Functional dynamics and selectivity of two parallel corticocortical pathways from motor cortex to layer 5 circuits in somatosensory cortex

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ABSTRACT

Long-range corticocortical pathways mediate direct interactions between the primary motor cortex (M1) and the somatosensory cortex (S1) and are likely critical for context-dependent sensory processing and sensorimotor integration. In the rodent whisker system, projections from M1 to S1 may be necessary for interpreting touch signals in the context of ongoing movement to drive behavior. However, understanding the function of these interareal interactions requires knowledge about the physiological properties of the synapses themselves and how specific classes of neurons integrate those signals. Here, we combined optogenetics and retrograde labeling with *in vitro* electrophysiology to characterize the synaptic properties of the connections between M1 and layer 5 (L5) intratelencephalic (IT) and pyramidal tract (PT) neurons in S1 of the mouse (both sexes) and how these two classes of neurons integrate those inputs. We found that M1 excitatory inputs to L5 IT cells depressed but had slow time courses that resulted in summation at the soma, whereas inputs to L5 PT cells facilitated and had faster time courses, resulting in less temporal summation at the soma. Differences in hyperpolarization-activated current (Ih) could partially explain the differences in subthreshold synaptic responses between L5 neurons. Functionally, we found that high-frequency M1 activity coupled more effectively with backpropagating action potentials within a narrow time window in PT neurons to trigger bursts at the soma. Our findings highlight the synaptic and cellular dynamics of two parallel pathways underlying the interactions between M1 and specific L5 circuits in S1.

INTRODUCTION

The remarkable ability of the mammalian neocortex to execute complex brain functions depends on long-range interactions between functionally related neocortical regions. Moreover, growing evidence indicates layer 5 (L5) pyramidal neurons, the primary output cells of the neocortex (Wise and Jones, 1977), are a major target of these projections (Petreanu et al., 2009; Dembrow et al., 2015; Kim et al., 2015; Kinnischtzke et al., 2016; Young et al., 2021), and that their activities may play a significant role during defined cortical computations (Xu et al., 2012; Cichon and Gan, 2015; Takahashi et al., 2016; Ranganathan et al., 2018; Takahashi et al., 2020; Mohan et al., 2023). In the rodent whisker sensorimotor system, the whisker primary somatosensory (S1) and motor cortex (M1) are massively interconnected (White and DeAmicis, 1977; Porter and White, 1983; Miyashita et al., 1994; Cauller et al., 1998), and their coordinated activities are critical for normal sensation, motor execution, and sensorimotor integration (Kleinfeld et al., 2006; Diamond et al., 2008).

Neural activity in M1 codes for various whisking parameters (Carvell et al., 1996; Brecht et al., 2004; Hill et al., 2011; Friedman et al., 2012; Huber et al., 2012; Petreanu et al., 2012; Sreenivasan et al., 2016), and the projections from M1 are generally thought to communicate these motor signals to S1, influencing sensorimotor integration and processing in individual neurons (Fee et al., 1997; Petersen, 2019). In S1, the axons of M1 pyramdial neurons arborize in L5/6 and ascend to ramify in L1 (Veinante and Deschenes, 2003). Although previous studies indicate that M1 axons make excitatory connections onto L5 pyramidal neurons in S1 (Petreanu et al., 2009; Rocco-Donovan et al., 2011; Zagha et al., 2013; Kinnischtzke et al., 2014; Kinnischtzke et al., 2016; Martinetti et al., 2022), understanding the nature of M1 influences requires information about the physiological properties of the connections and how L5 cells integrate those signals. A further complication is that L5 pyramidal neurons are grouped into two classes based on their axonal projections (Harris and Shepherd, 2015; Moberg and Takahashi, 2022). Intratelencephalic (IT) neurons project predominately to other neocortical areas, whereas pyramidal tract (PT) neurons project primarily to subcortical structures. In addition, IT and PT neurons have distinct biophysical membrane properties and morphological features that can significantly influence synaptic integration (Wise and Jones, 1977; Magee, 1999; Williams and Stuart, 2000; Hattox and Nelson, 2007; Groh et al., 2010; Morishima et al., 2011; Sheets et al., 2011; Dembrow et al., 2015; Harnett et al., 2015; Kinnischtzke et al., 2016; Anastasiades et al., 2018). They also appear to differ in the efficacy of action potentials to actively backpropagate into their dendrites (Stuart and Sakmann, 1994; Schiller et al., 1995; Larkum et al., 1999b; Grewe et al., 2010),

which may have important implications for how somatically generated action potentials interact with M1 input (Larkum et al., 1999a). Given these differences and their roles in corticocortical and cortical-subcortical communication, determining the physiological properties of M1 synapses and how L5 neurons integrate those signals is critical for understanding the nature of M1 influences on S1 output.

We previously found that long-range excitatory projections from M1 to S1 generally display synaptic facilitation (Martinetti et al., 2022), a form of short-term plasticity that enhances synaptic transmission when activated repetitively (Jackman and Regehr, 2017). However, we also found that some L5 cells receive depressing M1 synaptic inputs, reducing synaptic strength with activity (Zucker and Regehr, 2002). These differences and the two classes of L5 pyramidal neurons in S1 suggest that M1 synaptic properties could depend on the postsynaptic neuron and its long-range projection target. To test this, we combined optogenetic strategies and retrograde labeling techniques to excite M1 axons within S1, characterize their synaptic properties, and investigate how L5 IT and PT neurons integrate M1 signals. Our results support this hypothesis and suggest that M1 to S1 synaptic circuits are tailored to the divergent functions of the distinct L5 output neurons of S1.

MATERIALS AND METHODS

Animals. All experiments were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC). For this study, we used the following mouse lines: CD1 (ICR) (Charles River: 022) and Ai14 (Jackson Labs: 007908). Homozygous Ai14 mice were bred with CD1 (ICR) mice, resulting in heterozygous experimental mice for the Cre reporter allele. Animals were group-housed, maintained on a 12:12 hour light-dark cycle, and provided food and water ad libitum. We used both male and female mice in this study.

Stereotactic Virus Injections. All experiments followed previously described methods for stereotactic injections (Crandall et al., 2017; Martinetti et al., 2022). Briefly, mice (postnatal day 19-60, mean = 26 days) were anesthetized with a Ketaset-Dexdomitor mixture diluted in sterile saline (Ketaset, 70 mg/kg; Dexdomitor, 0.25 mg/kg; intraperitoneally). Once deeply anesthetized, eyes were covered with a thin layer of ophthalmic ointment to prevent drying (Patterson Veterinary Artificial Tears), and the mouse was head fixed into a digital stereotaxic setup with a warming base to maintain body temperature (Stoelting). Next, a small craniotomy

was made over the injection site, through which viruses or retrograde tracers were pressure injected into the brain via a glass micropipette attached to a Picospritzer pressure system (typically, 0.1-0.2 µL per injection site over 5-10 min). For optogenetic stimulation of M1 axons/terminals, an adeno-associated virus (AAV2) encoding genes for hChR2(H134R)-EYFP fusion proteins (rAAV2/hSyn-hChR2[H134R]-eYFP-WPREpA, AV4384, 3.1-3.5 x 10^{12} viral genomes/ml, University of North Carolina Viral Vector Core) was injected unilaterally into M1, as previously described (Martinetti et al., 2022). For retrograde labeling of PT neurons in S1 projecting to subcortical targets, mice were injected during the same procedure with either cholera toxin subunit B conjugated to Alexa Fluor 647 (CTB647; C34778 Thermo-Fischer), red fluorescent latex microsphere (Retrobeads, Lumafluor), or AAVretro-Cre (pAAV-Ef1a-mCherry-IRES-Cre, 55632-AAVrg, 1.1-3.5 x 10^{12} viral genomes/ml, Addgene) into the ipsilateral posterior medial nucleus of the thalamus (POM), the ipsilateral superior colliculus (SC), or the contralateral spinal trigeminal nucleus (SP5). For retrograde labeling of IT neurons in S1, mice were injected with CTB647 into either M1 (co-injection with the AAV2) or secondary somatosensory cortex (S2). Following injections, the pipette was left in place for an additional 5 min before being slowly withdrawn from the brain, and the scalp closed with a surgical adhesive (GLUture). After surgery, mice were given Antisedan (2.5 mg/kg) to reverse the effects of the Dexdomitor and allowed to recover on a heating pad for 1-2 h before returning to their home cage. Stereotaxic coordinates were relative to bregma: M1 = 1.25 mm lateral, 0.9 and 1.3 mm anterior, and 0.40 and 1.0 mm depth; S2 = 3.6 and 4.2 lateral, 1.1 mm posterior, and 1.95 and 1.16 mm depth; POM = 1.40 mm lateral, 1.27 mm posterior, and 2.84 mm depth; SC = 1.10 mm lateral, 3.23 mm posterior, and 2.25 mm depth; SP5 = 1.8 mm lateral, 6.4 mm posterior, and 4.1 mm depth.

