

Traumatic brain injury disrupts state-dependent functional cortical connectivity in a mouse model

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Traumatic brain injury (TBI) is the leading cause of death in young people and can cause cognitive and motor dysfunction and disruptions in functional connectivity between brain regions. In human TBI patients and rodent models of TBI, functional connectivity is decreased after injury. Recovery of connectivity after TBI is associated with improved cognition and memory, suggesting an important link between connectivity and functional outcome. We examined widespread alterations in functional connectivity following TBI using simultaneous widefield mesoscale GCaMP7c calcium imaging and electrocorticography (ECoG) in mice injured using the controlled cortical impact (CCI) model of TBI. Combining CCI with widefield cortical imaging provides us with unprecedented access to characterize network connectivity changes throughout the entire injured cortex over time. Our data demonstrate that CCI profoundly disrupts functional connectivity immediately after injury, followed by partial recovery over 3 weeks. Examining discrete periods of locomotion and stillness reveals that CCI alters functional connectivity and reduces theta power only during periods of behavioral stillness. Together, these findings demonstrate that TBI causes dynamic, behavioral state-dependent changes in functional connectivity and ECoG activity across the cortex.

Key words: calcium imaging; controlled cortical impact; functional connectivity; in vivo imaging; mesoscale imaging.

Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability among trauma-related injuries (Rubiano et al. 2015). Following moderate to severe TBI, there is significant mortality and less than a quarter of patients experience functional recovery in a 5-yr postinjury window (Program 2019). TBI causes cognitive and motor dysfunction (Arciniegas et al. 2002; Lutkenhoff et al. 2020; Walker and Pickett 2007), can lead to post-traumatic epilepsy (PTE) (Annegers et al. 1998; Salazar et al. 1985; Temkin 2009), and increases risk for neurodegenerative diseases (Graham and Sharp 2019; Ritzel et al. 2023), all conditions associated with network dysfunction (Cantu et al. 2015; Castellanos et al. 2011). Although multiple molecular and cellular changes are known to occur after TBI, the functional impact of TBI on neuronal activity and circuit function remains poorly understood. Elucidating how the brain responds to injury on a network level is thus a key goal

for identifying biomarkers of adverse outcomes and developing targeted therapeutic interventions.

Functional connectivity is a measure describing the short-latency temporal correlation of neuronal activity between brain regions and can provide information about how networks behave at baseline, during task performance, and after different patho-physiologic perturbations including TBI (Castellanos et al. 2010; Cordes et al. 2001; Guggisberg et al. 2008; Hutchison et al. 2013; Sporns 2014; Yang et al. 2021). Functional connectivity can be measured using electroencephalography (EEG), electrocorticography (ECoG), magnetoencephalography (MEG), magnetic resonance imaging (MRI), and mesoscale imaging (Benisty et al. 2024; Castellanos et al. 2011; Castellanos et al. 2010; Cordes et al. 2001; Cramer et al. 2023; Guggisberg et al. 2008; Lau et al. 2014; Lim et al. 2015; Lohani et al. 2022; Vanni et al. 2017). MEG and fMRI analysis of contusional TBI patients reveals decreased functional connectivity in damaged tissue compared to contralateral

uninjured tissue and to controls (Castellanos et al. 2010; Guggisberg et al. 2008; Han et al. 2014). Recovery of functional connectivity after TBI is associated with improved cognition and memory both globally and within networks responsible for measurable cognitive functions (Castellanos et al. 2011; Castellanos et al. 2010; Rigon et al. 2016). Functional connectivity patterns of TBI patients who underwent rehabilitation were more similar to healthy controls than to non-rehabilitated TBI patients, although deficits remained, and showed better functional recovery (Castellanos et al. 2010).

To assay functional connectivity in a clinically relevant mouse model of TBI, we developed a novel approach to perform *in vivo* widefield cortical mesoscale imaging with simultaneous ECoG in mice after controlled cortical impact (CCI). CCI is an established rodent model of TBI that primarily models contusion and hemorrhage, and leads to circuit reorganization, *in vitro* hyperexcitability, and post-traumatic epilepsy with a similar incidence to what occurs in patients with comparable injury severity (Alessandri et al. 2003; Annegers et al. 1998; Cantu et al. 2015; Di Sapia et al. 2021; Hunt et al. 2009; Koenig et al. 2019; Lighthall 1988; Lighthall et al. 1990; Xiong et al. 2013). Among patients with severe TBI, up to 35% experience intracerebral contusion and are the most neurologically impaired, have the highest healthcare impact, and the greatest societal impact (Bullock et al. 2006; Mandera et al. 1999). Mesoscale imaging allows *in vivo* widefield imaging of the cortical surface in awake, behaving animals (Barson et al. 2020; Lohani et al. 2022; Ma et al. 2016; Vanni et al. 2017). We quantified neuronal activity by using neuronal expression of the fluorescent reporter GCaMP7c, a genetically encoded calcium indicator (Zhang et al. 2023).

Not only is functional connectivity altered with injury, but it is highly dynamic across brain states. When at rest, cortical functional connectivity is highest, and it is reduced during increases in attention and motor-demanding tasks (Hermundstad et al. 2013; Lau et al. 2014; West et al. 2022). Measuring functional connectivity during different brain states enables a more complete understanding of how discrete brain regions are activated, recruited, and coordinated across states. However, little is known about how functional connectivity changes as a function of brain state in the injured brain. Therefore, we carried out mesoscale imaging while mice were free to run on a wheel and tracked their behavioral state (locomotion versus stillness) to understand state-dependent functional connectivity in the injured brain.

We report that CCI disrupts coherent cortical activity most profoundly immediately after injury in regions proximal to the lesion including somatosensory, parietal, and primary visual cortices. This is followed by a partial recovery during the weeks that follow. We also identify state-dependent changes in both ECoG power and functional activity during locomotion versus stillness that are disturbed by CCI. Interestingly, the biggest effects of CCI on functional connectivity are seen during stillness, with much smaller changes in functional connectivity during movement. Together, we report a novel approach to carry out mesoscale imaging with simultaneous ECoG after CCI and show temporally, regionally, and state-specific changes in functional connectivity in the injured rodent brain.

Materials and methods

Animals

Male CD-1 mice (bred in house from Charles River Labs stock, strain code 022) were kept on a 12-h light/dark cycle with access to food and water *ad libitum*. Following surgeries, mice were singly

housed. All procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

Focal viral injections

Cortical expression of the calcium sensor GCaMP7c was attained via focal viral injection of AAV1 under the human synapsin (hsyn) promoter for broad neuronal expression (#105321, Addgene) (Dana et al. 2019). CD-1 male mice (P42–56) were stereotactically injected in both hemispheres using three injection sites/hemisphere (coordinates): $(+/-2, 1.5, 0.75)$, $(+/-2, 3.5, 0.75)$, and $(+/-1.75, 6, 0.75)$ ($\lambda + x, +y, -z$) mm. Mice were anesthetized with isoflurane (4% induction, 2% maintenance) and body temperature was maintained on a heating pad at 37°C. Buprenorphine (0.1 mg/kg) was injected subcutaneously. A small incision was made, and burr holes drilled. Around 5×10^{10} viral gene copies were injected per site (1 μ L per site, 1:1 dilution with saline). The skin incision was closed using surgical sutures (#359551, McKesson). Mice were monitored closely until they resumed normal behavior and then were monitored daily for 3 days to ensure recovery. Mice were used for mesoscale implant 14–21 days following injection.

Surgical procedures

For implantation of mesoscale window and ECoG, anesthesia was induced, body temperature maintained, and analgesic administered as above. The skin and fascia overlying the skull from the interparietal bone to the nasal bone and between the temporal muscles was removed. The skull was thinned using a 0.7 mm spherical burr (FST) and dental drill anterior/posteriorly from the cross point between the median line connecting the frontal poles to lambda and laterally to the temporal muscles. The skull was appropriately thin when pliable under curved forceps and vasculature could be easily visualized. Next, mice were randomly selected to receive either CCI or sham injury. For CCI, a 5 mm craniectomy was made over the left somatosensory cortex using a dental drill and trephine. CCI was delivered using a Leica Benchmark Stereotaxic Impactor (3 mm piston, 1 mm depth, 400 ms dwell time, 3.5 m/s velocity). The craniectomy was sealed with cyanoacrylate (3 M). Sham mice received skull thinning but no craniectomy or CCI. Clear dental cement (C&B Metabond, Parkell) was applied, and a custom cut glass window covering the craniectomy and entire thinned skull was implanted (diamond scribe, VWR; No 1 gold seal cover glass, Thermo Scientific). Burr holes were drilled and ECoG electrodes implanted (#8403, Pinnacle) bilaterally over the somatosensory cortex laterally to the window (coordinates): $(+/-4.5, 4)$ ($\lambda + x, +y$) mm. Ground and reference electrodes were implanted in intraparietal bone. ECoG electrodes were soldered to a headmount (#8402, Pinnacle). Cold cure dental cement (Teets Denture Material, Cooralite Dental Manufacturing) mixed 2:1 with black tempera paint powder (Jack Richeson & Co.) was used to secure ECoG electrodes, custom head bar, and the glass window and to decrease optical interference. The head bar was anchored posterior to the window over ground and reference electrodes. Animals recovered on a warming pad until they resumed normal behavior and then were monitored daily to ensure postsurgical recovery.

Immunohistochemistry

Mice were deeply anesthetized and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.4 M phosphate buffer. Brains were extracted, postfixed in PFA for 24 h, and then cryopreserved in 30% sucrose. Coronal slices (40 μ m) were made on a Thermo Fisher Scientific Microm

HM 525 cryostat. Free-floating sections were blocked in 10% goat serum (GS), 5% bovine serum albumin (BSA), and 0.2% TWEEN20-PBS (PBST) at room temperature for 1 h. Sections were immunolabeled with primary antibodies in 5% GS/1% BSA/0.2% PBST. Primary antibodies were used as follows: Iba-1 (1:1000; Wako 019-19,741), glial fibrillary acidic protein (GFAP, 1:500; IF03L-100UG), green fluorescence protein (GFP, 1:1000; Abcam ab13970). Secondary antibodies were used at a ratio of 1:500 in 5% GS/1% BSA/0.2% PBST (anti-rabbit-Alexa-Fluor-555, Invitrogen A27039; anti-mouse-Alexa-Fluor-647, Invitrogen A21235; anti-chicken-Alexa-Fluor, Abcam ab150169). Images were collected for analysis using a Keyence epifluorescence microscope with a 10× objective from a single plane of focus per section, and composite images of hemi-cortex were made. Illumination intensity and exposure time were kept constant across all image collection. For image analysis, regions of interest (ROIs) were drawn using Image J software (NIH) in primary somatosensory cortex. Three sections per animal with one ROI per section were quantified. For CCI-injured mice, ROIs were drawn on the cortex ipsilateral to injury from the midline to beyond the lateral edge of the cavitory lesion superficial to CA3. ROIs were drawn in identical cortical regions in shams. Quantification of Iba-1+ cell density within each ROI and fluorescence intensity of Iba-1+ cells were performed using CellProfiler. Global GFAP expression within each ROI was quantified using a custom MATLAB script to determine the percent area that was GFAP positive (Cramer et al. 2023). GFP was quantified using the mean fluorescence intensity within ROIs. Representative images for Fig. 3 were collected using a Nikon A1R confocal microscope with a 40× oil objective (Olympus).

Widefield imaging and ECoG

Widefield calcium imaging was performed using a high-speed high-resolution Andor Zyla sCMOS camera with 470 nm and 535 nm alternating excitation (mounted light emitting diodes or LEDs, ThorLabs) to image GCaMP7c and hemodynamic reflectance, respectively (Balbi et al. 2017; Vanni et al. 2017). Emitted light from the 470 nm LED passed through a 460/50 nm excitation filter (ET460/50 m, Chroma) and from the 530 nm LED through an absorptive filter (NE10A, ThorLabs). Light then passed through (from 530 nm source) or was reflected off (from 470 nm source) a 495 nm dichroic mirror (T495lpxr, Chroma), a 470/530 nm dual excitation filter (CT470/530×, Chroma), and a 525/36 nm emission filter (ET525/36 m, Chroma). Images were acquired at 256 × 256 resolution, and each channel was acquired at 33 Hz with 5 ms exposure time. ECoG recording was performed using a PowerLab 8/35 (ADInstruments) at a sampling rate of 1000 Hz recorded in LabChart 8 (ADInstruments). Mice were head-fixed on a custom cylindrical running wheel and allowed to acclimate for 30 min prior to each imaging session (Heiney et al. 2014; Shuman et al. 2020). Mice did not undergo head fixation prior to 3 days postinjury (DPI) to allow for postsurgical healing. A magnetic encoder (MAE3, US Digital) was used to record rotational movement of the wheel. ECoG was recorded throughout the entire session and images were collected over 30 min (6 × 75 s imaging periods). Sessions were performed during the light phase of the 12-h light/dark cycle at 3, 5, 7, 14, and 21 DPI.

