*PAD is not correlated with inhibition of the monosynaptic reflex (MSR)*. Shortly after Frank and Fortes discovered that the leg extensor muscle MSR is inhibited by a conditioning of a flexor nerve in cats (PBST; like Fig. 6)[36,37], Eccles proposed the concept of presynaptic inhibition mediated by this conditioning depolarizing of the proprioceptive sensory axon terminals in the MSR pathway (PAD), simply because the MSR inhibition and PAD are somewhat correlated in time[14]. However, in retrospect PAD is far too brief to account for the much longer inhibition caused by this conditioning[38,39], and some flexor nerve conditioning (a single PBST pulse) inhibits the MSR (Fig. 1 of Eccles, 1961[14]), even though it does not cause PAD in the extensor proprioceptors of the MSR at all[40].	*PAD is correlated with nodal spike facilitation and facilitation of the MSR.* PAD causes facilitation of the MSR, explaining this correlation. When PAD is large and evokes axonal spikes, these cause post activation depression (detailed above) that should also be correlated with PAD, but is not due to presynaptic inhibition. Also, barbiturates used by Eccles and others potentiated $GABA_A$ receptor currents.
1959 - 1993	*Postsynaptic inhibition inevitably accounts for part of the inhibition of the MSR by flexor nerve conditioning*. In his initial short report Frank (1959)[36] correctly suggested that the early inhibition of the MSR by flexor nerve conditioning might be partly postsynaptic (rather than presynaptic), on motoneuron distal dendrites. Others dismissed postsynaptic inhibition because the decay times of the EPSP does not always change when the EPSP is reduced by conditioning, which they proposed indicated that there was no postsynaptic change in conductance in distal dendrites [26,41]. However, this method is likely not very sensitive, due to variability in unitary EPSP time course. Also, anatomically ~70% of $GABA_{axo}$ contacts on afferent terminals also contact motoneurons (in a triad), so postsynaptic inhibition is likely inevitable[42,43].	*Postsynaptic inhibition masks facilitation of the MSR by nodal facilitation.* We find evidence for long lasting postsynaptic inhibition on distal motoneuron dendrites during nerve conditioning stimulation (including postsynaptic reductions in Tau, Vm and unitary EPSP heights and single MU firing). Crucially, minimizing postsynaptic inhibition requires using a small conditioning stimulation when looking for MSR facilitation, unlike previous studies [17].
1961 -	*Self-facilitation during repeated MSR testing reduces the possibility of observing facilitation with subsequent conditioning stimuli, leaving only inhibitory actions of*	*Self-facilitation masks facilitation of the MSR by a conditioning stimulation.* To observe

2014	*conditioning.* Eccles and others knew that the same proprioceptive nerve stimulation that activates the MSR also depolarizes these proprioceptive afferents (PAD self-activation)[14]. Thus, just the act of repeatedly testing the MSR to find the average MSR prior to conditioning pre-activates PAD and produces self-facilitation of the MSR, reducing the headroom to observe changes in the MSR following a separate nerve conditioning stimulation that produces PAD. However, at the time it was not known that repeated nerve stimulation causes a tonic buildup of GABA and a tonic PAD that alters sensory transmission and MSR even at long repetition intervals of many seconds. Thus, Eccles and others used short test intervals (1 s) and strong maximal MSR test stimuli [13,14,17], presumably assuming that there would be no interaction between test stimuli, which is not the case. In retrospect, these short test intervals and strong test stimuli must have preactivated tonic GABA, leaving little headroom to observe facilitation of the MSR (facilitation), and leaving mainly only inhibitory action possible.	facilitation of the MSR by a conditioning stimuli that produces a PAD it is important to use long test intervals (5 - 10 s) and small MSR test intensities (1.1xT) to minimize self activation of a tonic PAD prior to conditioning. While experimentally troublesome, self facilitation during repetitive activation is actually one of the main functions of PAD, allowing sensory axons to faithfully transmit spikes to motoneurons at high frequencies that would otherwise produce sodium spike inactivation.