In vitro slice preparation and live slice imaging. After allowing 21±2 days for ChR2 expression, acute coronal brain slices containing S1 ipsilateral to the M1 injection were prepared for *in vitro* recording and optical stimulation, as previously described (Crandall et al., 2010; Crandall et al., 2015; Martinetti et al., 2022). Briefly, mice were deeply anesthetized by inhalation of isoflurane before being decapitated. After decapitation, the brain was immediately removed and submerged in an ice-cold (~4˚C) oxygenated (95% O_2, 5% CO_2) cutting solution containing the following (in mM): 3 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$, 10 glucose and 234 sucrose. Brain slices (300 µm thick) were prepared using a vibrating tissue slicer (VT1200S, Leica Microsystems) and then transferred to an incubation chamber filled with warm (32˚C) oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. Slices were

allowed to recover at 32°C for 20 min and then at room temperature for a minimum of 40 min. Before recording, all live brain slices were imaged using a Zeiss Axio Examiner.A1 microscope equipped with a 2.5x objective (Zeiss EC plan-Neofluar) and an Olympus XM101R camera with cellSens software. Bright-field and fluorescence images were taken to confirm the accuracy of the injections, tissue health, and the overall expression of M1 axons/terminals and retrogradely filled cells in S1. We only recorded slices obtained from mice without signs of damage to the brain or off-target injections.

In vitro electrophysiological recordings and data acquisition. Brain slices were transferred to a submersion-type recording chamber continuously perfused (2-3 mL/min) with warm (32±1 ˚C) oxygenated ACSF containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 1.2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose. Neurons were visualized using infrared differential interference contrast optics (IR-DIC) and fluorescence microscopy using a Zeiss Axio Examiner.A1 microscope equipped with a 40x water immersion objective (Zeiss, W plan-Apo 40x/1.0 NA) and video camera (Olympus, XM10-IR). All electrophysiological recordings were made from the soma using borosilicate glass patch pipettes (tip resistance 3-6 MΩ) filled with a potassium-based internal solution containing (in mM): 130 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris and 14 phosphocreatine-K (pH 7.25, 290 mOsm). In some experiments, neurobiotin (0.25%, w/v; Vector Laboratories) was added to the internal solution to fill individual cells for subsequent morphological reconstruction. Cells were filled for 20-60 min before placing the slice in 4% paraformaldehyde in 0.1 M phosphate buffer solution. All voltages were corrected for a -14 mV liquid junction potential.

Electrophysiological data were acquired and digitized at 20 kHz using Molecular Devices hardware and software (MultiClamp 700B amplifier, Digidata 1550B4, pClamp 11). Signals were low-pass filtered at 10 kHz (current-clamp) or 3 kHz (voltage-clamp) before digitizing. During whole-cell recordings, the pipette capacitances were neutralized, and series resistances were compensated online (100% for current-clamp and 60-80% for voltage-clamp). Series resistances were continually monitored and adjusted during experiments to ensure sufficient compensation. ZD7288 (Tocris Cat. No. 1000) was prepared as recommended by the manufacturer and diluted in ACSF just before use. To minimize the off-target effects of the agent, a low concentration of ZD7288 (10 μM) was bath applied for a maximum period of 5 minutes (Harnett et al., 2015; Hsu et al., 2018) before subsequent experimental tests.

Photostimulation. ChR2 was optically excited using a high-power white light-emitting diode (LED) (Mightex LCS-5500-03-22) driven by a Mightex LED controller (SLC-AA02-US). Collimated light was reflected through a single-edge dichroic beam-splitter (Semrock FF660-

FDi02) and then a high magnification water immersion objective (Zeiss, W-Plan-Apo 40x/1.0 NA), resulting in an estimated spot diameter of ~1500 um and maximum LED power at the focal plane of ~32 mW (~18 mW/mm^2). Optical stimuli were delivered as 0.5 ms flashes and were directed at ChR2-expressing axons/terminals by centering the light over the cell bodies. LED on/off times were fast (< 50 µs) and of constant amplitude and duration when verified with a fast photodiode (Thorlabs, DET36A). The LED intensity was typically adjusted to evoke 100-200 pA EPSC in the recorded neuron when held in voltage-clamp at -94 mV, near the reversal potential for inhibition (Martinetti et al., 2022).

Histology, imaging, and neuronal reconstruction. Tissue for histology was prepared from acute coronal brain slices, as previously described (Crandall et al., 2017; Martinetti et al., 2022). Briefly, 300 µm thick coronal brain slices containing S1 were transferred to a 4% paraformaldehyde in 0.1 M phosphate buffer solution overnight at 4°C (18–24 hr). The next day, slices were changed to a 30% sucrose in 0.1 M phosphate buffer solution until re-sectioned (4°C; 2–3 days). Individual brain slices were re-sectioned at 50-100 µm using a freezing microtome (Leica SM2010 R). Sections were washed 2 times in 0.1 M phosphate buffer followed by 3 washes in 0.1 M phosphate buffer with 0.15 M NaCl, pH 7.4 (PBS, 5 min/wash). After washing, sections were incubated for 1 hr at room temperature in a blocking solution containing 0.1% Tween, 0.25% Triton X-100, 10% normal goat serum in PBS. Sections were then incubated using Streptavidin/Biotin Blocking Kit (Vector Labs, Cat # SP-2002), 30 minutes in streptavidin solution, followed by 30 minutes in biotin solution with a brief rinse of PBS after each. Sections were then incubated with Alexa Fluor 647-conjugated streptavidin (Thermo-Fisher Scientific, Cat #S21374, 1:1000, 2mg/ml) solution prepared in blocking solution for 3 hrs with rotation at room temperature. Following incubation, sections were washed 3 times in PBS and 2 times in 0.1 M phosphate buffer solution (10 min/wash) before being mounted and cover-slipped using Vectashield Vibrance w/DAPI (Vector Laboratories H-1800). Fluorescent conjugates of streptavidin were stored and prepared as recommended by the manufacturer. Neurobiotin-filled cells were initially identified and assessed using a Zeiss Axio Imager.D2 fluorescence microscope. If suitable, confocal image stacks of labeled neurons were taken using a Nikon A1 Laser Scanning Confocal Microscope with a 20X Plan apochromat Lambda D NA 0.8 objective and laser excitation 405nm, 488nm, 561nm, and 647nm. Image brightness and contrast were adjusted offline using Fiji software (Schindelin et al., 2012). All filled cells were reconstructed in three dimensions using the Simple Neurite Tracer Plugin within FIJI software.

Data analysis. Analysis of electrophysiological data was performed in Molecular

Devices Clampfit 11, Microsoft Excel, and Igor Pro. The synaptic responses to optical stimulation were recorded from postsynaptic neurons in whole-cell voltage-clamp and current-clamp, and the amplitude of an evoked EPSC/EPSP was measured relative to a baseline (1-10 ms) before the stimulus. The maximum amplitude of an evoked EPSP was measured relative to the pre-train baseline (10 ms). The amplitude was typically measured over the 10 ms immediately after stimulus onset, but in some cases, a 4.5 to 5 ms window was used. Intrinsic physiological properties were measured from rest using previously described methods (Crandall et al., 2017). Bursts were defined as 3 or more action potentials riding upon a slow depolarizing envelope with a mean inter-spike frequency greater than 150 Hz (Connors et al., 1982; McCormick et al., 1985). Cell depth measurements were calculated as the normalized distance from pia to white matter across the cortical column.

Statistical analysis. Statistical comparisons were performed in OriginPro 2019. The Shaprio-Wilk test was first applied to determine whether the data had been drawn from a normally distributed population; in this case, parametric tests were used (Paired t-test or two-sample t-test). If the assumption of normality was invalid, nonparametric tests were used (Wilcoxon paired signed-rank test or Mann-Whitney U test). A one-way analysis of variance (ANOVA) was used for multiple comparisons, and a two-way ANOVA was used to compare short-term synaptic dynamics across 20 Hz trains. All tests were two-tailed. All the data are presented as mean ± SEM, and statistical significance was defined as $p < 0.05$.

RESULTS

L5 IT and PT neurons in S1 respond differently to M1 stimulation.