Mesoscale data analysis

Preprocessing of imaging and ECoG data

All analyses were conducted using custom-written scripts in MATLAB version 2022b. ECoG data was imported into MATLAB from LabChart 8 and included ECoG from two channels, locomotion data, and timing of alternating LED triggering. Imaging trials were

identified based on LED triggering (66 Hz for 75 s) and aligned with ECoG. Locomotion was identified from stillness by setting a minimum value for the integrated voltage change over 100 ms as detected by the running wheel encoder. Images were separated into fluorescence (470 nm excitation) and reflectance (530 nm excitation). Images were spatially aligned to bregma and the cross point between the median line connecting the frontal poles (Xiao et al. 2021). A mask was used to define cortical boundaries and applied to the full imaging stack.

Normalization and hemodynamic correction

To normalize fluorescence (F) and reflectance (R) data, $\Delta F/F_0$ and $\Delta R/R_0$ for each pixel was calculated using $(F - F_0)/F_0$, where F is the raw unfiltered signal and F_0 is the mean of the full image stack (Vanni et al. 2017; Wekselblatt et al. 2016). This was repeated for reflectance. To correct for hemodynamic artifacts, normalized reflectance was subtracted from normalized fluorescence as $\Delta F/F_0 - \Delta R/R_0$. $\Delta F/F_0 - \Delta R/R_0$ values were used for all subsequent analyses (Vanni et al. 2017).

Postprocessing analysis of imaging data

For anatomic mapping, images were aligned and anatomic landmarks registered as above. Ten cortical ROIs (5/hemisphere) were mapped onto images using Paxinos and Franklin's stereotactic coordinates including: secondary motor cortex, motor barrel cortex, primary somatosensory area forelimb, parietal cortex, primary visual cortex. The ROIs were small (radius = 3 pixels) ensuring they were entirely within the indicated anatomic regions. The three ROIs located proximal to CCI are (bregma + x, +y mm): primary somatosensory area for forelimb (+/-2, -0.83), medial parietal association cortex (+/-1, -1.67), primary visual cortex (+/-2.5, -2.69). For assessment of total neuronal activity, a trace from each ROI was generated representing fluorescent activity over time and the coefficient of variation (CV) of each trace calculated. This was repeated for all imaging sessions to generate a mean CV value for each brain region at every timepoint. Pearson's correlation coefficients between $\Delta F/F_0 - \Delta R/R_0$ signals were computed using the "corrcoef" function (MATLAB) which is amplitude independent (Xiao et al. 2021). Correlation coefficients were displayed as connectivity matrices. This process was repeated for all imaging trials and all behaviorally defined segments at least 8 s in length of locomotion and stillness within each imaging trial. To eliminate effects of state transition, we removed the first 1.5 s and last 0.5 s of each period of stillness or locomotion (Lohani et al. 2022). We measured interhemispheric connectivity by computing the correlation coefficient between left primary motor cortex and all 5 contralateral regions. This was repeated for the nine remaining regions and the mean across all regions was taken. For intrahemispheric connectivity, the correlation coefficient between regions within each hemisphere was computed and the mean taken.

Postprocessing analysis of ECoG data

To quantify ECoG power, raw ECoG signal was bandpass filtered using the "bandpower" function (MATLAB) into five bands: total (1–100 Hz), low (2–6 Hz), theta (6–8.3 Hz), alpha (8–12 Hz), gamma (30–70 Hz), and high gamma (80–120 Hz) (Klaft et al. 2020). Bands were normalized to total power. Power from these bands was identified from the entire ECoG recording, the first half, and the second half to determine any effect due to acclimation to the wheel, and each imaging trial.

Statistics and reproducibility

Statistical analyses were conducted using custom-written scripts in MATLAB and RStudio (2022 July 2 Build 576). Figures were created in Prism 8 (GraphPad) and on BioRender.com. Three animals were used in each treatment group. For all experiments, we performed linear mixed modeling (LMM) to measure statistical differences due to animal injury status, state, and DPI (fixed effects) while accounting for intra- and inter-animal variability (random effects) (Aarts et al. 2014; Koenig et al. 2019). Dependent variables included GFAP fluorescence, Iba1 density (Fig. 2), $\Delta F/F_0$ (Fig. 4), connectivity (Figs. 5 and 7), and ECoG power (Fig. 6). The “lmer” function (RStudio) was used for running LMM with lme4, lmerTest, matrix, and stats packages. For LMM, t values > 1.96 and < -1.96 were considered statistically significant. Example code is as follows: `corrcoef=lmer(corr_coef ~ injury_status * dpi + (1|trial_num:mouse_num) + (1|mouse_num), data=Connect_Interhem, na.action="na.omit")`. In this example from Fig. 5, we tested the data (Connect_Interhem) for an effect of injury (injury_status) and time postinjury (dpi) on functional connectivity (corr_coef). Inter-animal (1|mouse_num) and intra-animal (1|trial_num:mouse_num) variability were considered. Error bars indicate standard error of mean (SEM). In Figs. 5 and 7, average differences in connectivity matrices were determined using a Wilcoxon rank sum test (unpaired) or Wilcoxon signed rank test (paired) and multiple comparisons controlled for using a Benjamini–Yekutieli correction (Lohani et al. 2022). No animals were excluded from analysis. Post hoc power analysis was performed using G*Power (3.1.9.7) on the data in Fig. 5A to confirm that sample sizes are sufficient given the effect sizes observed (Faul et al. 2009; Faul et al. 2007). Input parameters: effect size $f = 30.246$, $\alpha = 0.05$, total sample size = 6, number of groups = 2, number of measurements = 18, corr among rep measures = 0.05. Output parameters: noncentrality parameter $\lambda = 10,400.06$, critical $F = 7.7$, numerator $df = 1$, denominator $df = 4$, power = 1. Statistical results are reported in their entirety in Supplementary Tables 1 and 2.

Results

Simultaneous mesoscopic imaging and ECoG in CCI-injured mice

To perform widefield cortical imaging in the CCI model of TBI, we developed a novel surgical and imaging approach for simultaneous GCaMP mesoscale imaging and ECoG in awake mice following TBI. Our protocol allows mice to run freely on a running wheel to analyze state-specific changes in brain activity during stillness and motion. Focal cortical injections (three/hemisphere) of the neuronal calcium indicator GCaMP7c (AAV1-syn-jGCaMP7c), selected for its high contrast and low baseline fluorescence (Zhang et al. 2023), were made in adult male CD-1 mice. This resulted in wide reporter expression throughout both cortical hemispheres including somatosensory, retrosplenial, motor, and visual cortices. Two weeks later, mice received sham or CCI injury and were implanted with a cortical window, ECoG electrodes, and a head bar for head fixation (Fig. 1A). Our implant approach does not require any modification of CCI parameters and allows optical access to the dorsal surface of the cortex including the site of injury. Beginning 3 DPI to allow time for recovery after surgery, mice were head-fixed on a custom-made mesoscale imaging microscope with a running wheel and allowed to run freely during imaging and ECoG recording. Mice were imaged and ECoG recorded 3, 5, 7, 14, and 21 DPI.

Images were anatomically registered using visually identifiable anchoring coordinates (Franklin and Paxinos 2013; Xiao et al. 2021). Fluorescence and reflectance data were separated and normalized (Fig. 1B and D) (Silasi et al. 2016). Reflectance was subtracted from fluorescence to account for hemodynamic fluctuations (Vanni et al. 2017). All analyses utilized this normalized and corrected signal to account for small differences in baseline fluorescence due to focal viral injections and curvature of the brain. ECoG signal was temporally aligned with imaging data and behavior state was categorized as locomotion or stillness (Fig. 1C and E). Throughout the cortex in sham and injured animals, large, spatially specific calcium signals were seen, indicative of neuronal activity. Mice spontaneously transitioned between stillness and locomotion, and movement state corresponded to fluctuations in calcium activity and ECoG spectrogram. In ECoG traces, there was prominent theta activity during periods of locomotion. Thus, we demonstrate that the CCI model of TBI can be integrated with widefield calcium imaging and ECoG.

Mesoscale implant slightly increases GFAP but not Iba1 immunolabeling after CCI

We next asked if implanting a mesoscale window altered the response to CCI, as quantified by changes in glial reactivity. Glial cells, including astrocytes and microglia, mount the primary response to brain insult and contribute to secondary injury effects following TBI (Kumar et al. 2017; Shandra et al. 2019). After injury, astrocytes can become reactive and cause inflammation, alter the blood–brain barrier, and form a glial scar (Burda et al. 2016; Burda and Sofroniew 2014; Myer et al. 2006). Microglia are activated, recruited to the site of injury, secrete inflammatory molecules, and are associated with postinjury cognitive deficits (Csuka et al. 1999; Semple et al. 2010; Witcher et al. 2021). To address whether mesoscale implant alters glial reactivity after CCI, we quantified the expression of markers for astrocyte (GFAP) and microglia (Iba1) reactivity.

Animals underwent CCI or sham injury, and half received subsequent mesoscale implant. Three weeks later, brains were prepared for immunolabeling with GFAP and Iba1 as a proxy for astrocytosis (as assayed by total area of GFAP immunohistochemical staining) and microglial activation (as measured by the number of Iba1+ cells and the intensity of Iba1 staining per cell), respectively (Fig. 2A). We found a significant increase in GFAP expression and Iba1+ cell number in CCI mice, as compared to shams, in both implanted and un-implanted groups, consistent with abundant glial reactivity after TBI (GFAP: $t_{(8)} = 2.39$, $P = 0.04$ effect of implant; $t_{(8)} = 5.19$, $P = 8 \times 10^{-4}$ effect of injury; $t_{(8)} = 2.35$, $P = 0.04$ interaction effect, LMM) (Iba1: $t_{(8)} = 3.18$, $P = 0.01$ effect of injury, LMM; Fig. 2D and E; Supplementary Table 1) (Burda et al. 2016). Mesoscale implant caused a small but significant increase in GFAP expression, but not number of Iba1+ cells, in CCI-injured mice alone. It is only in the context of a concurrent CCI that window implantation increases GFAP expression. Window implantation alone does not elicit an increase in GFAP.

Similarly, when comparing the Iba1+ intensity within each cell, CCI but not implant caused a significant increase ($t_{(8)} = 4.42$, $P = 2 \times 10^{-3}$ effect of injury, LMM; Fig. 2F). This shows that CCI significantly increased GFAP and Iba1 immunoreactivity, consistent with reactive astrocytosis and microglial activation after injury. The addition of the mesoscale implant caused a small but significant increase in GFAP but not Iba1 immunolabeling in the injured cortex, indicating that mesoscale implant slightly increased astrocyte reactivity after CCI.