1980	*Sensory axon terminal potentials at motoneurons are consistent with spike failure.* Early efforts to examine how spikes propagated to sensory axon terminals employed extracellular recordings (EC) near the motoneurons, called terminal potentials (Sypert et al. 1980)[44]. However, unlike EC recordings from near conducting axons (Fig. 2b), these terminal potentials lacked much of the obvious negative field associated with the action potential, and instead had a prominent positive field, followed by a smaller negative field (Extended Data Fig. 10 and Sypert[44]). This positive field has been shown in other axons to be indicative of spike propagation failure and result from the passive axonal current caused by the last non-failing node, similar to a FP, as demonstrated in motor axon recording[45,46]. Indeed, we found that even dorsal horn recordings could exhibit this positive field if the nearby dorsal root conduction is blocked with a microinjection of TTX (Extended Data Fig. 10d). Sypert[44] went on to show that with PAD evoked by nerve conditioning this positive terminal potential field was decreased, and incorrectly interpreted this as evidence for decreased spike conduction and thus supposed it was due to presynaptic inhibition.	*Positive terminal potential fields are decreased with PAD, indicative of decreased conduction failure, consistent with Sypert[44].* There is a small negative field that follows the positive field in terminal potential recordings, representing spikes that actually reach the terminals. We quantified negative field and found it to increase with PAD, consistent again with increased spikes conducting to motoneurons (Extended Data Fig. 10). Blocking activity in the spinal cord with glutamate antagonists, which would include blocking $GABA_{axo}$ circuit activity, decreased this negative field.
1988 - 1998	*$GABA_B$ receptors cause presynaptic inhibition and related RDD.* Decades, after Eccles popularized the notion of $GABA_A$ mediated presynaptic inhibition, Curtis (1998)[30,38] concluded that the late part of the inhibition of the MSR by flexor nerve conditioning is instead $GABA_B$ receptor mediated, since it is reduced by the $GABA_B$ antagonist CGP55845 (as Fink also showed[16]), and as is RDD[47]. RDD is a rate dependent depression in the MSR during repeated testing. We suggest that RDD is partly mediated by a build up of GABA released by GABAergic neurons onto the terminals during this repeated MSR testing, though activity dependent homosynaptic depression likely also contributes[48].	*$GABA_B$ mediated presynaptic inhibition masks facilitation of the MSR by $GABA_A$ receptors. $GABA_B$ receptors* are located on the terminals, and produce presynaptic inhibition (Fig. 5) and RDD (Bennett and Hari, unpublished results), which are reduced by $GABA_B$ antagonists (CGP55845) or silencing $GABA_{axo}$ neurons.
1990 - 1998	*$GABA_A$ receptors have direct postsynaptic inhibitory effects on many spinal neurons, making the actions of $GABA_A$ antagonists difficult to attribute to presynaptic inhibition*. By the 1990s Redman and others tried to confirm the role of $GABA_A$ receptors in presynaptic inhibition by locally applying the $GABA_A$ antagonists bicuculline to the spinal cord, and indeed found this drug or other antagonists reduced the inhibition of the MSR by flexor nerve conditioning [17,30,38,49]. However, we now know that this is indirectly due to bicuculline causing a widespread disinhibition of the spinal cord (including loss of post activation depression, detailed above) that leads to a convulsive spinal cord with very long lasting polysynaptic reflexes evoked by the nerve conditioning or the MSR testing itself, making pre and postsynaptic actions hard to distinguish. Further, we know that $GABA_A$ receptors mediate dorsal root reflexes and associated post activation depression of the MSR (see above point), and thus bicuculline and picrotoxin likely reduce the inhibition of the MSR via reducing post activation depression (see above), rather than changing presynaptic inhibition.	*$GABA_A$ receptor antagonists reduce the MSR, by reducing nodal facilitation.* Postsynaptic $GABA_A$ receptors have potent inhibitory actions on many spinal neurons involved in polysynaptic reflexes. However, minimizing these polysynaptic reflexes (by using weak test stimuli and blocking NMDA receptors, Fig 5c) reveals a direct inhibition of the MSR by $GABA_A$ antagonists, as does optogenetically silencing $GABA_{axo}$ neuron, consistent with $GABA_A$ receptors facilitating rather than inhibiting sensory transmission.
1990 - 1995	*PAD recorded in the dorsal roots cannot arise from terminal GABA receptors, due to spatial attenuation on the axon.* With advent of detailed anatomical and computer models of sensory axons[50-52] it became clear that signals like spikes or PAD are attenuated over short distances in axons, < 200 µm. This implies that PAD recorded on or near the DR is unlikely to bare any relation to terminal presynaptic inhibition, despite claims to the contrary[2,13,26,49].	*Space constant λ_S of sensory axons is about 90 µm.* Thus, the PAD recorded in the dorsal root must arise from GABA receptors at or near nodes, and not bear any relation to GABA action at the terminals 1000 µm away.