To investigate M1 synaptic responses in L5 pyramidal neurons of S1 with distinct cortical or subcortical targets, we injected fluorescent retrograde tracers in combination with an adeno-associated virus (AAV2) carrying genes for a Channelrhodospin-2/enhanced yellow fluorescent protein (ChR2/EYFP) into M1 of Ai14 reporter mice *in vivo*. For most experiments, a far-red cholera toxin subunit B (CTB647) was injected into the ipsilateral M1 or secondary somatosensory cortex (S2) to label IT neurons or an AAVretro-Cre (Tervo et al., 2016) was injected into the ipsilateral posterior medial nucleus of the thalamus (POM), ipsilateral superior colliculus (SC), or contralateral spinal trigeminal nucleus (SP5) to label PT neurons. We chose CTB647 to label IT neurons because we observed minimal labeling in L5 using AAVretro-Cre (data not shown). Three weeks after injections, retrogradely labeled cells and ChR2/EYFP-expressing M1 axons/terminals were visible within S1 (**Figure 1A**). M1 terminal arbors were

densest in L1 and L5/6 of S1, consistent with previous studies (Veinante and Deschenes, 2003; Martinetti et al., 2022). Examining the labeled neurons revealed significant electrophysiological, anatomical, and morphological differences consistent with those previously reported for identified L5 IT and PT populations (Wise and Jones, 1977; Chagnac-Amitai et al., 1990; Mason and Larkman, 1990; Kasper et al., 1994; Hattox and Nelson, 2007; Groh et al., 2010; Morishima et al., 2011; Oberlaender et al., 2012; Kinnischtzke et al., 2016). Specifically, the input resistance of PT cells was smaller, input capacitance was larger, and membrane time constant was shorter when compared to IT cells (**Figure 1B, Table 1**). PT neurons had a more depolarized resting membrane potential, lower spike threshold, lower rheobase, and narrower spikes than IT cells. In addition, while most IT neurons could be classified as regular spiking (RS) neurons (96%), exhibiting robust spike frequency adaptation, many PT neurons (40.5%) could be characterized as intrinsically bursting (Connors et al., 1982; McCormick et al., 1985), responding to positive current injections with high-frequency bursts of action potentials. Anatomically, PT neurons were also located primarily in the lower part of L5 (L5b). Lastly, while both cell types had L1 projecting apical dendrites, PT neurons had large diameter apical dendrites with extensive branching in L1 (i.e., thick-tufted), whereas IT neurons had smaller diameter apical dendrites and less branching in L1 (i.e., thin-tufted) (**Figure 1C**).

To compare the synaptic properties of M1 inputs onto L5 IT and PT neurons in S1, we used whole-cell voltage clamp recordings to measure monosynaptic excitatory currents evoked by full-field photostimulation of M1 axons/terminals while holding the membrane potential near the reversal for inhibition (-94 mV). To control for variability in ChR2 expression across slices and mice, we compared synaptic dynamics of the M1 synapses onto each L5 neuron to those contacting L2/3 RS neuron because our previous work has shown that light stimulation of M1 arbors evokes facilitating synaptic excitation in these neurons (Martinetti et al., 2022). Pairs were recorded sequentially and in varying order from cells in the same slice and column. When using 20 Hz optical stimuli (paired-pulse and trains), we found stimulation drove short-latency (~2 ms) monosynaptic excitatory postsynaptic currents (EPSCs) with short-term synaptic dynamics that depended on the class of L5 neuron. EPSCs recorded in M1 and S2 projecting (M1p and S2p, respectively) neurons remained relatively unchanged or depressed weakly, while SCp, SP5p, and POMp responses exhibited synaptic facilitation (**Figure 2**). Although short-term plasticity was significantly different for both M1p and S2p neurons compared to control L2/3 RS cells, M1 responses in all 3 classes of PT neurons facilitated similarly to L2/3 neurons, increasing 57-86% across trains. Collapsing the data from

multiple subclasses into IT and PT groups revealed that despite equivalent synaptic strength, when normalized to their paired L2/3 RS cell, the short-term synaptic dynamics of the M1-evoked EPSCs were significantly different between L5 IT and PT neurons (**Figure 3A, B**).

To rule out the possibility that CTB caused abnormal short-term dynamics, we compared M1-evoked EPSCs in CTB-labeled M1p cells across multiple layers in S1. In contrast to M1p cells in L5, the EPSCs of M1p cells in L2/3 and L6 showed clear short-term facilitation (**Figure 3C, 3D**). Moreover, the magnitude of the short-term facilitation was similar to that observed in L5 PT neurons, increasing 70-82% across trains (**Figure 3B, 3D**). Therefore, CTB does not appear to cause abnormal short-term dynamics in L5 IT neurons.

These data confirm that long-range synapses from M1 to L5 of S1 excite both IT and PT neurons but reveal target-cell-specific differences in short-term plasticity. Specifically, the M1 to IT synapse displays modest frequency-dependent depression, whereas the M1 to PT synapse shows robust frequency-dependent facilitation. Furthermore, we show that M1 excitatory responses in IT neurons outside L5 undergo activity-dependent facilitation, suggesting short-term depression is unique for M1 synapses onto L5 IT neurons.

Integration of M1 synaptic inputs to L5 IT and PT neurons in S1

To determine the functional impact of differences in synaptic properties and intrinsic physiology, we next examined how L5 cells integrate M1 synaptic activity by recording their responses in current-clamp. Optical stimuli were again delivered in 20-Hz trains to active ChR2-expressing M1 arbors in S1 while controlling for the amplitude of the first synaptic potential (~2-3 mV). Furthermore, all cells were injected with a negative current to hold their membrane potential near the reversal for inhibition to minimize any impact of di-synaptic inhibition without adding $GABA_A$ and $GABA_B$ receptor blockers to the external solution. Note that all L5 IT data was collected from only M1p cells from this point forward. When recording from the soma, we found that trains of M1 stimulation evoked EPSPs in L5 M1p neurons that depressed weakly, whereas the EPSPs in all three classes of PT neurons facilitated (**Figure 4A-D**). This pattern is highly consistent with the synaptic currents described above. However, within the train, we observed significantly more summation in M1p neurons than all PT neurons (% summation by 10th pulse: M1p: 38.7 ± 5.6 %; SCp: 4.5 ± 2.5 %; SP5p: 5.7 ± 2.8 %; POMp: 1.5 ± 2.8 %; $p < 1.82E-6$, one-way ANOVA with a post hoc Bonferroni test; **Figure 4, bottom**). Thus, L5 PT and IT neurons integrate M1 inputs differently, with IT neurons showing greater somatic temporal summation of M1-evoked EPSPs.

We also found that individual EPSPs decayed much slower and had greater half-widths in M1p than in all PT neurons and that this difference in time course was consistent throughout the train (**Figure 5A, 5B**). Previous studies have shown that hyperpolarizion-actived cation (HCN) channels underlying h-current (Ih) can significantly impact the time course and summation of subthreshold PSPs in pyramidal neurons (Magee, 1999; Williams and Stuart, 2000; Sheets et al., 2011; Dembrow et al., 2015; Harnett et al., 2015; Anastasiades et al., 2018). Furthermore, work in the prefrontal cortex suggests Ih contributes to differences in the time course of subthreshold potential observed between distinct classes of L5 pyramidal neurons (Dembrow et al., 2015; Anastasiades et al., 2018). If Ih is involved in increasing the decay and decreasing the overall temporal integration of M1 synaptic potentials in L5 PT neurons, then blocking HCN channels should broaden individual EPSPs and enhance the overall summation to a greater extent than for IT neurons. Indeed, performing similar M1 stimulation experiments in the presence of ZD-7288 (10 μM) to block HCN channels (Harris and Constanti, 1995; Magee, 1999; Williams and Stuart, 2000; Kole et al., 2006; Harnett et al., 2015) revealed that PT neurons displayed more than a 2-fold increase in half-width, decay, and summation, as compared to only 1.3-1.6-fold for IT neurons (**Figure. 5C-F**). ZD-7288 did not affect the relative amplitude of M1-evoked EPSPs in PT neurons (Control: 4.9 ± 0.9 mV, ZD: 4.6 ± 0.7 mV, $p = 0.40168$, Wilcoxon paired signed-rank test), but did increase the amplitude in IT neurons (Control: 5.9 ± 0.4 mV, ZD: 7.8 ± 7.8 mV, $p = 0.02068$, Paired t-test; data not shown). Therefore, the weaker temporal summation of M1 EPSPs in L5 PT than IT neurons is at least partially attributable to a difference in their h-currents.

The effect of coupling M1 inputs with backpropagating action potentials

Excitatory M1 synapses not only target the basal dendrites of L5 pyramidal neurons but are also found on their apical tuft in L1 (Petreanu et al., 2009), where they can interact with backpropagating action potentials (bAPs) to trigger large amplitude calcium spikes, leading to high-frequency burst firing (Stuart and Sakmann, 1994; Schiller et al., 1995; Larkum et al., 1999b; Larkum et al., 1999a; Grewe et al., 2010). It is also clear from the present findings that the magnitude and time course of M1-evoked responses depends on the pattern of presynaptic activity. As such, short trains of M1 synaptic input might couple more effectively with bAPs to trigger bursts of action potentials in the soma of L5 neurons. To test this, we next performed current-clamp recordings from the somata of labeled L5 IT (M1p) and PT (POMp) neurons to examine how optical M1 stimuli couple with somatic action potentials. We chose to test the POMp neurons as our PT population because these cells showed the highest

propensity to burst when depolarized with positive current injections (**Table 1**). All cells were injected with direct current to produce a similar resting membrane potential at the soma (approx. -77 mV), and synaptic inhibition was intact throughout the recording. When M1 axons were activated in isolation using a single, low-intensity light pulse, somatic responses were always subthreshold (2.6 ± 0.4 mV, n = 17 cells; **Figure 6a**). Short 20 Hz trains of the same light intensity were also subthreshold, rarely generating somatic APs (only 1 of 17 cells or 2 of 99 total trials; **Figure 6B**), whereas brief positive current injections at the soma (1-35 ms, typically 5 ms; 0.35-1.85 nA) reliably evoke single APs (98 of 99 total trials; **Figure 6C**). For L5 M1p neurons, combining a single or short train of M1 synaptic input and a somatic AP did not generate a burst (0 of 7 cells, or 0 of 38 total trials) or significantly change their overall spike output ($Soma_{inj}$: 1.0 ± 0.0 APs; $+LED_{single}$: 1.1 ± 0.1 APs; $+LED_{train}$: 1.0 ± 0.0 APs; **Figure 6D-G**).