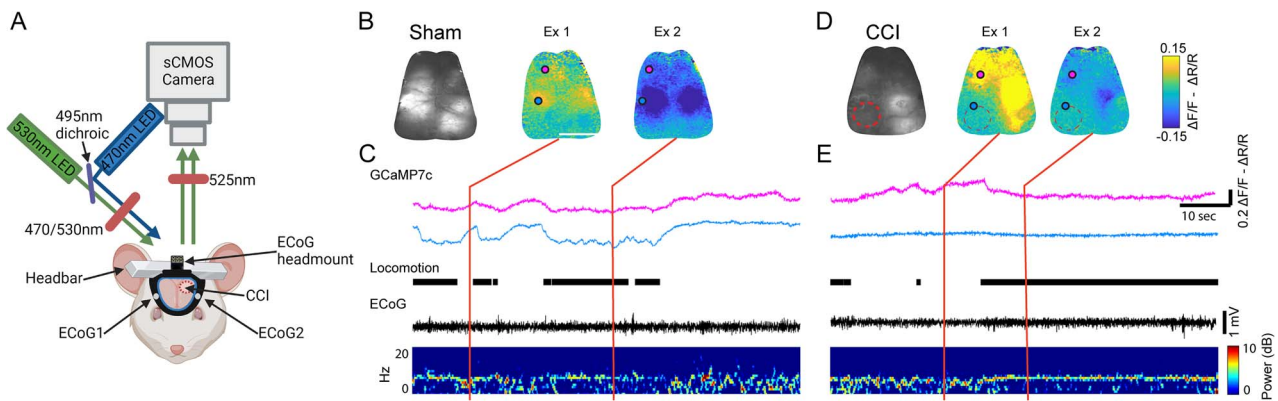


Fig. 1. Implant design, imaging setup, and spatiotemporal cortical dynamics of neuronal activity and ECoG. (A) Diagram of the implant & imaging setup including camera, LEDs, and ECoG recording equipment. (B) Baseline image of a sham implanted mouse (left) and example normalized images showing fluctuations in neuronal (GCaMP7c) activity during stillness (ex 1) and during movement (ex 2). Overlaid circles indicate anatomic regions from which activity is quantified in the traces below. Top circle (pink) indicates secondary motor cortex (M2) and bottom circle (blue) indicates primary somatosensory area, forelimb (SSC). Scale bar = 3 mm. (C) From top to bottom, sample traces of GCaMP7c activity from M2 (top, pink) and SSC (bottom, blue), times of locomotion (black bar) for the animal shown in B, ECoG, and corresponding spectrogram. Red lines indicate temporal alignment with ex 1 and 2. (D) Same as B but of a CCI-injured mouse. Red dotted circles indicate location of CCI. (E) As in C but for the CCI animal indicated in D. These example images come from 21 days after CCI or sham surgery.

GCaMP7c expression is not significantly altered by CCI

To test whether CCI altered the expression of GCaMP7c, we performed CCI and sham surgeries on animals focally injected with GCaMP7c and then used immunohistochemistry and epifluorescence imaging to quantify GCaMP7c expression independent of neuronal activity. 21 days postsurgery, brains were prepared for immunohistochemical analysis of reporter expression using an anti-GFP antibody. GFP immunoreactivity was quantified in cortical layers I–III corresponding to the area of cortex that was imaged during mesoscale imaging (Fig. 3A and B) (Ma et al. 2016). There were no significant differences in GFP immunoreactivity between CCI and sham mice ($t_{(4)} = -1.09$, $P = 0.34$, LMM). There were also no significant differences in GFP immunoreactivity in layers IV–VI (data not shown). Even though this shows that there were not large-scale changes in GFP expression, we wanted to examine GFP expression immediately adjacent to the site of injury. We quantified both the number of GFP+ neurons and the intensity of GFP within neuronal soma in ROIs within 200 μm of the injury border but found no significant effect of CCI (Supplementary Fig. 1). There was, however, a nonsignificant trend toward decreased numbers of GFP+ cells and GFP fluorescence in individual neuronal soma compared to shams. We saw persistent GCaMP7c expression in injured animals adjacent to the site of injury 3 weeks after injury (Fig. 3C). This supports that any changes in GCaMP7c activity both distal and proximal to injury are not due to decreased reporter expression, but rather occur due to changes in neuronal activity.

Neuronal activity is decreased proximal to CCI and increased in the contralateral cortex

To determine whether CCI affected total neuronal activity during imaging, the CV of GCaMP7c fluorescence was quantified in each imaging session across multiple cortical regions. CV allows a simple quantification that integrates both the dynamic range and the total ongoing activity during imaging (Larsch et al. 2013). ROI were placed bilaterally to include motor, somatosensory, parietal, and visual cortices. CCI border was marked ensuring ROIs proximal to CCI were perilesional, not in the injury itself (Fig. 4A). A mean CV value for each brain region at every timepoint was generated

(Fig. 4B). Following CCI, GCaMP7c CV was significantly decreased over all timepoints examined in the three brain regions most proximal to CCI as compared to identical regions in sham-injured mice ($t_{(43)} = -6.12$, $P = 2.5e-7$, LMM; Fig. 4C).

Interestingly, this decrease in GCaMP7c CV was limited to cortical areas just outside the CCI and was not seen in regions anterior to injury ($t_{(429)} = 9.23$, $P < 2e-16$ effect of brain region; $t_{(429)} = 5.66$, $P = 2.84e-8$ effect of time; $t_{(429)} = 4.1$, $P = 5.9e-5$ interaction effect, LMM; Fig. 4D). To confirm that the perilesionally decreased CV is due to suppressed activity, the mean $\Delta F/F_0$ proximal to injury was quantified and found to be decreased in CCIs compared to shams (Supplementary Fig. 2A). The minimum $\Delta F/F_0$, which reflects the basal fluorescence during periods of minimal neuronal activity, was closer to zero in CCI mice, again consistent with a smaller CV (Supplementary Fig. 2B). Additionally, the minimum raw fluorescence was not different between shams and CCIs (Supplementary Fig. 2C). Reflectance also did not affect CV as it varied little between and within imaging sessions regardless of animal injury status (Supplementary Fig. 3), suggesting a true decrease in GCaMP7c $\Delta F/F_0$ CV in CCIs that is not caused by elevated basal fluorescence signal or hemodynamic effect. Finally, regions contralateral to CCI had an increased GCaMP7c CV when compared to matching regions in sham-injured mice at all timepoints ($t_{(56)} = 2.96$, $P = 4.5e-3$ effect of injury, LMM; Fig. 4E). Overall, GCaMP7c CV was consistently decreased in somatosensory, parietal, and primary visual cortices ipsilateral to injury, and increased in contralateral somatosensory, parietal, and primary visual cortices following CCI.

CCI regionally impairs functional cortical connectivity that partially recovers over time

We next asked whether CCI alters functional connectivity, as measured by coherent GCaMP7c activity across brain regions. Interhemispheric connectivity, quantified using the strength of correlated neuronal activity across hemispheres, is an important readout of functional connectivity and is altered with TBI (Rigon et al. 2016). We measured interhemispheric connectivity by mapping the same 10 ROIs onto the cortex as in Fig. 4 and computed the correlation coefficient between all regions. We found that CCI significantly decreased interhemispheric functional connectivity

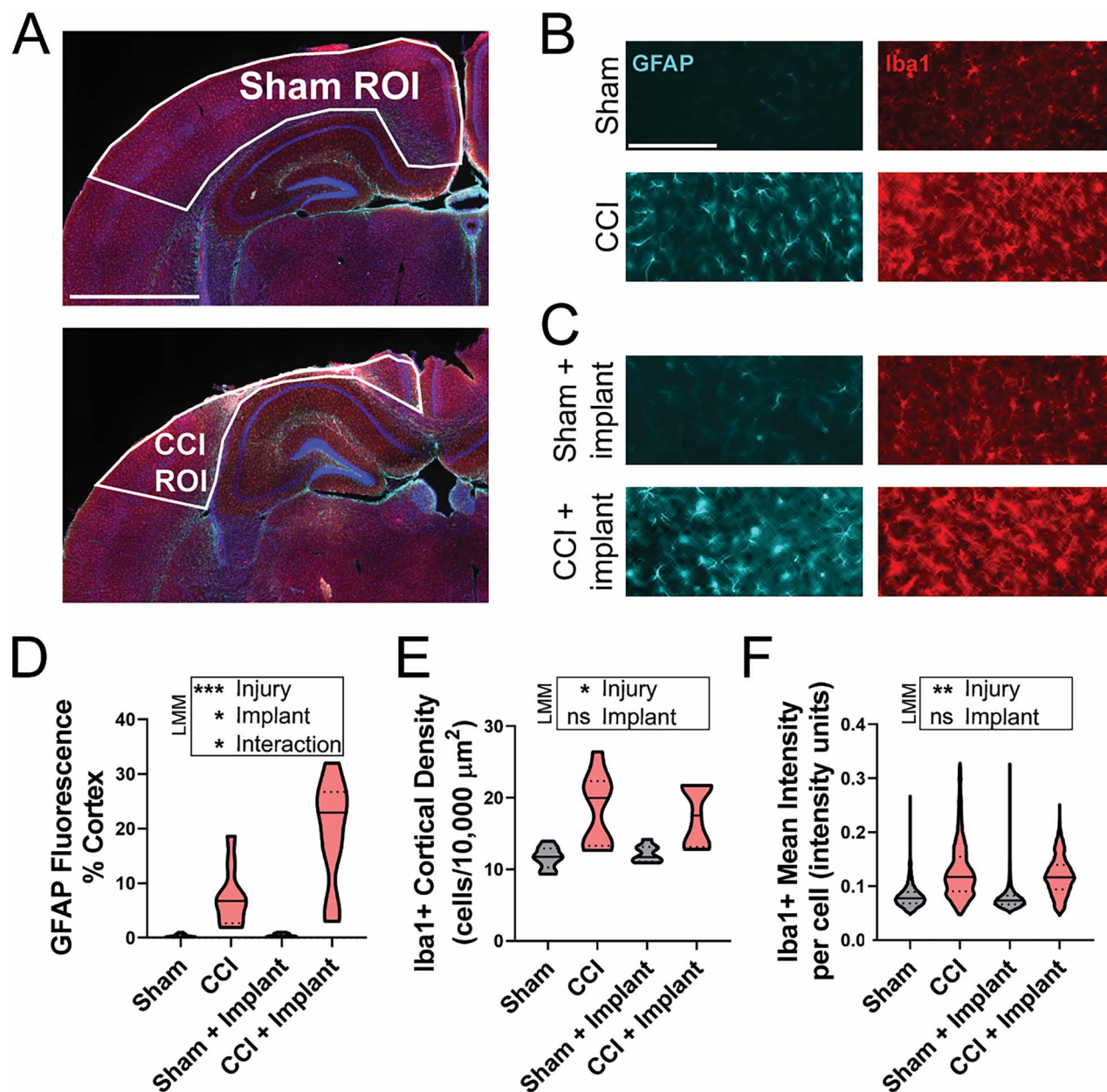


Fig. 2. Mesoscale implant slightly increases GFAP but not Iba1 immunolabeling after CCI. (A) Example ROIs drawn in cortex of a sham (top) and a CCI (bottom) mouse brain section that were used for image quantification in D–F. Scale bar = 1,000 μm . (B) Representative fluorescent images from somatosensory cortex of non-implanted sham and CCI mice 21 days postsurgery immunolabeled for GFAP (left, cyan) and Iba1 (right, red). For CCI mice, images are from cortex ipsilateral to injury. Scale bar = 100 μm . (C) Representative fluorescent images of sham and CCI mice implanted with a cranial window 21 days postsurgery immunolabeled for GFAP (left, cyan) and Iba1 (right, red). (D) Injury and mesoscale implant both significantly increased GFAP expression in cortex, quantified as area of cortex that was GFAP positive. (E) Mean cortical density of Iba1+ cells were significantly increased by injury with no apparent effect of mesoscale implant. (F) Mean Iba1+ cell fluorescence intensity was increased by injury, but not by mesoscale implant. Total number of cells are as follows: Sham: 20,064, CCI: 14,378, sham + implant: 18,700, CCI + implant: 13,940. For all plots, $n = 3$ animals per group, three sections per animal. LMM: *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$. In violin plots, median is indicated with a solid line, quartiles are demarcated with dashed lines.