1994 - 1999	*Shunting inhibition produced by axon terminal $GABA_A$ receptors is not adequate to produce presynaptic inhibition of the MSR*. Numerous invertebrate studies proposed that the conductance from $GABA_A$ receptors in terminals caused a reduction in spike height via its shunting action that contributed to presynaptic inhibition with nerve conditioning[53]. However, the effects of conditioning on spikes was small and terminals were not actually recorded from. Subsequently modelling considerations led to the conclusion that shunting inhibition is not adequate to produce presynaptic inhibition	*$GABA_A$ receptors only slightly decrease spikes by shunting conductances, and otherwise assist nodal spike conduction in proprioceptive axons.* In non-failing secure spikes in sensory axons $GABA_A$ receptors lower the threshold for spike activation (rheobase) and speed the spikes, the latter

	and calcium was somehow involved[51], possibly further implicating $GABA_B$ receptors, as we see. Considering our estimated space constant λ_S of ~90 µm, the small shunting inhibition of the spike height (1 mV) we observe is very unlikely to prevent the spike produced at a given node from activating a downstream neighbouring node, since nodes are ~50 µm apart, leading to only about a 50% reduction in spike height at the downstream node (to ~40 mV, unless of course the node is failing), which is well above that needed to initiate a full nodal spike. Thus, spike propagation is very unlikely to be blocked by shunting inhibition. Also, terminal boutons are mostly on unmyelinated axons without sodium channels (passive, 3rd order), and so a 1% reduction in the spike arising from the last/closest node on the 2nd order branch will have little effect on the terminal depolarization (1%), ruling out substantial shunting inhibition of transmitter release from the terminal.	by decreasing the time constant of the axon (RC). They do decrease the spike, but only by about 1%, consistent with shunting being unlikely to inhibit spike transmission to motoneurons. However, this does not rule out densely expressed $GABA_A$ receptors causing shunting and presynaptic inhibition in cutaneous afferents, as previously suggested[27,54,55].
1994 - 1998	*Sodium spike inactivation from axon terminal $GABA_A$ receptor depolarization is not adequate to produce presynaptic inhibition.* Early poor quality recordings from sensory axons (resting near -50 mV from penetration injury)[56] led to the prevailing view that spike failure with depolarization (PAD) was much more common than we now find with better recordings (resting near -70 mV, Extended Data Fig. 3b). Further, Redman later questioned this view, and it seems unlikely for the MSR pathway[17,31].	*Physiological PAD depolarizations do not block proprioceptive sensory axons spikes, and instead prevent them from failing in the MSR pathway.* However, this does not rule out $GABA_A$ receptors causing spike inactivation in other axons[27].
1995 - 1998	*Physiological $GABA_A$ receptor activation is unlikely to produce branch point failure in the sensory axons of the MSR pathway.* Over the years sensory axon conduction failure has been occasionally noted from indirect observations[23,54,57-62]. Wall and others[50,54,55] questioned whether this failure could be increased by GABA. However, Wall thought GABA should inhibit rather than assist spikes by inactivating sodium channels. However, Wall was misled by two issues. First, at the time low quality recordings from sensory axons may have led to the misconception that spike failure with physiological depolarizations (like PAD) was common[56], unlike what we observe. To be fair, Wall was studying cutaneous, as well as proprioceptive, afferents, which are more densely innervated by GABA receptors[27], making spike inactivation by PAD more likely[54]. Second, by this time $GABA_A$ and associated PAD had been firmly entrenched as synonymous with presynaptic inhibition.	*$GABA_A$ receptors help prevent branch point failure and thus facilitate sensory transmission in the MSR.* Computer simulations by Walmsley and others [50,51] have led to the conclusion that physiological $GABA_A$ receptor conductances cannot stop spikes from propagating past a node. Instead, we report here that they help prevent spike failure near branch points, including in our computer simulations.
1996 - 2018	*Lack of $GABA_A$ receptors on proprioceptive Ia axon terminals*. Extrasynaptic α5 $GABA_A$ receptors are lacking at most proprioceptive axon terminals in the ventral horn[27]. Synaptic $GABA_A$ receptors also appear to be lacking from these terminals, though only indirectly studied[13,63,64]. $GABA_B$ receptor immunolabelling had not been investigated in these Ia afferents, though is has in others (Aβ)[65].	*$GABA_A$ receptors are mostly at nodes, whereas $GABA_B$ receptors are at terminals in large proprioceptive afferents*.