In contrast, short trains of M1 synaptic input coupled with a somatic AP could elicit a high-frequency burst of 3-4 APs (3.3 ± 0.3 APs; frequency: 213.4 ± 13.8 Hz) at the soma of some POMp neurons (4 of 10 cells or 23 of 61 total trials), resulting in a significantly greater mean spike output than pairing a somatic AP and a single M1 synaptic input ($Soma_{inj}$: 1.0 ± 0.0 APs; $+LED_{single}$: 1.1 ± 0.1 APs; $+LED_{train}$: 2.0 ± 0.3 APs; **Figure 6D-G**). For most POMp neurons, coupling a single light pulse (same intensity) and a somatic action potential was ineffective in evoking a high-frequency burst (observed bursting in only 1 of 10 cells or 1 of 61 total trials; **Figure 6G**). Consistent with the faster time course of M1-evoked EPSPs for PT neurons, we also found that the optimal time for generating a burst with a short train of M1 stimuli was very narrow, typically 0-4 ms after the somatic AP (or 2-6 ms when accounting for a 2 ms synaptic latency; **Figure 7**). For intervals of 10 ms or greater, the probability of eliciting a burst with a train of M1 input fell quickly (**Figure 7B, 7C**).

DISCUSSION

One of the most prominent structural features of the neocortex is the presence of numerous corticocortical pathways interconnecting areas (Rockland and Pandya, 1979; Felleman and Van Essen, 1991). While it is generally thought that these pathways convey contextual information, such as attention, expectation, and action command (Gilbert and Li, 2013), we are only beginning to understand how these inputs influence their target regions. Realizing the nature of these influences requires information about the physiological properties of the connections themselves and how specific cell types integrate those signals. Here, we show

that M1 synaptic inputs to distinct classes of L5 neurons in S1 are dramatically different. M1 synaptic currents in IT cells displayed short-term depression, but responses summated at the soma due to the longer time course of their subthreshold potentials. In contrast, inputs to PT cells facilitated with repetitive stimulation and had faster time courses, resulting in less temporal summation at the soma. Differences in Ih could partially explain the differences in kinetics and temporal summation. We also found differences in the functional impact of M1 input. Specifically, during high-frequency trains of synaptic inputs, EPSPs later in trains coupled more effectively with bAPs within a narrow time window in PT cells than IT neurons to generate somatic bursts. Thus, L5 IT and PT neurons integrate M1 inputs with distinct temporal dynamics. These physiological differences in the connections between M1 and S1 L5 circuits may be specializations related to their particular functions during sensorimotor processing.

Selective optogenetic stimulation of axons has been used to study postsynaptic targets, synapse locations, strength, and the impact of M1 input on various excitatory and inhibitory cells in S1 (Petreanu et al., 2009; Lee et al., 2013; Zagha et al., 2013; Kinnischtzke et al., 2014; Kinnischtzke et al., 2016; Shen et al., 2022). However, our understanding of their short-term dynamics has lagged due to the complications with using opsins to study synapses (Jackman et al., 2014). Recently, our lab has addressed these challenges and found that M1-evoked responses in S1 facilitated with repetitive activity. While this is true for most M1 to S1 connections, we found responses in a subset of L5 neurons that displayed short-term depression (Martinetti et al., 2022).

The present study is the first to demonstrate that this diversity in short-term dynamics depends on the postsynaptic L5 neuron and their axonal projection target, with M1 synapses onto IT cells depressing and those onto PT cells facilitating. The relevance of such variable short-term dynamics in corticocortical projections is unclear. Previous work has suggested that depressing synapses provide a dynamic gain-control mechanism that enables them to respond proportionally to the percent change in firing frequency rather than the absolute change (Abbott et al., 1997). Thus, during periods of elevated M1 activity, such as bouts of whisking, depressing signals may enable L5 IT cells to encode relative changes in M1 firing. In contrast, since facilitating synapses act as high-pass filters (Jackman and Regehr, 2017), PT cells may be less responsive to transient stimuli but ideally suited to transmit and amplify salient high-frequency M1 signals.

Importantly, M1 inputs onto IT neurons in other layers facilitated with repetitive activity, indicating that short-term depression is a unique property of synapses connecting with L5 IT

neurons and not a general rule of all M1 connections to IT cells in S1. These findings provide physiological evidence for cell-type and layer-specific corticocortical connection specificity at a synaptic physiology level. These results complement previous findings of target-cell-specific short-term dynamics in local cortical circuits (Markram et al., 1998; Reyes et al., 1998; Beierlein et al., 2003; Lefort and Petersen, 2017) and highlight how the specificity and synaptic dynamics of connections also govern long-range neuronal circuits underlying the interactions between cortical areas.

The diversity in short-term dynamics found here is consistent with earlier studies investigating the pathways linking secondary and primary sensory cortices and the callosal pathway within the prefrontal cortex (Covic and Sherman, 2011; De Pasquale and Sherman, 2011; Lee et al., 2014). However, two of these studies (Covic and Sherman, 2011; De Pasquale and Sherman, 2011) suggest that corticofugal neurons receive depressing inputs, contrasting the present results. We also found that M1 input strength was similar across L5 neurons, including M1p cells projecting back to M1 (looped neurons), consistent with previous studies examining the pathways linking M1 to S1 and the retrosplenial cortex to the secondary motor cortex (Kinnischtzke et al., 2016; Yamawaki et al., 2016). Conversely, in visual cortices, long-range corticocortical inputs preferentially target looped L5 IT neurons (Young et al., 2021). Whether these discrepancies are due to methodological differences or represent distinct connectivity features between these pathways is unclear. Indeed, recent studies using similar optical strategies have begun revealing key differences between corticocortical pathways (Naskar et al., 2021; Martinetti et al., 2022; Shen et al., 2022). Thus, further studies are needed to determine if corticocortical synapses onto L5 neurons follow general or specialized rules depending on the area and projection type.

In addition to receiving inputs with distinct temporal dynamics, we also found L5 neurons integrate M1 signals differently. Specifically, the EPSP decay times and half-widths were longer in IT neurons than PT cells, resulting in robust temporal summation during trains. This evidence is the first of our knowledge to demonstrate how distinct L5 neurons in S1 respond to repetitive M1 inputs and complement the differences in input summation reported for L5 neurons in the prefrontal cortex (Dembrow et al., 2015). The differences imply that IT neurons behave more as temporal integrators, summating temporally dispersed M1 synaptic events over a broad time window, whereas PT neurons behave as coincidence detectors, summating temporally coincident inputs over a narrow time window (Konig et al., 1996). Functionally, it has been proposed that neurons operating as temporal integrators and coincidence detectors are well suited to perform rate and synchrony coding, respectively

(Ratte et al., 2013).

Differences in the attenuating effects exerted by Ih are partially responsible for the cell-type-specific differences in EPSP kinetics and temporal summation observed in this study. Of course, we cannot exclude the possibility that M1-mediated feedforward inhibition also influenced the time course of EPSPs (Pouille and Scanziani, 2001; Gabernet et al., 2005; Mittmann et al., 2005). Furthermore, we cannot rule out additional physiological properties, such as differential synapse locations, expression of synaptic receptors, or other voltage-activated ion channels (Kumar and Huguenard, 2003; Petreanu et al., 2009; Lafourcade et al., 2022). Nevertheless, our results are consistent with work highlighting the importance of Ih current in normalizing temporal summation in L5 pyramidal neurons (Magee, 2000; Williams and Stuart, 2000; Berger et al., 2001; Harnett et al., 2015) and the idea that PT neurons express higher levels of Ih compared to IT cells (Dembrow et al., 2010; Sheets et al., 2011; Dembrow et al., 2015; Anastasiades et al., 2018).

M1 axons projecting to S1 terminate densely in L1, where they make connections with the apical tuft dendrites of L5 neurons (Cauller et al., 1998; Veinante and Deschenes, 2003; Petreanu et al., 2009). Although single, local depolarizing events in the dendrites undergo significant voltage attenuation (Rall, 1967; Williams and Stuart, 2002), distal dendritic depolarization coupled with single bAPs can trigger dendritic calcium spikes and somatic bursts (Larkum et al., 1999a). Such somatodendritic coupling has been proposed as a cellular mechanism through which L5 neurons implement translaminar input associations (Larkum, 2013). Consistent with this hypothesis and recent work (Shen et al., 2022), we showed that M1 synaptic inputs triggered bursting in PT neurons when paired with temporally precise somatic spikes. Moreover, we demonstrate that the probability of generating somatic bursts significantly increased with high-frequency input (most likely due to the presynaptic facilitation) while maintaining a narrow time window for somatodendritic coupling. This frequency-dependent boosting and the narrow time window for burst initiation suggest that S1 PT neurons favor high-frequency, coincident M1 inputs. In contrast, under the same experimental conditions, we found that the coincidence of bAPs with either low- or high-frequency M1 input did not change the output of IT cells, indicating they are less sensitive to synchronous M1 events. Altogether, these results demonstrate that the impact of M1 inputs on spiking output is markedly different between L5 PT and IT cells, and that synaptic facilitation can influence dendritic integration and output of PT neurons.