($t_{15} = -6.64$, $P = 9.2e - 6$ effect of injury; $t_{170} = 6.0$, $P = 1.18e - 8$ interaction effect, LMM; Fig. 5A). This was evaluated 3, 5, 7, 14, and 21 days postinjury. To determine if changes in functional connectivity were driven by changes in the injured versus contralateral hemispheres, we also calculated intrahemispheric connectivity. We computed the average functional connectivity between all regions in the left hemisphere and separately for the right hemisphere (Fig. 5B). Interestingly, CCI reduced functional connectivity only in the injured hemisphere ($t_{(5)} = -6.22$, $P = 2.3e - 3$ effect of injury; $t_{(172)} = 8.39$, $P = 1.7e - 14$ interaction

effect, LMM). Connectivity in the injured hemisphere significantly recovered over time, more than doubling over the 21 days following injury. There was no significant decrease in connectivity in the hemisphere contralateral to injury after TBI, only a small effect of time indicating some variability in connectivity in the contralateral cortex over time ($t_{(172)} = -2.43$, $P = 0.02$ effect of time, LMM). We also performed inter and intrahemispheric connectivity analysis with larger ROIs by doubling the radius to assess sensitivity of our analysis to the size of the selected ROIs. Doubling the radius of the ROI had no effect on any measures of functional connectivity

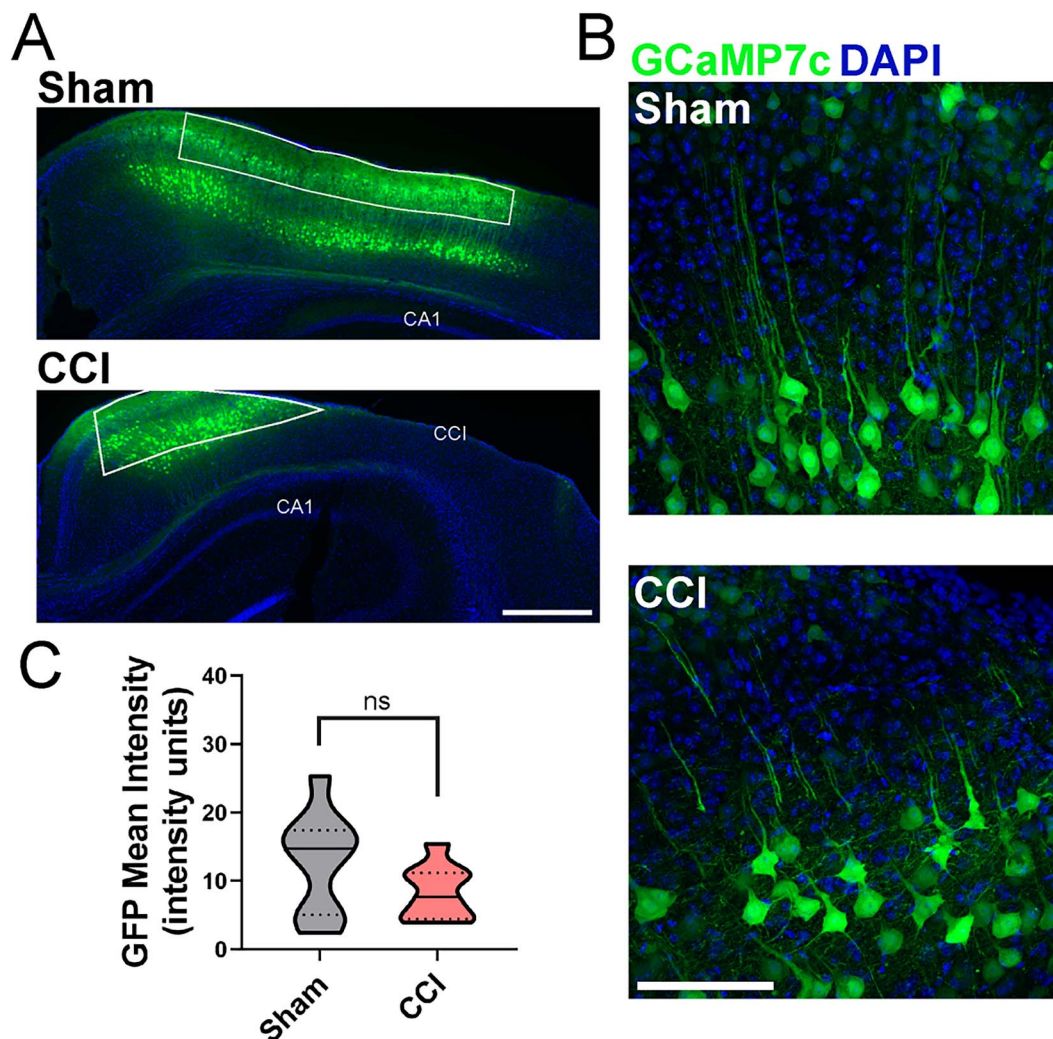


Fig. 3. GCaMP7c expression is not significantly altered by CCI. (A) Representative fluorescent images of sham (top) and CCI (bottom) injured mice 21 days postsurgery immunolabeled for GFP (green). Example ROIs drawn in the cortices were used for image quantification in B. CA1 and the CCI location with cortical thinning are labeled. scale bar = 500 μ m. (B) Representative confocal images of GFP-expressing cells in layers I–III in a sham (top) and CCI (bottom) mouse. Note that the CCI representative image is perilesional. Scale bar = 100 μ m. (C) Mean GFP fluorescence intensity in layers I–III was not significantly affected by CCI. For plot, $n = 3$ animals per group, three sections per animal. LMM: Ns is not significant. In violin plot, median is indicated with a solid line, quartiles are demarcated with dashed lines.

(data not shown). Therefore, we proceeded with the original ROIs in subsequent experiments to ensure anatomic accuracy and specificity. Together, these data indicate that CCI drives largely focal disruptions in functional connectivity that recover over time.

To determine whether specific functional connections are disrupted by CCI, we generated connectivity matrices displaying the functional connectivity between each region and every other region. The connectivity matrices in sham mice showed that the functional connectivity across the brain was stable over all timepoints examined (Fig. 5C). Following CCI, the disruption of functional connectivity occurred most notably in the three regions proximal to injury (somatosensory, parietal, and primary visual cortices), but gradually recovered over time for some connections (Fig. 5D). These connectivity matrices were consistent both across repeated imaging sessions for individual animals and between animals (Supplementary Fig. 4). We next quantified the difference in functional connectivity between sham and CCI mice over all timepoints and found that all regions proximal to CCI were significantly decreased compared to corresponding regions in shams at 3, 5, and 7 days postimplant (Fig. 5E, top row). By 14 days,

some regions near CCI were no longer significantly different from shams, specifically in somatosensory and parietal cortices. Interestingly, we found several regions in which functional connectivity was increased in CCI animals, including areas contralateral and anterior to the region of injury (Fig. 5E, bottom row).

Given the region-specific recovery of functional connectivity in somatosensory and parietal cortices, we further examined brain areas closest to CCI to determine if these regions displayed unique recovery trajectories over time. We computed the mean functional connectivity between the indicated region proximal to CCI (left somatosensory, parietal, and primary visual cortices) and all other ROIs for each imaging trial (Supplementary Fig. 5). In all three regions, CCI connectivity increased over the 21 days but to different extents with parietal cortex activity most closely approaching sham levels ($t_{(4)} = -4.41$, $P = 9.4e - 3$ somatosensory; $t_{(4)} = -5.1$, $P = 5.4e - 3$ parietal; $t_{(5)} = -9.25$, $P = 1.6e - 4$ visual; effect of injury for all, LMM). This increase in connectivity proximal to CCI further supports our findings that connectivity deficits are driven by focal injury effects. Together, these findings show that functional connectivity is significantly disrupted by CCI, and gradually recovers over time.

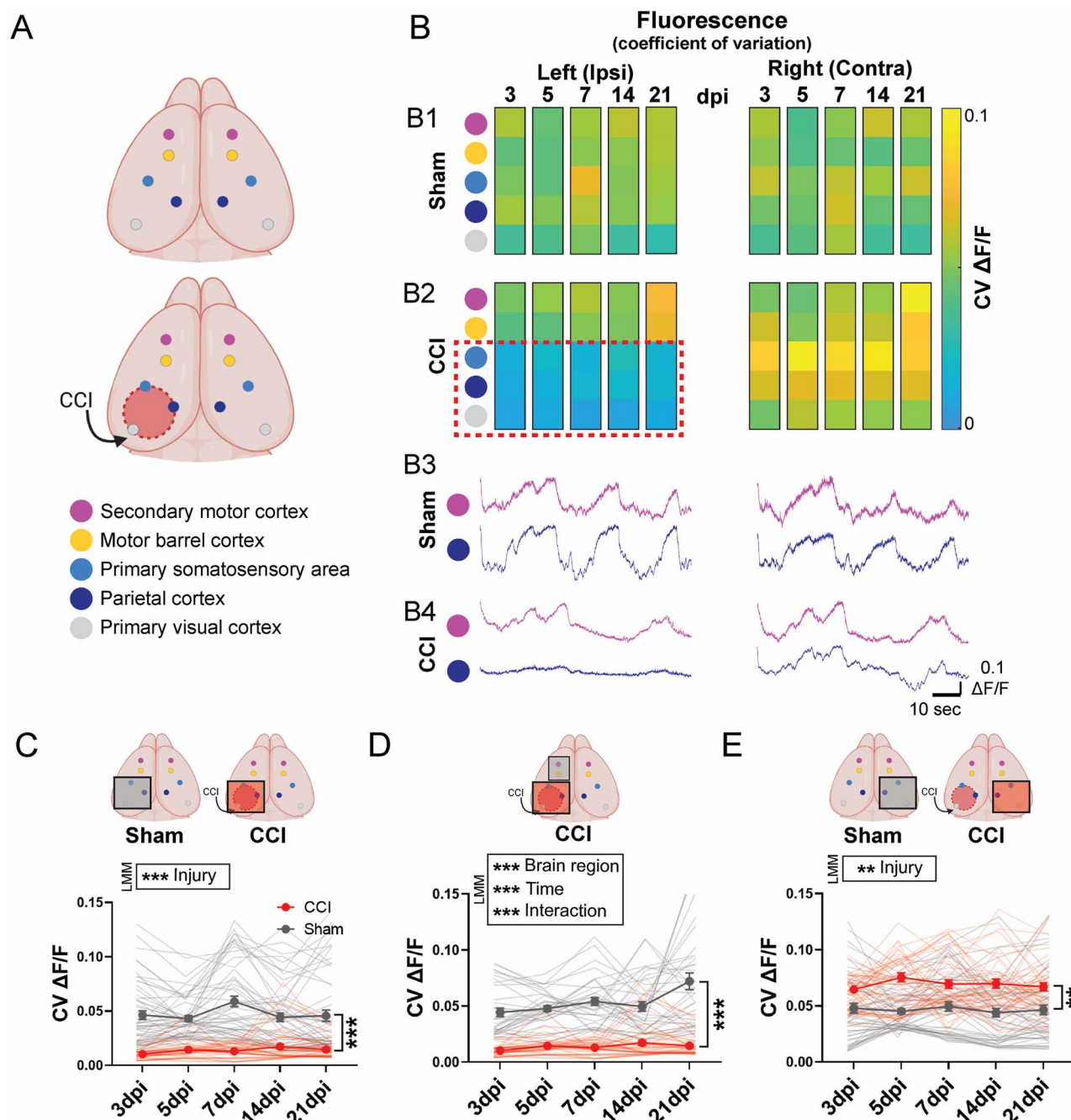


Fig. 4. Neuronal activity is decreased proximal to CCI and increased in the contralateral cortex. (A) Schematic of location of ROIs in sham (top) and CCI (bottom). Colors correspond with panels in B and are anterior to posterior: Secondary motor cortex (pink), motor barrel cortex (yellow), primary somatosensory area forelimb (light blue), parietal cortex (dark blue), and primary visual cortex (gray). (B) CV of $\Delta F/F_0$ in the regions mapped in a in sham (B1) and CCI (B2) mice. Red dashed box indicates perilesional regions. B3. Sample $\Delta F/F_0$ traces from a single imaging session 3dpi from sham and CCI (B4) mice. (C) Mean CV of $\Delta F/F_0$ from the three ROIs proximal to CCI (demarcated with red box) compared to identical regions in sham mice (ROIs within gray box). Lines indicate all replicates contributing to the mean. (D) Mean CV of $\Delta F/F_0$ from the three ROIs proximal to CCI (red box, bottom) compared to ROIs anterior to CCI (gray box, top). (E) Mean CV of $\Delta F/F_0$ from ROIs contralateral to CCI (red box) compared to identical regions in sham mice (gray box). For C–E, LMM: *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$. Error bars indicate SEM. Dpi, days postinjury. Ipsi, ipsilateral. contra, contralateral.