2005 - 2014	*GABAergic innervation of axons.* Recently, GAD2 expressing GABAergic neurons were identified that make axoaxonic connections onto presynaptic terminals of proprioceptive axons[13,42,64] (termed $GABA_{axo}$ here). Previously, Walsmley found GABAergic P-boutons contacting nodes of these axons. Subsequently, Kolta and Zytnicki again found GABAergic contacts near branch points of mammalian afferents[55,66], as did Cattaert in crayfish[53].	*A key role of $GABA_{axo}$ neurons is to innervate proprioceptive afferent nodes via $GABA_A$ receptors and ventral terminals via $GABA_B$ receptors, producing nodal facilitation and presynaptic inhibition, respectively*.
2018	*$GABA_{axo}$ neuron activation by sensory conditioning does not depolarize proprioceptive axon terminals*. Direct recordings from the fine terminals of proprioceptive afferents reveal that during sensory conditioning the terminal is not depolarized during the long PAD recorded on dorsal roots[27].	*$GABA_{axo}$ neuron activation depolarizes nodes*. Dorsally located nodes produce the PAD recorded in dorsal roots.

Supplementary Table 2. Resources used in Methods.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-α_5 $GABA_A$ receptor subunit	OriGene Tech.	TA338505
Rabbit anti- α_1 $GABA_A$ receptor subunit	Sigma-Aldrich	06-868
Guinea pig anti-α_2 $GABA_A$ receptor subunit	Synaptic Systems	224 104
Chicken anti-γ_2 $GABA_A$ receptor subunit	Synaptic Systems	224 006
Rabbit anti-$GABA_{B1}$ receptor subunit	Synaptic Systems	322 102
Mouse anti-NF200 (Neurofilament 200)	Sigma-Aldrich	N0142
Guinea pig anti-VGLUT1 (Vesicular glutamate transporter 1)	Sigma-Aldrich	AB5905
Rabbit anti-Caspr (Contactin associated protein)	Abcam	ab34151
Mouse anti-Caspr (Contactin associated protein)	NeuroMab	K65/35
Chicken anti-MBP (Myelin basic protein)	Abcam	ab106583
Chicken anti-VGAT (Vesicular inhibitory amino acid transporter)	Synaptic Systems	131 006
Rabbit anti-VGAT	Sigma-Aldrich	AB5062P
Rabbit anti-EYFP (Enhanced yellow fluorescent protein)	Biorbyt	orb256069
Goat anti-RFP (Red fluorescent protein; binds tdTom)	Biorbyt	orb334992
Rabbit anti-RFP (Red fluorescent protein; binds tdTom)	MBL Int.	PM005
Rabbit anti-GFP (Green fluorescent protein)	ThermoFisher Sc.	A11122
Mouse anti-Pan Sodium Channel (binds all Na_V types)	Sigma-Aldrich	S8809
Goat anti-rabbit Alexa Fluor 555	ThermoFisher Sc.	A32732
Goat anti-rabbit Alexa Fluor 647	Abcam	ab150079
Goat anti-rabbit Pacific orange	ThermoFisher Sc.	P31584
Goat anti-mouse Alexa Fluor 647	ThermoFisher Sc.	A21235
Goat anti-mouse Alexa Fluor 488	ThermoFisher Sc.	A11001
Goat anti-mouse Alexa Fluor 555	ThermoFisher Sc.	A28180
Goat anti-guinea pig Alexa Fluor 647	ThermoFisher Sc.	A21450
Goat anti-chicken Alexa Fluor 405	Abcam	ab175674
Donkey anti-goat Alexa Fluor 555	Abcam	ab150130
Donkey anti-rabbit Alexa Fluor 488	ThermoFisher Sc.	A21206
Streptavidin-conjugated Alexa Fluor 488	Jackson immunoR.	016-540-084
Streptavidin-conjugated Cyanine Cy5	Jackson immunoR.	016-170-084
Guinea pig anti-GAD2/GAD65	Synaptic Systems	198 104
Guinea pig anti-Neurofilament M (NFM),	Synaptic Systems	171 204