The results we describe here indicate that the M1 to S1 corticocortical projection comprises two parallel but dynamically distinct systems of synaptic excitation in L5, each

defined by the short-term plasticity of its synapses, the postsynaptic cell type targeted, and the integration of its inputs. Because of their unique dynamic properties, distinct L5 projection neurons are likely to be differentially engaged by patterns of M1 activity that vary in frequency and timing. Our work complements previous *in vivo* work demonstrating the importance of motor-associated synaptic inputs and active dendritic processing in generating L5 activity as well as the differences in activity between IT and PT neurons during active whisker-mediated behavior (Xu et al., 2012; Manita et al., 2015; Ranganathan et al., 2018; Takahashi et al., 2020; de Kock et al., 2021).

FIGURE LEGENDS

Figure 1. ChR2-EYFP expression in M1 projection neurons and retrograde identification of distinct L5 projection neurons in the mouse S1. **A**, AAV-encoding ChR2-EYFP was injected into the ipsilateral M1 to label their axonal projections in S1. Retrograde fluorescent tracers (CTB-647, rAAV-Cre, or microspheres) were also injected into M1 or S2 to label subclasses of IT neurons, or SC, SP5, or POM to label different subclasses of PT neurons. EYFP expression in M1 axons/terminals (far left image) and retrogradely labeled neurons in L5 were evident 3 weeks post-injection. **B-C**, Whole-cell recordings (**B**) and neurobiotin filling (**C**) of L5 pyramidal neurons not only revealed that labeled cells were healthy and had apical dendrites that terminated in tufts near the pia but also confirmed the physiological and morphological differences between L5 IT and PT neurons (Succesful fills with apical tufts: n = 3 M1p, 4 S2p, 3 SCp, 1 SP5p, and 8 POMp neurons).

Figure 2. L5 projection neurons in S1 are excited differently by long-range M1 inputs. **A-E**, Top: Average EPSCs evoked by two optical stimuli (LED, Purple arrows, 0.5 ms pulse duration) delivered at a 50 ms interval (20 Hz). Data are shown from L2/3 neurons paired to various L5 IT and PT subtypes, including L5 M1p (**A**), L5 S2p (**B**), L5 SCp (**C**), L5 SP5p (**D**), L5 POMp (**E**). Vertical scale bars, 50 pA (**A-E**). Bottom: Summary of paired-pulse ratios for a 50-ms interstimulus interval. Asterisks denote a significant difference from responses evoked in control L2/3 RS neurons (M1p: 1.05 ± 0.06, L2/3: 1.33 ± 0.05, n = 14 pairs, 9 mice; p = 0.0073, paired t-test; S2p: 0.91 ± 0.07, L2/3: 1.20 ± 0.10, n = 6 pairs, 5 mice; p = 0.00329, paired t-test; SCp: 1.32 ± 0.12, L2/3: 1.45 ± 0.06, n = 8 pairs, 4 mice; p = 0.34802, paired t-test; SP5p: 1.30 ± 0.10, L2/3: 1.51 ± 0.11, n = 8 pairs, 4 mice; p = 0.18343, Wilcoxon paired signed-rank test; POMp: 1.36 ± 0.05, L2/3: 1.30 ± 0.0.11, n = 11 pairs, 7 mice; p = 1.0, Wilcoxon paired signed-rank test). **F-J**, Top: Average EPSCs evoked by a 20 Hz train of optical stimuli recorded in a single L5 M1p neuron (**F**), a L5 S2p neuron (**G**), a L5 SCp neuron (**H**), a L5 SP5p neuron (**I**), and a L5 POMp neuron (**J**). Vertical scale bars, 100 pA (**F-J**). Bottom: Summary of EPSC amplitudes plotted as a function of stimulus number within 20 Hz trains for all L2/3–L5 pairs (normalized to first responses) (M1p vs. L2/3: p = 2.88E-20; S2p vs. L2/3: p = 4.37E-10; SCp vs. L2/3: p = 0.18018; SP5p vs. L2/3: p = 0.24162; POMp vs. L2/3: p = 0.09125; two-way ANOVA, stim. 2–10). EPSCs were recorded at -94 mV in voltage-clamp, near the reversal for inhibition, and the light intensity for each cell was set to obtain an initial peak of 100-200 pA. Values are represented as mean ± SEM.

Figure 3. Synaptic responses during repetitive M1 activation are less facilitating for L5 IT than L5 PT neurons. **A**, L5 IT and PT neurons receive similar strength M1 inputs. EPSCs of both cells were normalized to control L2/3 RS neurons (IT: 14 cells, 11 mice; PT: 14 cells, 10 mice; p = 0.66247, Mann-Whitney U test). Bars represent the median (**A**). **B**, Summary of short-term plasticity for all L5 IT (M1p and S2p) and PT neurons (SCp, SP5p, and POMp) to a pair of 20 Hz optical stimuli (left) and a 20 Hz optical stimulus train (right) (data combined from Figure 2) (PPR: L5 IT: 1.01 ± 0.05, n = 20 cells, 15 mice; L5 PT: 1.33 ± 0.06, n = 27 cells, 15 mice; p = 1.89E-4, Mann-Whitney U Test; Train: p = 1.53E-30, two-way ANOVA, stim. 2–10). **C**, Average EPSCs evoked by a 20 Hz train of optical stimuli recorded in a single L2/3 and L6 M1p neuron labeled with CTB. **D**, Summary of EPSC amplitudes plotted as a function of stimulus number within 20 Hz trains for CTB-labeled M1p neurons in L2/3 (n = 8 cells, 3 mice), L5 (n = 14 cells, 9 mice), and L6 (n = 9 cells, 4 mice) (normalized to first responses) (* indicates p < 2.23E-18, two-way ANOVA, stim. 2–10). L5 M1p data from Figure 2. Values are represented as mean ± SEM (**B, D**).

Figure 4. Trains of M1 synaptic potentials showed less temporal summation in L5 PT than L5 IT neurons. **A-D**, Top: Average EPSPs evoked by a 20 Hz train of optical stimuli recorded in a single L5 M1p neuron (**A**), L5 SCp neuron (**B**), L5 SP5p neuron (**C**), and L5 POMp neuron (**D**). Responses were recorded at -94 mV in current-clamp, near the reversal for inhibition, and the light intensity was set to obtain an initial sub-threshold EPSP of ~3 mV. Bottom: Summary plots showing the average EPSP amplitude and summation (maximal depolarization) as a function of stimulus number within trains (normalized to the first response). (M1p: p = 3.18E-7; SCp: p = 0.58747; SP5p: p = 0.31794; POMp: p = 0.98772, two-way ANOVA, stim. 2–10). Values are represented as mean ± SEM.

Figure 5. Blockade of Ih channels enhanced the time course and temporal summation of M1 synaptic potentials more in L5 PT than IT neurons. **A**, Average of the first EPSP in a train, scaled to match the amplitude, evoked by an optical stimulus recorded in a single L5 M1p, SCp, SP5p, and POMp neuron. **B**, Summary plots showing that decay time of the first and fifth EPSP in a train were greater for L5 M1p than SCp, SP5p, and POMp neurons (First EPSP decay tau: M1p: 24.6 ± 2.4 ms, n = 6 cells, 3 mice; SCp: 15.1 ± 1.6 ms, n = 9 cells, 4 mice; SP5p: 16.6 ± 1.1 ms, n = 6 cells, 2 mice; POMp: 13.5 ± 1.4 ms, n = 5 cells, 5 mice; * indicates p < 0.03, one-way ANOVA with a post hoc Bonferroni test; Fifth EPSP decay tau: M1p: 26.2 ± 2.8 ms; SCp: 16.6 ± 2.0 ms; SP5p: 15.4 ± 0.7 ms; POMp: 14.4 ± 1.0 ms; * indicates p < 0.009,

one-way ANOVA with a post hoc Bonferroni test). C, Average EPSPs recorded under control conditions and in the presence of ZD7288 (10 μM) for a single L5 IT (M1p) and PT (SCp) neuron. D, Summary plots showing that the increase in EPSP half-width and decay tau was more significant in L5 PT than IT neurons (Half-width: $p = 0.0294$; Decay tau: $p = 0.01534$, two-sample t-test). C, Average EPSPs evoked by a 20 Hz train of optical stimuli under control conditions and in the presence of ZD7288 (10 μM) for a single L5 M1p and SCp neuron. D, Summary plot showing the change in the integral of EPSP trains for L5 IT and PT neurons ($p = 0.06$, two-sample t-test). Responses were recorded at -94 mV, near the reversal for inhibition, in current-clamp, and the light intensity was set to obtain an initial sub-threshold EPSP of ~5 mV. Values are represented as mean ± SEM.