Injury decreases ECoG theta power during stillness but not locomotion

We next examined ECoG data collected during imaging. Due to known state-dependent changes in brain activity, we separated the data into times of locomotion and stillness (Greene et al. 2018; McFarland et al. 1975). Stillness recapitulates aspects of resting state in human fMRI studies (Poulet and Crochet 2018;

Rocchi et al. 2022) and low arousal state in rodent studies (Vinck et al. 2015). Movement is considered a higher arousal state and results in distinct circuit changes (Lohani et al. 2022). We first focused on ECoG theta power (6–8 Hz), which is known to be modulated by state as well as TBI. To control for potential injury-induced changes in animal behavior, we determined the percent time animals spent moving during each imaging session. There

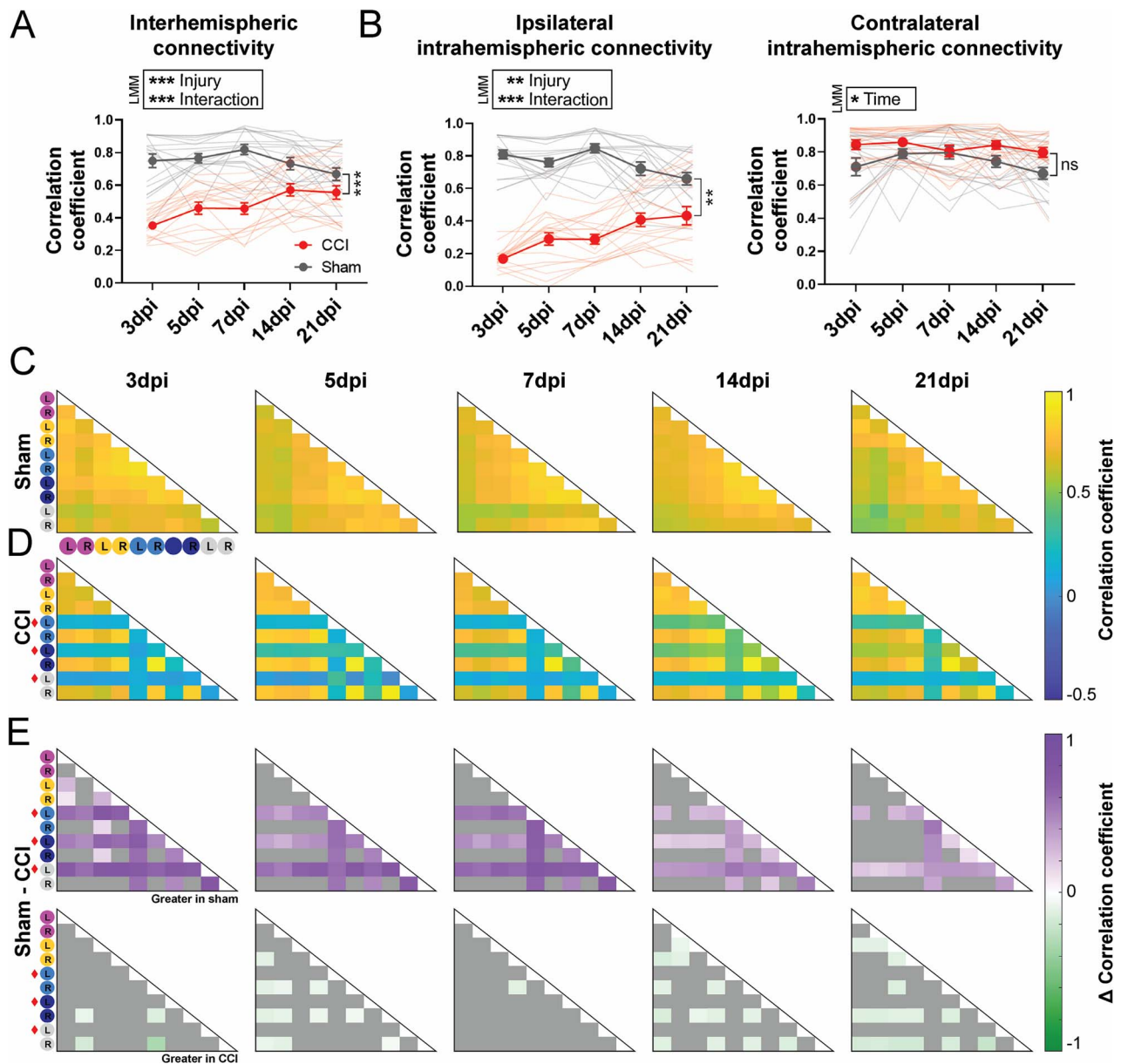


Fig. 5. CCI regionally impairs functional cortical connectivity that partially recovers over time. (A) Interhemispheric connectivity, as calculated by computing the correlation coefficient between anatomic regions in the left versus the right hemisphere. Regions are anterior to posterior: Secondary motor cortex, motor barrel cortex, primary somatosensory area forelimb, parietal cortex, and primary visual cortex. Lines indicate each imaging session from all mice contributing to the mean. (B) Intrahemispheric connectivity in ipsilateral (left) and contralateral (right) hemispheres between the five anatomic regions indicated in A. connectivity is significantly decreased in the ipsilateral hemisphere but not in the contralateral. for A–B, $n = 3$ animals per group, six trials per animal per timepoint. LMM: * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$. Error bars indicate SEM. (C) Average connectivity matrices over time in sham and CCI (D) mice. This is a point-to-point matrix with each square displaying the correlation coefficient of each anatomic ROI compared to every other ROI. Anatomic ROIs are indicated along the outside of the matrices, marked with an L or R to indicate which hemisphere they originate from, and are anterior to posterior: Secondary motor cortex (pink), motor barrel cortex (yellow), primary somatosensory area forelimb (light blue), parietal cortex (dark blue), and primary visual cortex (gray). Areas proximal to CCI are indicated with a red diamond. For all conditions in C and D, $n = 3$ animals per group, six imaging trials per timepoint. (E) Average differences in correlation coefficient between sham and CCI are shown for connections that are significantly decreased (top row) or increased (bottom row) with injury ($P < 0.01$, Benjamini–Yekutieli corrected Wilcoxon rank sum test [unpaired]). Nonsignificant values are shown in gray. Dpi, days postinjury.

was no effect of injury on the total amount of time animals spent moving ($t_{(11)} = -0.85$, $P = 0.45$ effect of injury; $t_{(22)} = 2.0$, $P = 0.06$ effect of time; $t_{(22)} = 0.5$, $P = 0.62$ interaction effect, LMM; Fig. 6B) or on the total distance traveled or average speed (Supplementary Fig. 6).

To quantify ECoG during each state, segments of locomotion or stillness were identified, and state transitions removed. In both sham and CCI animals, increased theta power was associated

with locomotion ($t_{(274)} = 15.6$, $P < 2e - 16$, effect of state, LMM; Fig. 6A). We next examined theta power during locomotion and stillness over all timepoints. There was no difference in theta power during locomotion between CCI and sham mice, but theta power did increase slightly over time in CCI mice ($t_{(11)} = -1.9$, $P = 0.08$ effect of injury; $t_{(275)} = 3.2$, $P = 1.6e - 3$ effect of time, LMM; Fig. 6C and E). During stillness, however, theta power was significantly decreased in CCI animals compared to shams ($t_{(5)} = -3.1$,

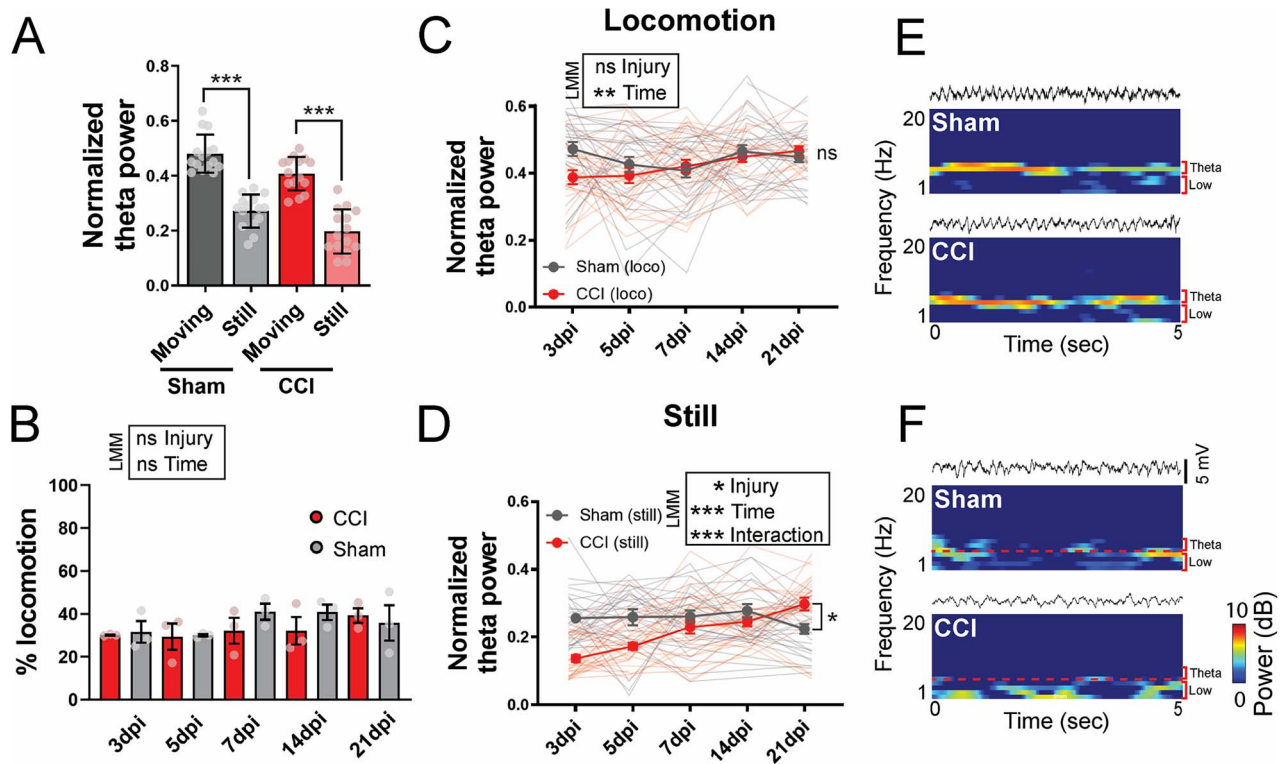


Fig. 6. Injury decreases ECoG theta power during stillness but not locomotion. (A) Quantification of theta power in sham and CCI mice from the ipsilateral (left) hemisphere during locomotion and stillness shows a significant decrease in theta power during stillness. Individual points show the average of each mouse at each timepoint. LMM: ***indicates $P < 0.001$. (B) Percent time spent moving throughout entire recording session was not significantly different between sham and CCI mice. LMM: ns is not significant. (C) Theta Power during locomotion alone shows no significant differences between CCI and sham mice (ECoG power is average of ipsilateral and contralateral electrodes). (D) Theta Power during stillness alone shows a significant decrease in CCI mice compared to sham. For all conditions, $n = 3$ animals per group, 3–15 instances of locomotion or stillness at each timepoint. LMM: *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$, ns is not significant. Error bars indicate SEM. (E) Sample ECoG traces from the ipsilateral (left) somatosensory cortex and corresponding spectrograms for sham (top) and CCI (bottom) mice 3dpi during locomotion show a strong theta band (5–8 Hz). (F) Sample ECoG traces from the ipsilateral (left) somatosensory cortex and spectrograms for sham (top) and CCI (bottom) mice 3dpi during stillness show lower theta power in CCI mice than in sham. For E and F, theta and low power bands are noted to the right of spectrograms. loco indicates locomotion. Dpi, days postinjury.

$P = 0.03$ effect of injury; $t_{(269)} = 4.4$, $P = 1.5e - 5$ effect of time; $t_{(269)} = -4.3$, $P = 9e - 11$ interaction effect, LMM; Fig. 6D and F). This stillness-specific decrease in theta power in CCI mice returned to normal by 21 days postinjury. Finally, we examined the remaining ECoG bands including low power (2–6 Hz), alpha power (8–12 Hz), gamma power (30–70 Hz), and high gamma power (80–120 Hz). We observed both state and injury-dependent differences in low power, and a small nonsignificant trend toward an injury-dependent difference in high gamma power during movement (Supplementary Fig. 7). The remaining bands (alpha and gamma) had very little difference between CCI and sham groups or state effects. In summary, CCI affected ECoG theta power during stillness but not locomotion, suggesting that in vivo studies of brain activity after injury would benefit from state-dependent analysis.

Locomotion and stillness engage distinct functional connectivity states that are disrupted by CCI

Given our findings of state-dependent and injury-induced changes on ECoG as well as the literature suggesting state-dependent modulation of functional connectivity (Hermundstad et al. 2013; Lau et al. 2014; Lohani et al. 2022), we quantified changes in functional connectivity during locomotion and stillness in CCI and sham-injured mice. We first determined the interhemispheric correlation coefficients during locomotion and stillness. In sham mice, functional connectivity was significantly

higher during stillness than locomotion at all timepoints examined ($t_{(265)} = -4.3$, $P = 2.7e - 5$ effect of state, $t_{(265)} = -0.13$, $P = 0.9$ effect of time, LMM; Fig. 7A). In CCI-injured animals, this state-dependent difference in interhemispheric functional connectivity was lost, but connectivity increased for both states over time after injury ($t_{(271)} = -2.0$, $P = 0.051$ effect of state, $t_{(271)} = 6.1$, $P = 4.2e - 9$ effect of time, LMM; Fig. 7D).