Chemicals, Peptides, and Recombinant Proteins		
M.O.M (Mouse on Mouse Immunodetection Kit)	Vector	BMK-2202
Experimental Models: Organisms/Strains		
$Gad2^{CreER}$ mouse: *Gad2*$^{tm1(cre/ERT2)Zjh}$/J	The Jackson Laboratory	Stock# 010702
$Vglut1^{cre}$ mouse: B6;129S-*Slc17a7*$^{tm1.1(cre)Hze}$/J	The Jackson Laboratory	Stock# 023527
$R26^{LSL\text{-}ChR2\text{-}EYFP}$ mouse: B6;129S-*Gt(ROSA)26Sor*$^{tm32(CAG\text{-}COP4*H134R/EYFP)Hze}$/J	The Jackson Laboratory	Stock# 012569
$R26^{LSL\text{-}Arch3\text{-}GFP}$ mouse: B6;129S-*Gt(ROSA)26Sor*$^{tm35.1(CAG\text{-}aop3/GFP)Hze}$/J	The Jackson Laboratory	Stock# 012735
$R26^{LSL\text{-}tdTom}$ mouse crossed with $Gad2^{CreER}$ mice: B6.Cg-*Gt(ROSA)26Sor*$^{tm14(CAG\text{-}tdTomato)Hze}$/J	The Jackson Laboratory	Stock# 007914
$R26^{LSL\text{-}tdTom}$ mouse crossed with $Vglut1^{Cre}$ mice: B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J	The Jackson Laboratory	Stock# 007909
EIIa-cre mice crossed with Gabra5-floxed mice	Dr. Pearce	
Oligonucleotides		

5’ -> ACG TTT CCT GTC CCT GTG TG -> 3’ Common for Gad2^{CreER} mice	Integrated DNA technologies	11400
5’ -> AGG CAA ATT TTG GTG TAC GG -> 3’ Mutant for Gad2^{CreER} mice	Integrated DNA technologies	oIMR9074
5’ -> CAG ACG CTG CAG TCT TTC AG -> 3’ Wild type for Gad2^{CreER} mice	Integrated DNA technologies	oIMR3346
5’ -> ACA TGG TCC TGC TGG AGT TC -> 3’ Mutant Forward for ChR2 mice	Integrated DNA technologies	oIMR9102
5’ -> GGC ATT AAA GCA GCG TAT CC -> 3’ Mutant Reverse for ChR2 mice	Integrated DNA technologies	oIMR9103
5’ -> AAG GGA GCT GCA GTG GAG TA -> 3’ Wild type Forward for ChR2 mice	Integrated DNA technologies	oIMR9020
5’ -> CCG AAA ATC TGT GGG AAG TC -> 3’ Wild type Reverse for ChR2 mice	Integrated DNA technologies	oIMR9021
5’ -> CTG TTC CTG TAC GGC ATG G -> 3’ Mutant Forward for both tdTom mouse strains	Integrated DNA technologies	oIMR9105
5’ -> GGC ATT AAA GCA GCG TAT CC -> 3’ Mutant Reverse for both tdTom mouse strains	Integrated DNA technologies	oIMR9103
5’ -> AAG GGA GCT GCA GTG GAG TA -> 3’ Wild type Forward for both tdTom mouse strains	Integrated DNA technologies	oIMR9020
5’ -> CCG AAA ATC TGT GGG AAG TC -> 3’ Wild type Reverse for both tdTom mouse strains	Integrated DNA technologies	oIMR9021
5’ -> CTT CTC GCT AAG GTG GAT CG -> 3’ Mutant Forward for Arch3 mice	Integrated DNA technologies	12178
5’ -> CAC CAA GAC CAG AGC TGT CA -> 3’ Mutant Reverse for Arch3 mice	Integrated DNA technologies	12179
5’ -> TCC CAA AGT CGC TCT GAG TT -> 3’ Wild type Forward for Arch3 mice	Integrated DNA technologies	oIMR8713
5’ -> CTT TAA GCC TGC CCA GAA GA -> 3’ Wild type Reverse for Arch3 mice	Integrated DNA technologies	12177
5’ -> ATG AGC GAG GAG AAG TGT GG -> 3’ Common for VGLUT1^{cre} mice	Integrated DNA technologies	17904
5’ -> CCC TAG GAA TGC TCG TCA AG -> 3’ Mutant reverse for VGLUT1^{cre} mice	Integrated DNA technologies	12231
5’ -> GTG GAA GTC CTG GAA ACT GC -> 3’ Wild type reverse for VGLUT1^{cre} mice	Integrated DNA technologies	17905
Software and Algorithms		
Leica Application Suite X software	Leica Microsystems	
Clampfit 8.0	Axon Instruments	

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