Figure 6. Coupling a short train of M1 synaptic inputs with a single spike at the soma increases the spike output and burst probability in L5 PT but not IT neurons. A-B, Subthreshold EPSPs evoked by a single optical stimulus (A) or the 5th pulse of a 20 Hz train of stimuli (B) for a single L5 M1p and POMp neuron. A single EPSP or the first in a train only produced a voltage response of 2-3 mV at the soma and never reached the threshold for either an action potential or calcium-mediate action potential. (C) A short threshold current injection (typically 5 ms) at the soma evoked a single action potential in both cell types. (D-E) Voltage responses to combining somatic action potential (used in C) with a single EPSP (used in A) or the EPSP evoked by the 5th pulse of a 20 Hz train (used in B) separated by an interval of 3-4 ms between the start of the somatic current injection and that of the light pulse. The mean synaptic latency from the onset of the light was 2.3 ± 0.1 ms (n = 17 cells; 11 mice). F-G, Group data summarizing the action potential output (F) and the fraction of cells that generated a burst (G) at the soma during coupling for each L5 cell type. Coupling a somatic action potential with the 5th pulse of an optical train produced significantly more spikes (* indicates $p < 0.006$, one-way ANOVA) and burst responses for the L5 POMp neurons. There was no difference in spike output ($p = 0.258$, one-way ANOVA) or the number of cells bursting for the M1p cells. The bars in F represent the means.

Figure 7. Coupling a train of M1 synaptic inputs with a somatic action potential requires precise timing. A , Voltage responses of a POMp neuron to combining a somatic action potential with the EPSP evoked by the 5th pulse of a 20 Hz train separated by different time intervals (-4, -2, 0, 2, 4, 6-8, 9-13, and 13+ ms). Optically evoked synapse responses had synaptic delays with short onset latencies (~2 ms). B-C, Plots show that this cell produced

more spikes and had a higher incidence of bursting when the onset of the 5th pulse was 0-6 ms after the somatic action potential.

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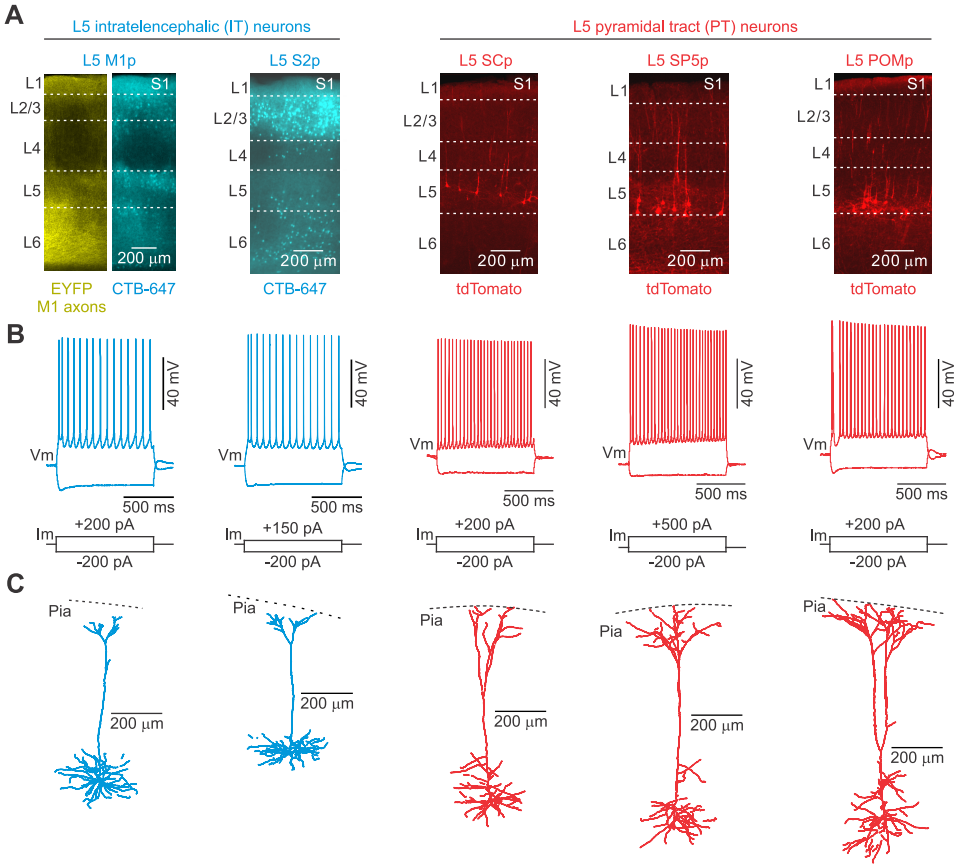
A
L5 intratelencephalic (IT) neurons
L5 pyramidal tract (PT) neurons
L5 M1p
L5 S2p
L5 SCp
L5 SP5p
L5 POMp
L1
L2/3
L4
L5
L6
S1
200 µm
EYFP
M1 axons
CTB-647
tdTomato
B
40 mV
Vm
500 ms
Im
+200 pA
+150 pA
+500 pA
-200 pA
C
Pia

Figure 1

Table 1 Electrophysiological properties of excitatory projection neurons in L5 of mouse primary somatosensory cortex

	L5 Intratelencephalic (IT) neurons		L5 Pyramidal Tract (PT) Neurons						
	M1p	S2p	SP5p	SCp	POMp	All ITs	All PTs	P-value	Test
Cell depth normalized to pia	0.52 ± 0.01	0.54 ± 0.01	0.65 ± 0.00	0.63 ± 0.01	0.62 ± 0.01	0.53 ± 0.01	0.63 ± 0	1.69E-12	MW test
RMP (mV)	-83.3 ± 1.2	-84.7 ± 1.3	-84.9 ± 0.7	-82.8 ± 1.1	-79.0 ± 0.5	-83.8 ± 0.9	-81.5 ± 0.5	0.02156	MW test
R_m (MΩ)	114.0 ± 10.1	87.4 ± 8.4	73.5 ± 4.8	65.4 ± 4.4	78.8 ± 5.5	105.2 ± 7.6	74.0 ± 3.1	1.38E-04	MW test
τ_m (ms)	17.5 ± 1.1	15.2 ± 1.7	13.0 ± 0.6	14.7 ± 1.1	15.4 ± 0.9	16.7 ± 0.9	14.5 ± 0.5	0.31490	t-test
C_m (pF)	166.0 ± 13.1	174.2 ± 12.9	184.0 ± 10.2	227.2 ± 13.4	212.4 ± 15.4	168.7 ± 9.6	207.3 ± 8.4	0.01140	MW test
Rheobase (pA)	100.9 ± 8.1	111.1 ± 11.1	121.1 ± 11.0	89.5 ± 8.2	72.2 ± 6.1	103.7 ± 6.5	89.2 ± 5.1	0.04767	MW test
Spike threshold (mV)	-58.1 ± 0.8	-59.3 ± 1.1	-61.0 ± 0.5	-62.5 ± 1.0	-63.5 ± 0.5	-58.5 ± 0.6	-62.6 ± 0.4	3.64E-06	MW test
Spike amplitude (mV)	90.0 ± 1.5	91.9 ± 1.6	92.5 ± 1.1	91.6 ± 1.3	96.5 ± 1.2	90.6 ± 1.1	94.2 ± 0.7	0.01243	t-test
Spike half-width (mV)	0.61 ± 0.02	0.69 ± 0.04	0.54 ± 0.01	0.51 ± 0.01	0.57 ± 0.03	0.63 ± 0.02	0.55 ± 0.01	7.69E-06	MW test
Max rate of rise (mV*ms)	591.5 ± 20.5	588.5 ± 17.4	632.8 ± 16.3	618.9 ± 19.0	660.2 ± 22.0	590.5 ± 14.7	642.6 ± 12.5	0.02296	t-test
Max rate of decay (mV*ms)	-132.6 ± 5.1	-116.2 ± 11.3	-152.0 ± 5.3	-164.4 ± 4.0	-167.4 ± 5.1	-127.1 ± 5.2	-162.7 ± 3.1	2.73E-07	MW test
RMP - threshold (mV)	24.5 ± 0.9	25.4 ± 1.5	23.9 ± 0.8	20.1 ± 1.1	15.6 ± 0.6	24.8 ± 0.8	18.9 ± 0.6	9.55E-07	t-test
Voltage sag amplitude (mV)	-3.3 ± 0.4	-3.4 ± 0.4	-3.4 ± 0.3	-3.4 ± 0.3	4.3 ± 0.3	-3.4 ± 0.3	-3.8 ± 0.2	0.14805	MW test
Percent with bursts	0.0 (0/19)	11.1 (1/9)	26.3 (5/19)	36.8 (7/19)	50.0 (18/36)	3.6 (1/28)	40.5 (30/74)		
	(n = 18-19 cells, 11 mice)	(n = 9 cells, 4 mice)	(n = 19 cells, 7 mice)	(n = 16-19 cells, 6 mice)	(n = 28-36 cells, 18 mice)	(n = 26-28 cells, 15 mice)	(n = 62-74 cells, 31 mice)		

* See methods for an explanation of how the electrophysiological parameters were defined/measured. Data shown as mean ± s.e.m.