To examine specific connections engaged in each state-dependent network, we generated separate connectivity matrices for locomotion and stillness. Similar to interhemispheric connectivity, we found a state-dependent change in functional connectivity in sham, but not CCI mice (Fig. 7B and E). We then calculated the difference between the average connectivity matrices in locomotion versus stillness to identify a functional signature of brain activity during stillness. In sham mice, there were multiple functional connections particularly between motor and somatosensory regions that showed increased functional connectivity during stillness (Fig. 7B). In CCI mice, however, there were no connections that showed increased functional connectivity during stillness (Fig. 7E).

We next generated network diagrams of stillness-specific connectivity (Fig. 7C and F). The line thickness between brain regions corresponds to the functional connectivity strength. In shams, network diagrams showed uniformly higher connectivity between brain regions during stillness compared to locomotion. After CCI, there was little difference in connectivity between locomotion

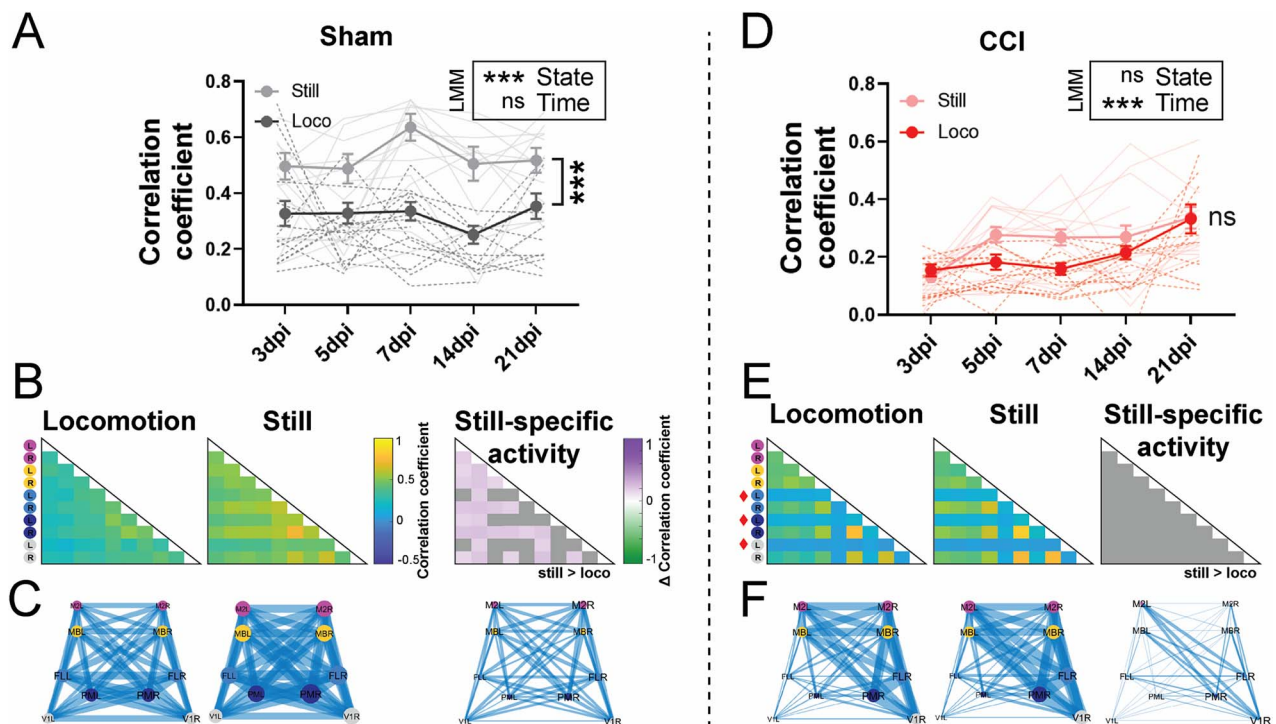


Fig. 7. Locomotion and stillness engage distinct functional connectivity states that are disrupted by CCI. (A) Interhemispheric correlation coefficients derived from mesoscale imaging in sham mice at all timepoints during locomotion and stillness show that stillness engages significantly higher connectivity across all timepoints. LMM: *** indicates $P < 0.001$, ns is not significant. (B) Average connectivity matrices (across all timepoints, all trials) in sham mice during locomotion (left) and stillness (middle). Right: Specific connections that are significantly increased during stillness in sham mice ($P < 0.01$, Benjamini–Yekutieli corrected Wilcoxon signed rank test (paired), $n = 3$ mice). Nonsignificant values are shown in gray. (C) Network diagrams indicating the strength of connectivity (the thicker the line the stronger the connectivity) in sham mice between the marked anatomic locations during locomotion (left), stillness (middle), and still-specific (still—Move) activity (right). (D) Interhemispheric correlation coefficients in CCI-injured mice at all timepoints during locomotion and stillness. There is no significant difference in connectivity between states in CCI mice. LMM: *** indicates $P < 0.001$, ns is not significant. (E) Average connectivity matrices (across all timepoints, all trials) in CCI mice during locomotion (left) and stillness (center). Areas proximal to CCI are indicated with red diamonds. Right: Average differences in correlation coefficient between stillness and locomotion in CCI mice ($P < 0.01$, Benjamini–Yekutieli corrected Wilcoxon signed rank test (paired), $n = 3$ mice). No connections showed state-dependent changes in CCI mice (gray). (F) Network diagrams in CCI mice during locomotion (left), stillness (middle), and still-specific (still—Move) activity (right). For all conditions, $n = 3$ animals per group, 3–15 instances of locomotion or stillness at each timepoint. Error bars indicate SEM. Loco indicates locomotion. Dpi, days postinjury.

and stillness. Together, these findings show that in sham-injured mice, functional cortical connectivity is higher during stillness than during locomotion, but in injured mice this state-dependent difference in connectivity is lost at all timepoints examined.

Discussion

We report a novel approach to perform mesoscale cortical imaging and ECoG after CCI, allowing a previously unattainable view of mouse brain function after TBI. First, we determined that mesoscale implant slightly increased astrocyte reactivity after TBI, but those changes were very small compared to the effect of injury itself. We then determined that GFP expression, measured by both GFP+ cell density and fluorescence intensity, was not significantly altered by injury despite a trend toward decreased expression in CCIs both proximal and distal to the CCI border. Next, we showed that CCI reduced neuronal activity and functional connectivity in regions proximal to injury. Functional connectivity, but not total neuronal activity, gradually returned over time proximal to injury. This suggests functional reorganization occurs in injured regions even while total neuronal activity remains low. These data are consistent with others' findings that the amplitude of activity and measurements of connectivity are independent (Benisty et al. 2024; Lohani et al. 2022). Interestingly, total activity and connectivity was increased in CCI

mice contralateral to injury, suggesting hyperconnectivity after TBI could contribute to hyper-synchronization associated with post-traumatic epilepsy. These findings suggest that functional connectivity changes dynamically throughout the entire cortex after injury with unique responses in ipsilateral and contralateral hemispheres.

Furthermore, we observed state-specific effects of injury on ECoG activity and functional connectivity, as well as a loss of functional state switching after TBI. Injury decreased ECoG theta power during periods of stillness but had no effect on periods of movement. On imaging in sham mice, functional connectivity was increased at rest and decreased during locomotion, however CCI eliminated this state-dependent change. By 21 days postinjury, CCI connectivity had returned to sham levels during locomotion, but injured animals never regained the state-dependent switch. Together, these findings point to an injury-induced disruption of resting state networks with much smaller effects on locomotion-specific networks, and a loss of state-dependent switching of functional connectivity (Fig. 8).

Our approach permits characterization of a movement state that is challenging to assess using human imaging modalities (e.g. fMRI) and enables study of a large area of cortex after TBI. The connectivity changes we observed in both hemispheres underscore the importance of including regions beyond the perilesional area. Additionally, human studies are limited in that

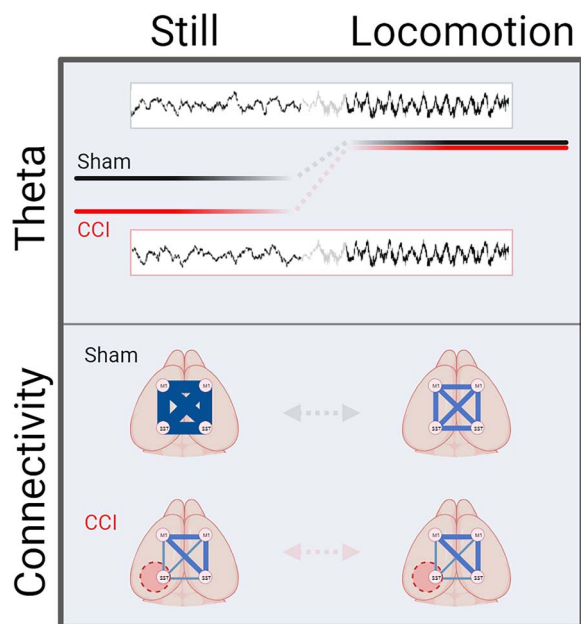


Fig. 8. TBI disproportionately alters activity during stillness compared to locomotion. During stillness, CCI decreases theta power and functional connectivity compared to sham levels. During locomotion, theta power is not significantly different from sham levels and functional connectivity is only slightly affected. Theta power and connectivity are altered during stillness but not during locomotion suggesting a state-specific effect of injury.

severity, injury region, and etiology differ drastically between patients. Recruiting patients immediately after injury to capture a consistent acute timepoint and following them over time is challenging. fMRI cannot be collected during most motor tasks such as locomotion given the restrictions of the imaging modality. Therefore, our approach uniquely allows quantification of state-specific functional connectivity at multiple timepoints after TBI.

Our findings recapitulate human studies that found arousal state affected both EEG and functional connectivity. EEG and ECoG are modulated by locomotion, particularly in the theta band. Hippocampal theta activity predicts locomotion onset (Greene et al. 2018), and theta dynamics are highly correlated with locomotion speed (McFarland et al. 1975). Locomotion-induced theta activity has not been well studied in the cortex. However, cortical neurons are at times phase-locked to hippocampal theta rhythm (Siapas et al. 2005), and theta oscillations in the motor cortex coordinate behavior with theta power altered upon locomotion onset (Igarashi et al. 2013). Our findings build on this, demonstrating in both CCI and sham-injured mice that locomotion corresponds to a significant increase in theta power.

In fMRI studies of healthy participants, functional connectivity decreased with task performance compared to resting state (Hermundstad et al. 2013). During locomotion, human functional connectivity measured via 248-channel EEG was decreased compared to standing particularly in sensorimotor cortex (Lau et al. 2014). This suggests that less coordinated input from the cortex is required for locomotion than for standing. State also alters functional connectivity in mice. Widefield cortical imaging demonstrated globally decreased connectivity between nearly all nodes examined during locomotion compared to rest (Lohani et al. 2022; West et al. 2022). Together, this indicates that both movement and task-dependent variations in state alter cortical activity in humans and in mice. Moving forward, it will be important to

collect data during multiple states to gain a more complete picture of cortical network activity and how it is modulated by disease. For example, theta relative power has been proposed as a biomarker of epileptogenesis following TBI (Milikovsky et al. 2017). Performing state-dependent analysis of theta power could improve the strength and accuracy of this biomarker.

In addition to state, EEG and functional connectivity are altered with TBI. In rodent models of TBI, theta power (Fedor et al. 2010; Lee et al. 2013; Ondek et al. 2020) and functional connectivity are decreased after TBI (Guggisberg et al. 2008; Han et al. 2014; Rigon et al. 2016). In patients with lesions, connectivity was decreased proximal to the lesion compared to intact contralateral regions and controls (Guggisberg et al. 2008). Functional connectivity was also predictive of recovery with patients who exhibited stronger interhemispheric functional connectivity performing better on cognitive tests (Rigon et al. 2016). Rehabilitated TBI patients had connectivity profiles more similar to healthy controls than to the non-rehabilitated group and scored higher on cognitive and memory indexes (Castellanos et al. 2010). These human and rodent studies indicate that functional connectivity may be an important measure to track disease severity, recovery, and response to rehabilitation. We found dynamic changes in ECoG power and functional connectivity particularly in the first week after injury, suggesting monitoring patients over several timepoints could provide additional insight into their recovery.