* All membrane potentials were corrected for a -14 mV liquid junction potential

* Two sample t Test (t-test); Mann-Whitney Test (MW test)

Table 1

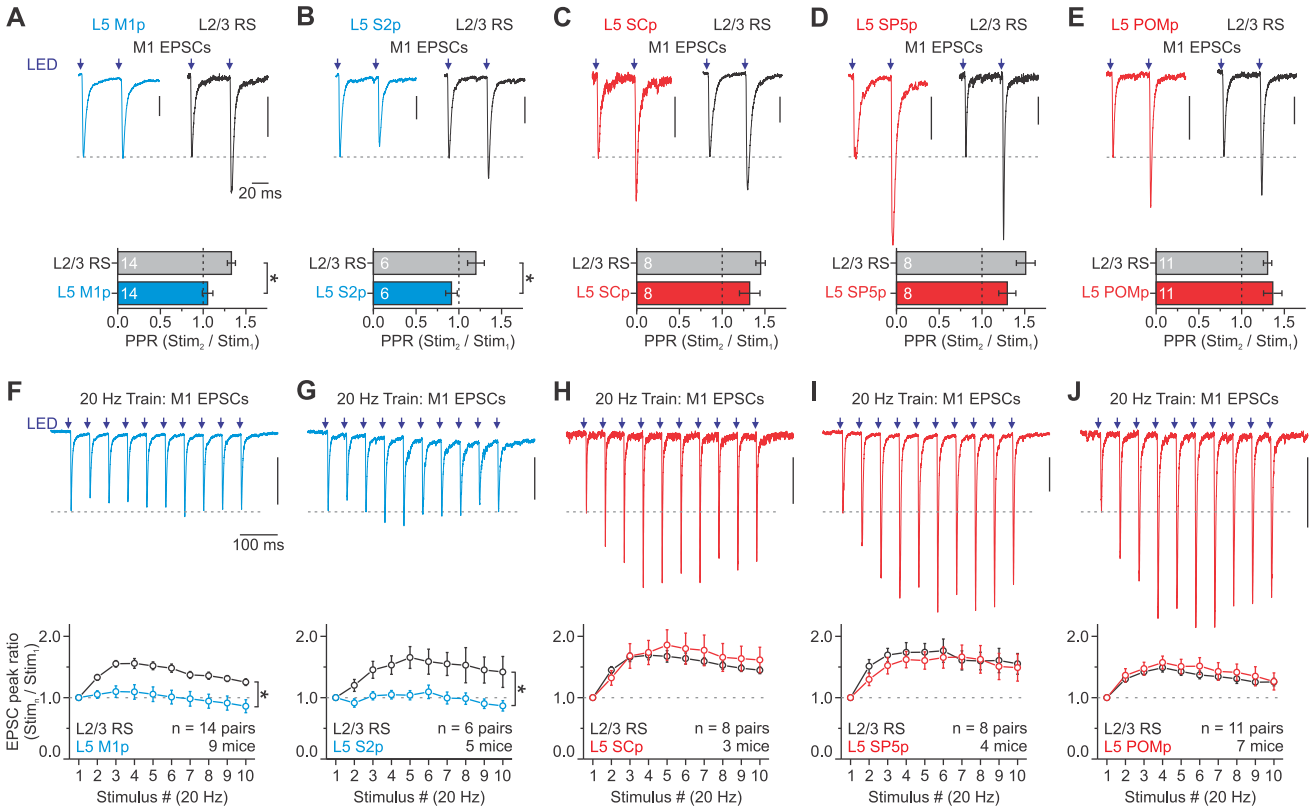
A
L5 M1p
L2/3 RS
M1 EPSCs
LED
20 ms
L2/3 RS 14
L5 M1p 14
PPR (Stim2 / Stim1)
B
L5 S2p
L2/3 RS
M1 EPSCs
L2/3 RS 6
L5 S2p 6
PPR (Stim2 / Stim1)
C
L5 SCp
L2/3 RS
M1 EPSCs
L2/3 RS 8
L5 SCp 8
PPR (Stim2 / Stim1)
D
L5 SP5p
L2/3 RS
M1 EPSCs
L2/3 RS 8
L5 SP5p 8
PPR (Stim2 / Stim1)
E
L5 POMp
L2/3 RS
M1 EPSCs
L2/3 RS 11
L5 POMp 11
PPR (Stim2 / Stim1)
F
20 Hz Train: M1 EPSCs
LED
100 ms
G
20 Hz Train: M1 EPSCs
H
20 Hz Train: M1 EPSCs
I
20 Hz Train: M1 EPSCs
J
20 Hz Train: M1 EPSCs
EPSC peak ratio (Stim2 / Stim1)
L2/3 RS
L5 M1p
n = 14 pairs
9 mice
L2/3 RS
L5 S2p
n = 6 pairs
5 mice
L2/3 RS
L5 SCp
n = 8 pairs
3 mice
L2/3 RS
L5 SP5p
n = 8 pairs
4 mice
L2/3 RS
L5 POMp
n = 11 pairs
7 mice
Stimulus # (20 Hz)

Figure 2

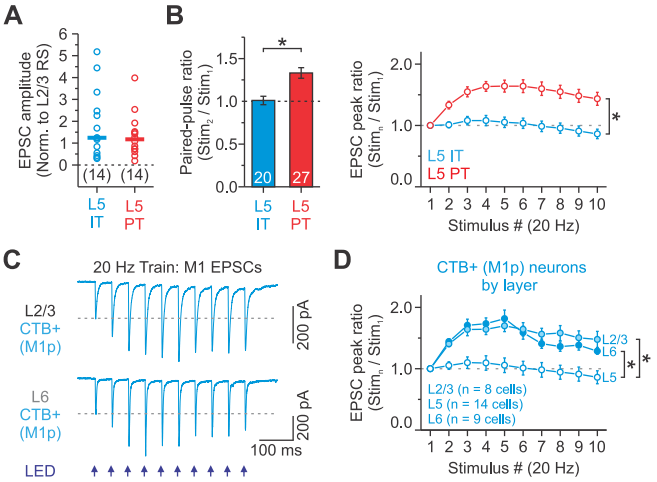
A
EPSC amplitude
(Norm. to L2/3 RS)
6
5
4
3
2
1
0
(14)
(14)
L5
IT
L5
PT
B
Paired-pulse ratio
(Stim2 / Stim1)
1.5
1.0
0.5
0.0
*
20
27
L5
IT
L5
PT
EPSC peak ratio
(Stimn / Stim1)
2.0
1.0
0.0
L5 IT
L5 PT
*
1 2 3 4 5 6 7 8 9 10
Stimulus # (20 Hz)
C
20 Hz Train: M1 EPSCs
L2/3
CTB+
(M1p)
200 pA
L6
CTB+
(M1p)
200 pA
100 ms
LED
D
CTB+ (M1p) neurons
by layer
EPSC peak ratio
(Stimn / Stim1)
2.0
1.0
0.0
L2/3
L6
L5
*
*
L2/3 (n = 8 cells)
L5 (n = 14 cells)
L6 (n = 9 cells)
1 2 3 4 5 6 7 8 9 10
Stimulus # (20 Hz)

Figure 3

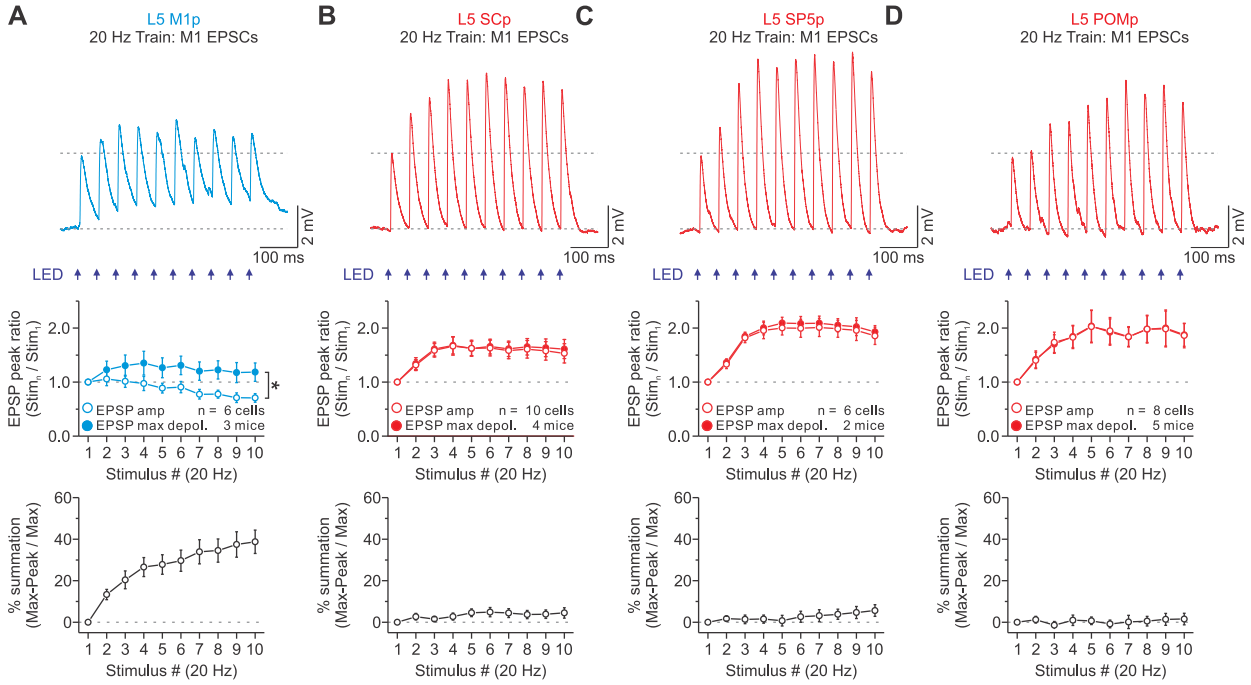
A
L5 M1p
20 Hz Train: M1 EPSCs
B
L5 SCp
20 Hz Train: M1 EPSCs
C
L5 SP5p
20 Hz Train: M1 EPSCs
D
L5 POMp
20 Hz Train: M1 EPSCs
2 mV
100 ms
LED
EPSP peak ratio (Stim$_n$ / Stim$_1$)
EPSP amp n = 6 cells
EPSP max depol. 3 mice
EPSP amp n = 10 cells
EPSP max depol. 4 mice
EPSP amp n = 6 cells
EPSP max depol. 2 mice
EPSP amp n = 8 cells
EPSP max depol. 5 mice
Stimulus # (20 Hz)
% summation (Max-Peak / Max)

Figure 4

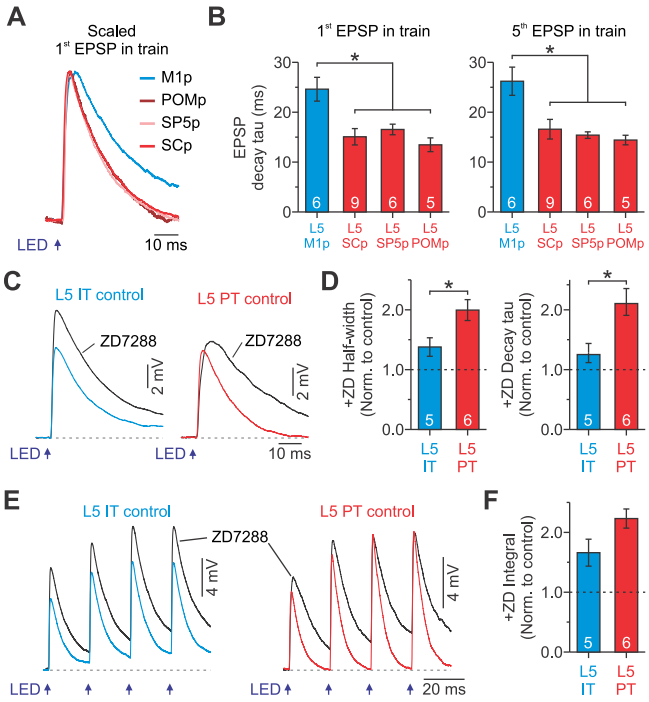
A
Scaled
1st EPSP in train
M1p
POMp
SP5p
SCp
LED
10 ms
B
1st EPSP in train
5th EPSP in train
EPSP
decay tau (ms)
30
20
10
0
*
6
9
6
5
L5
M1p
L5
SCp
L5
SP5p
L5
POMp
C
L5 IT control
L5 PT control
ZD7288
2 mV
LED
10 ms
D
+ZD Half-width
(Norm. to control)
+ZD Decay tau
(Norm. to control)
2.0
1.0
0.0
L5
IT
L5
PT
E
L5 IT control
L5 PT control
ZD7288
4 mV
LED
20 ms
F
+ZD Integral
(Norm. to control)

Figure 5

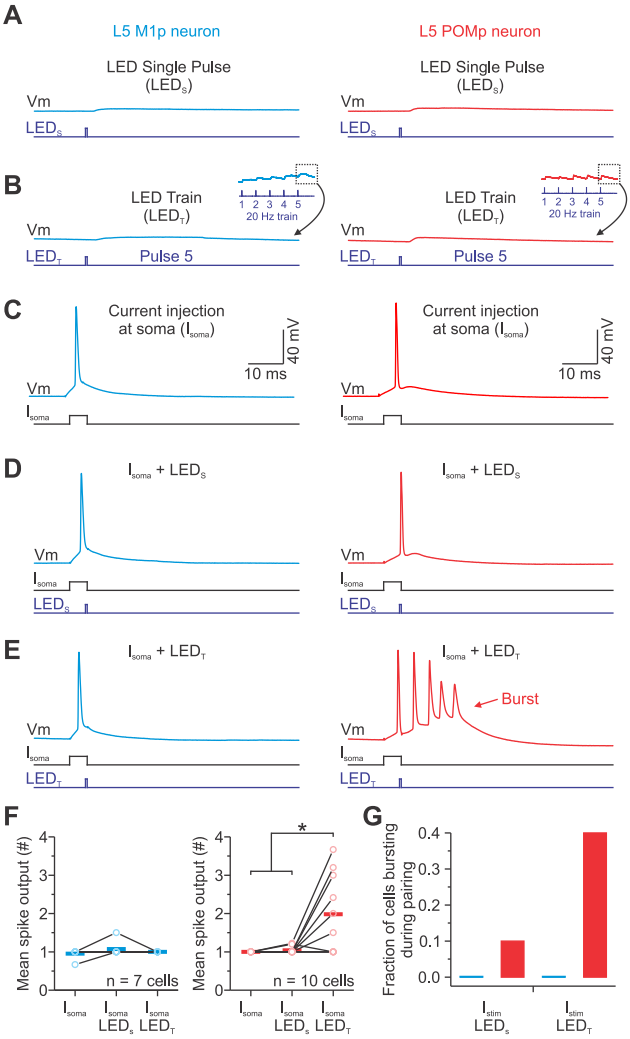
A
L5 M1p neuron
L5 POMp neuron
LED Single Pulse
(LEDS)
Vm
LEDS
B
LED Train
(LEDT)
1 2 3 4 5
20 Hz train
Pulse 5
LEDT
C
Current injection
at soma (Isoma)
40 mV
10 ms
Isoma
D
Isoma + LEDS
E
Isoma + LEDT
Burst
F
Mean spike output (#)
n = 7 cells
n = 10 cells
*
G
Fraction of cells bursting
during pairing
Istim
LEDS
LEDT

Figure 6

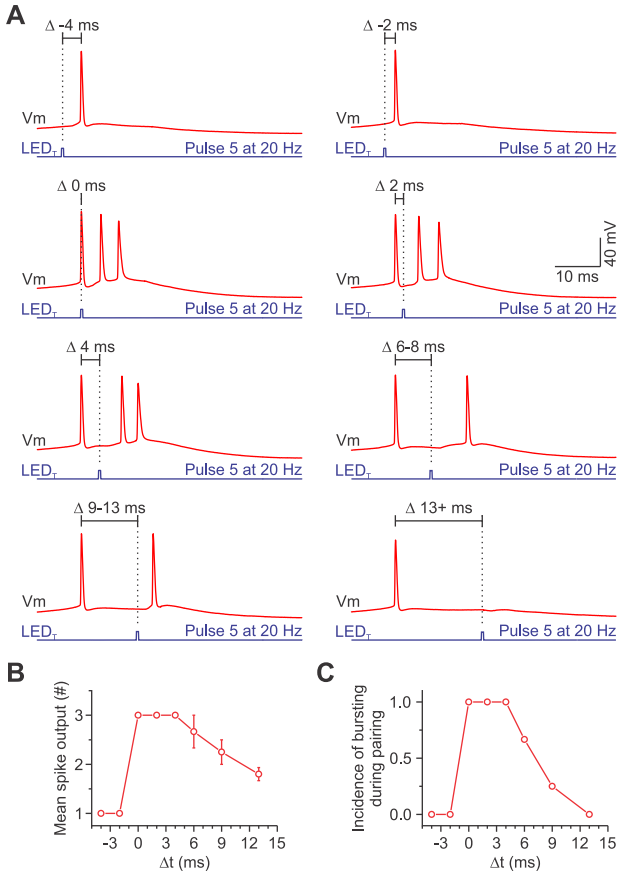
A
Δ -4 ms
Δ -2 ms
Δ 0 ms
Δ 2 ms
Δ 4 ms
Δ 6-8 ms
Δ 9-13 ms
Δ 13+ ms
Vm
LED_T
Pulse 5 at 20 Hz
40 mV
10 ms
B
Mean spike output (#)
Δt (ms)
C
Incidence of bursting during pairing
Δt (ms)

Figure 7