Beyond the commonly observed decrease in functional connectivity perilesionally, several rodent studies also observed focal increases in functional connectivity after TBI. This consistently occurred contralateral to injury in tandem with decreased connectivity ipsilateral to injury (Harris et al. 2016; Verley et al. 2019). Increased connectivity was observed in humans after mild TBI both between regions in existing brain networks and recruitment of regions not previously in-network, both thought to represent compensation after injury (Stevens et al. 2012). This could explain why we observed both increased total neuronal activity and connectivity contralateral to injury. Moving forward, it will be important to understand if contralateral connectivity has implications for functional recovery.

Putting our findings into the context of behavioral recovery, we observed significant increases in functional connectivity during time periods where behavioral deficits are still observed (Castellanos et al. 2010). However, connectivity never returned to sham levels in the cortex ipsilateral to injury. Additionally, the brain regions imaged in this study may not be involved in behaviors commonly tested, for example the Morris water maze or the novel object recognition test, both of which are largely hippocampal dependent (Broadbent et al. 2010; Vorhees and Williams 2006). Many of these studies also perform CCIs of varying severity by modulating both depth of injury from 0.5 mm to 2 mm and velocity of impact from 3.5 m/s to 5.25 m/s (Koenig et al. 2019; Washington et al. 2012; Zhao et al. 2012). More severe injuries were associated with more deficits in motor function, learning and memory, and depressive-like behavior. Finally, while improved functional connectivity correlates with increased performance on cognitive tests, it does not perfectly predict with how humans or rodents might perform on a battery of motor and cognitive tests.

Our study has several caveats. Our imaging approach is restricted to upper-layer cortical activity, and the model of CCI we employ damages the cortex (Lighthall 1988), limiting our ability to directly quantify activity in the site of injury. We therefore selected ROIs not directly within the injury site but on the border in the perilesional area. Additionally, GCaMP7c

expression is not altered by CCI perilesionally, allowing rigorous quantification of neuronal activity even after injury. Addressing the caveats of imaging neuronal activity after CCI is important because CCI models the sequelae of the most severe TBI patients with the highest symptom burden (Bullock et al. 2006; Mandera et al. 1999). Two ECoG electrodes were implanted in each mouse, one per hemisphere just lateral to the imaging window to maintain window clarity. We therefore cannot equate ECoG and imaging data with exact spatial precision. ECoG also lacks spatial resolution, however it did allow us to compare our findings with known changes in electrical activity across states ipsilateral and contralateral to injury. Development of transparent multi electrode arrays could allow us to record electrical activity with greater spatial resolution and image from the same regions. Additionally, during the development of our combined CCI/mesoscale imaging approach, we focused only on male mice. It will be important to include female mice in future studies as TBI occurs in both sexes (Thurman et al. 1999), and male and female mice differ in their response to TBI with sex-specific hormones potentially acting neuroprotectively in females (Rubin and Lipton 2019). It will also be important to implement a behavioral assessment of functional recovery that can be evaluated in tandem with functional connectivity. Employing behavioral tests alongside imaging and ECoG in male and female mice will allow us to begin testing functional effects and efficacy of therapeutic interventions. Additionally, further separating stillness epochs into low and moderate arousal states using pupil or facial motion could allow us to detect potential differences in how injured and sham animals engage in stillness.

Another limitation is the duration animals were followed for. PTE typically develops 8 or more weeks following injury, but here animals were imaged for only 21 days postinjury. Future studies will include imaging through 6 weeks postimplant, a duration where the cranial window remains transparent and stable, as well as 24/7 video-ECoG from 8 to 10 weeks post-CCI. This will allow for detection of PTE if present (Bolkvadze and Pitkanen 2012; Guo et al. 2013; Hunt et al. 2009, 2010; Szu et al. 2020). Finally, because this initial study was designed to validate mesoscale imaging in the injured brain, *n*-values are somewhat low (*n* = 3 male mice for all assays). Both statistical and power analysis, however, show that our study is sufficiently powered to detect changes in functional connectivity and ECoG after CCI. More subtle changes, with smaller effect sizes, may not be detected in this study. Additionally, CCI is a heterogeneous disease and animals respond differently. For example, some portion of animals will develop PTE after CCI, but our study is underpowered to detect subpopulations within measures such as functional connectivity recovery. Future studies with this now validated approach will include larger sample sizes and interventional approaches.

The most significant and novel finding in our study is demonstrating that both state and TBI alter functional cortical activity and that TBI has much greater effects on networks engaged during stillness as compared to during movement. In addition, we report dynamic recovery of connectivity after TBI driven by the ipsilateral hemisphere, along with increases in connectivity over sham levels in the contralateral hemisphere. Few previous studies have performed state-dependent analysis after TBI. A porcine model of pediatric TBI used fMRI to compare resting and stimulation-induced functional connectivity under anesthesia after injury (Simchick et al. 2021). Functional connectivity was decreased in TBI piglets compared to shams and state affected which networks were most decreased after injury (Simchick et al. 2021). While anesthesia drastically alters brain activity, this study

nonetheless emphasizes that state modulates the location and extent of brain regions affected by TBI. In summary, we report one of the first studies to perform awake state-dependent analysis of TBI-injured animals and identified state-specific changes in connectivity that were altered by CCI particularly when animals were at rest. Future studies will utilize mesoscale cortical imaging to help identify disease-relevant biomarkers that will allow for early and individualized therapeutic interventions.

Transparency, rigor, and reproducibility

Animals were randomized to experimental groups and all CCI and sham surgeries were performed when animals were 8–10 weeks of age. Sample sizes ensure power > 0.8 for $\alpha = 0.05$. ROI mapping and identification of periods of stillness and locomotion were automated to avoid experimenter bias. We used linear mixed modeling to account for inter- and intra-animal variability. Detailed statistical analyses are reported in [Supplementary Tables 1 and 2](#).

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Author contributions

SBT: conceptualization, methodology, software, investigation, writing—original draft. SC: methodology, validation. JM, MS, LB, TM, SH, MS: resources, writing—review & editing. MW: writing—review & editing. SQ: software, writing—review & editing. FN: formal analysis, writing—review & editing. RO, MJH, JAC: software, resources, writing—review & editing. MA, CD*: conceptualization, methodology, software, writing—original draft.

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Conflict of interest statement: None declared.

Data availability

Data generated in this study will be made available from the corresponding author on reasonable request.

References

- Aarts E, Verhage M, Veenliet JV, Dolan CV, van der Sluis S. A solution to dependency: using multilevel analysis to accommodate nested data. *Nat Neurosci*. 2014;17(4):491–496.
- Alessandri B, Heimann A, Filippi R, Kopacz L, Kempinski O. Moderate controlled cortical contusion in pigs: effects on multi-parametric neuromonitoring and clinical relevance. *J Neurotrauma*. 2003;20(12):1293–1305.
- Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med*. 1998;338(1):20–24.
- Arciniegas DB, Held K, Wagner P. Cognitive impairment following traumatic brain injury. *Curr Treat Options Neurol*. 2002;4(1):43–57.

- Balbi M, Vanni MP, Silasi G, Sekino Y, Bolanos L, LeDue JM, Murphy TH. Targeted ischemic stroke induction and mesoscopic imaging assessment of blood flow and ischemic depolarization in awake mice. *Neurophotonics*. 2017;4(3):035001.
- Barson D, Hamodi AS, Shen X, Lur G, Constable RT, Cardin JA, Crair MC, Higley MJ. Simultaneous mesoscopic and two-photon imaging of neuronal activity in cortical circuits. *Nat Methods*. 2020;17(1):107–113.
- Benisty H, Barson D, Moberly AH, Lohani S, Tang L, Coifman RR, Crair MC, Mishne G, Cardin JA, Higley MJ. Rapid fluctuations in functional connectivity of cortical networks encode spontaneous behavior. *Nat Neurosci*. 2024;27(1):148–158.
- Bolkvadze T, Pitkanen A. Development of post-traumatic epilepsy after controlled cortical impact and lateral fluid-percussion-induced brain injury in the mouse. *J Neurotrauma*. 2012;29(5):789–812.
- Broadbent NJ, Gaskin S, Squire LR, Clark RE. Object recognition memory and the rodent hippocampus. *Learn Mem*. 2010;17(1):5–11.
- Bullock MR, Chesnut R, Ghajar J, Gordon D, Hartl R, Newell DW, Servadei F, Walters BC, Wilberger J, Surgical Management of Traumatic Brain Injury Author G. Surgical management of traumatic parenchymal lesions. *Neurosurgery*. 2006;58(3 Suppl):S25–S46 discussion Si-iv.
- Burda JE, Bernstein AM, Sofroniew MV. Astrocyte roles in traumatic brain injury. *Exp Neurol*. 2016;275(3):305–315.
- Burda JE, Sofroniew MV. Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron*. 2014;81(2):229–248.
- Cantu D, Walker K, Andresen L, Taylor-Weiner A, Hampton D, Tesco G, Dulla CG. Traumatic brain injury increases cortical glutamate network activity by compromising gabaergic control. *Cereb Cortex*. 2015;25(8):2306–2320.
- Castellanos NP, Leyva I, Buldu JM, Bajo R, Paul N, Cuesta P, Ordonez VE, Pascua CL, Boccaletti S, Maestu F, et al. Principles of recovery from traumatic brain injury: reorganization of functional networks. *NeuroImage*. 2011;55(3):1189–1199.
- Castellanos NP, Paul N, Ordonez VE, Demuyneck O, Bajo R, Campo P, Bilbao A, Ortiz T, Del-Pozo F, Maestu F. Reorganization of functional connectivity as a correlate of cognitive recovery in acquired brain injury. *Brain*. 2010;133(Pt 8):2365–2381.
- Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *AJNR Am J Neuroradiol*. 2001;22(7):1326–1333.
- Cramer SW, Haley SP, Popa LS, Carter RE, Scott E, Flaherty EB, Dominguez J, Aronson JD, Sabal L, Surinach D, et al. Wide-field calcium imaging reveals widespread changes in cortical functional connectivity following mild traumatic brain injury in the mouse. *Neurobiol Dis*. 2023;176:105943.
- Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF- α , TGF- β 1 and blood-brain barrier function. *J Neuroimmunol*. 1999;101(2):211–221.
- Dana H, Sun Y, Mohar B, Hulse BK, Kerlin AM, Hasseman JP, Tsegaye G, Tsang A, Wong A, Patel R, et al. High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. *Nat Methods*. 2019;16(7):649–657.
- Di Sapia R, Moro F, Montanarella M, Iori V, Micotti E, Tolomeo D, Wang KKW, Vezzani A, Ravizza T, Zanier ER. In-depth characterization of a mouse model of post-traumatic epilepsy for biomarker and drug discovery. *Acta Neuropathol Commun*. 2021;9(1):76.
- Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using g*power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149–1160.
- Faul F, Erdfelder E, Lang AG, Buchner A. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–191.
- Fedor M, Berman RF, Muizelaar JP, Lyeth BG. Hippocampal theta dysfunction after lateral fluid percussion injury. *J Neurotrauma*. 2010;27(9):1605–1615.
- Franklin KBJ, Paxinos G. *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Amsterdam: Academic Press, an imprint of Elsevier; 2013.
- Graham NS, Sharp DJ. Understanding neurodegeneration after traumatic brain injury: from mechanisms to clinical trials in dementia. *J Neurol Neurosurg Psychiatry*. 2019;90(11):1221–1233.
- Greene AS, Gao S, Scheinost D, Constable RT. Task-induced brain state manipulation improves prediction of individual traits. *Nat Commun*. 2018;9(1):2807.
- Guggisberg AG, Honma SM, Findlay AM, Dalal SS, Kirsch HE, Berger MS, Nagarajan SS. Mapping functional connectivity in patients with brain lesions. *Ann Neurol*. 2008;63(2):193–203.
- Guo D, Zeng L, Brody DL, Wong M. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. *PLoS One*. 2013;8(5):e64078.
- Han K, Mac Donald CL, Johnson AM, Barnes Y, Wierzechowski L, Zonies D, Oh J, Flaherty S, Fang R, Raichle ME, et al. Disrupted modular organization of resting-state cortical functional connectivity in U.S. military personnel following concussive 'mild' blast-related traumatic brain injury. *NeuroImage*. 2014;84:76–96.
- Harris NG, Verley DR, Gutman BA, Thompson PM, Yeh HJ, Brown JA. Disconnection and hyper-connectivity underlie reorganization after tbi: a rodent functional connectomic analysis. *Exp Neurol*. 2016;277:124–138.
- Heiney SA, Kim J, Augustine GJ, Medina JF. Precise control of movement kinematics by optogenetic inhibition of purkinje cell activity. *J Neurosci*. 2014;34(6):2321–2330.
- Hermundstad AM, Bassett DS, Brown KS, Aminoff EM, Clewett D, Freeman S, Frithsen A, Johnson A, Tipper CM, Miller MB, et al. Structural foundations of resting-state and task-based functional connectivity in the human brain. *Proc Natl Acad Sci USA*. 2013;110(15):6169–6174.
- Hunt RF, Scheff SW, Smith BN. Posttraumatic epilepsy after controlled cortical impact injury in mice. *Exp Neurol*. 2009;215(2):243–252.
- Hunt RF, Scheff SW, Smith BN. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J Neurophysiol*. 2010;103(3):1490–1500.
- Hutchison RM, Womelsdorf T, Allen EA, Bandettini PA, Calhoun VD, Corbetta M, Della Penna S, Duyn JH, Glover GH, Gonzalez-Castillo J, et al. Dynamic functional connectivity: promise, issues, and interpretations. *NeuroImage*. 2013;80:360–378.
- Igarashi J, Isomura Y, Arai K, Harukuni R, Fukui T. A theta-gamma oscillation code for neuronal coordination during motor behavior. *J Neurosci*. 2013;33(47):18515–18530.
- Klaft ZJ, Duerrwald LM, Gerevich Z, Dulla CG. The adenosine A1 receptor agonist wag 994 suppresses acute kainic acid-induced status epilepticus in vivo. *Neuropharmacology*. 2020;176:108213.
- Koenig JB, Cantu D, Low C, Sommer M, Noubary F, Croker D, Whalen M, Kong D, Dulla CG. Glycolytic inhibitor 2-deoxyglucose prevents cortical hyperexcitability after traumatic brain injury. *JCI Insight*. 2019;5(1):19.

- Kumar A, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, Kumar A, Thom SR, Faden AI. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. *J Neuroinflammation*. 2017;14(1):47.
- Larsch J, Ventimiglia D, Bargmann CI, Albrecht DR. High-throughput imaging of neuronal activity in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA*. 2013;110(45):E4266–E4273.
- Lau TM, Gwin JT, Ferris DP. Walking reduces sensorimotor network connectivity compared to standing. *J Neuroeng Rehabil*. 2014;11(1):14.
- Lee DJ, Gurkoff GG, Izadi A, Berman RF, Ekstrom AD, Muizelaar JP, Lyeth BG, Shahlaie K. Medial septal nucleus theta frequency deep brain stimulation improves spatial working memory after traumatic brain injury. *J Neurotrauma*. 2013;30(2):131–139.
- Lighthall JW. Controlled cortical impact: a new experimental brain injury model. *J Neurotrauma*. 1988;5(1):1–15.
- Lighthall JW, Goshgarian HG, Pinderski CR. Characterization of axonal injury produced by controlled cortical impact. *J Neurotrauma*. 1990;7(2):65–76.
- Lim DH, LeDue JM, Murphy TH. Network analysis of mesoscale optical recordings to assess regional, functional connectivity. *Neurophotonics*. 2015;2(4):041405.
- Lohani S, Moberly AH, Benisty H, Landa B, Jing M, Li Y, Higley MJ, Cardin JA. Spatiotemporally heterogeneous coordination of cholinergic and neocortical activity. *Nat Neurosci*. 2022;25(12):1706–1713.
- Lutkenhoff ES, Wright MJ, Shrestha V, Real C, McArthur DL, Buitrago-Blanco M, Vespa PM, Monti MM. The subcortical basis of outcome and cognitive impairment in tbi: a longitudinal cohort study. *Neurology*. 2020;95(17):e2398–e2408.
- Ma Y, Shaik MA, Kim SH, Kozberg MG, Thibodeaux DN, Zhao HT, Yu H, Hillman EM. Wide-field optical mapping of neural activity and brain haemodynamics: considerations and novel approaches. *Philos Trans R Soc Lond Ser B Biol Sci*. 2016;371(1705):20150360.
- Mandera M, Zralek C, Krawczyk I, Zycinski A, Wencel T, Bazowski P. Surgery or conservative treatment in children with traumatic intracerebral haematoma. *Childs Nerv Syst*. 1999;15(5):267–269 discussion 270.
- McFarland WL, Teitelbaum H, Hedges EK. Relationship between hippocampal theta activity and running speed in the rat. *J Comp Physiol Psychol*. 1975;88(1):324–328.
- Milikovskiy DZ, Weissberg I, Kamintsky L, Lippmann K, Schefenbauer O, Frigerio F, Rizzi M, Sheintuch L, Zelig D, Ofer J, et al. Electro-corticographic dynamics as a novel biomarker in five models of epileptogenesis. *J Neurosci*. 2017;37(17):4450–4461.
- Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain*. 2006;129(Pt 10):2761–2772.
- Ondek K, Pevzner A, Tercovich K, Schedlbauer AM, Izadi A, Ekstrom AD, Cowen SL, Shahlaie K, Gurkoff GG. Recovery of theta frequency oscillations in rats following lateral fluid percussion corresponds with a mild cognitive phenotype. *Front Neurol*. 2020;11:600171.
- Poulet JFA, Crochet S. The cortical states of wakefulness. *Front Syst Neurosci*. 2018;12:64.
- Rigon A, Duff MC, McAuley E, Kramer AF, Voss MW. Is traumatic brain injury associated with reduced inter-hemispheric functional connectivity? A study of large-scale resting state networks following traumatic brain injury. *J Neurotrauma*. 2016;33(11):977–989.
- Ritzel RM, Li Y, Jiao Y, Lei Z, Doran SJ, He J, Shahrer RA, Henry RJ, Khan R, Tan C, et al. Brain injury accelerates the onset of a reversible age-related microglial phenotype associated with inflammatory neurodegeneration. *Sci Adv*. 2023;9(10):eadd1101.
- Rocchi F, Canella C, Noei S, Gutierrez-Barragan D, Coletta L, Galbusera A, Stuefer A, Vassanelli S, Pasqualetti M, Iurilli G, et al. Increased fmri connectivity upon chemogenetic inhibition of the mouse prefrontal cortex. *Nat Commun*. 2022;13(1):1056.
- Rubiano AM, Carney N, Chesnut R, Puyana JC. Global neuro-trauma research challenges and opportunities. *Nature*. 2015;527(7578):S193–S197.
- Rubin TG, Lipton ML. Sex differences in animal models of traumatic brain injury. *J Exp Neurosci*. 2019;13:1179069519844020.
- Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam head injury study. *Neurology*. 1985;35(10):1406–1414.
- Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. *J Cereb Blood Flow Metab*. 2010;30(3):459–473.
- Shandra O, Winemiller AR, Heithoff BP, Munoz-Ballester C, George KK, Benko MJ, Zuidhoek IA, Besser MN, Curley DE, Edwards GF 3rd, et al. Repetitive diffuse mild traumatic brain injury causes an atypical astrocyte response and spontaneous recurrent seizures. *J Neurosci*. 2019;39(10):1944–1963.
- Shuman T, Aharoni D, Cai DJ, Lee CR, Chavlis S, Page-Harley L, Vetere LM, Feng Y, Yang CY, Mollinedo-Gajate I, et al. Breakdown of spatial coding and interneuron synchronization in epileptic mice. *Nat Neurosci*. 2020;23(2):229–238.
- Siapas AG, Lubenov EV, Wilson MA. Prefrontal phase locking to hippocampal theta oscillations. *Neuron*. 2005;46(1):141–151.
- Silasi G, Xiao D, Vanni MP, Chen AC, Murphy TH. Intact skull chronic windows for mesoscopic wide-field imaging in awake mice. *J Neurosci Methods*. 2016;267:141–149.
- Simchick G, Scheulin KM, Sun W, Sneed SE, Fagan MM, Cheek SR, West FD, Zhao Q. Detecting functional connectivity disruptions in a translational pediatric traumatic brain injury porcine model using resting-state and task-based fmri. *Sci Rep*. 2021;11(1):12406.
- Sporns O. Contributions and challenges for network models in cognitive neuroscience. *Nat Neurosci*. 2014;17(5):652–660.
- Stevens MC, Lovejoy D, Kim J, Oakes H, Kureshi I, Witt ST. Multiple resting state network functional connectivity abnormalities in mild traumatic brain injury. *Brain Imaging Behav*. 2012;6(2):293–318.
- Szu JI, Chaturvedi S, Patel DD, Binder DK. Aquaporin-4 dysregulation in a controlled cortical impact injury model of posttraumatic epilepsy. *Neuroscience*. 2020;428:140–153.
- Temkin NR. Preventing and treating posttraumatic seizures: the human experience. *Epilepsia*. 2009;50(Suppl 2):10–13.
- Thurman DJ, Alverson C, Browne D, Dunn KA, Guerrero J, Johnson R, Johnson V, Langlois J, Pilkey D, Sniezek JE, Toal S. Report to Congress: Traumatic Brain Injury in the United States. Centers for Disease Control and Prevention. Atlanta, GA, 1999. https://www.cdc.gov/traumaticbraininjury/pubs/tbi_report_to_congress.html.
- Vanni MP, Chan AW, Balbi M, Silasi G, Murphy TH. Mesoscale mapping of mouse cortex reveals frequency-dependent cycling between distinct macroscale functional modules. *J Neurosci*. 2017;37(31):7513–7533.
- Verley DR, Torolira D, Hessell BA, Sutton RL, Harris NG. Cortical neuromodulation of remote regions after experimental traumatic brain injury normalizes forelimb function but is temporally dependent. *J Neurotrauma*. 2019;36(5):789–801.

- Vinck M, Batista-Brito R, Knoblich U, Cardin JA. Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. *Neuron*. 2015;86(3):740–754.
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc*. 2006;1(2):848–858.
- Walker WC, Pickett TC. Motor impairment after severe traumatic brain injury: a longitudinal multicenter study. *J Rehabil Res Dev*. 2007;44(7):975–982.
- Washington PM, Forcelli PA, Wilkins T, Zapple DN, Parsadanian M, Burns MP. The effect of injury severity on behavior: a phenotypic study of cognitive and emotional deficits after mild, moderate, and severe controlled cortical impact injury in mice. *J Neurotrauma*. 2012;29(13):2283–2296.
- Wekselblatt JB, Flister ED, Piscopo DM, Niell CM. Large-scale imaging of cortical dynamics during sensory perception and behavior. *J Neurophysiol*. 2016;115(6):2852–2866.
- West SL, Aronson JD, Popa LS, Feller KD, Carter RE, Chiesl WM, Gerhart ML, Shekhar AC, Ghanbari L, Kodandaramaiah SB, et al. Wide-field calcium imaging of dynamic cortical networks during locomotion. *Cereb Cortex*. 2022;32(12):2668–2687.
- Witcher KG, Bray CE, Chunchai T, Zhao F, O'Neil SM, Gordillo AJ, Campbell WA, McKim DB, Liu X, Dziabis JE, et al. Traumatic brain injury causes chronic cortical inflammation and neuronal dysfunction mediated by microglia. *J Neurosci*. 2021;41(7):1597–1616.
- Xiao D, Forsy BJ, Vanni MP, Murphy TH. Mesonet allows automated scaling and segmentation of mouse mesoscale cortical maps using machine learning. *Nat Commun*. 2021;12(1):5992.
- Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. *Nat Rev Neurosci*. 2013;14(2):128–142.
- Yang Z, Zhu T, Pompilus M, Fu Y, Zhu J, Arjona K, Arja RD, Grudny MM, Plant HD, Bose P, et al. Compensatory functional connectome changes in a rat model of traumatic brain injury. *Brain Commun*. 2021;3(4):fcab244.
- Zhang Y, Rozsa M, Liang Y, Bushey D, Wei Z, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, et al. Fast and sensitive gcamp calcium indicators for imaging neural populations. *Nature*. 2023;615(7954):884–891.
- Zhao Z, Loane DJ, Murray MG 2nd, Stoica BA, Faden AI. Comparing the predictive value of multiple cognitive, affective, and motor tasks after rodent traumatic brain injury. *J Neurotrauma*. 2012;29(15):2475–2489.