nature mental health

Article

https://doi.org/10.1038/s44220-024-00329-8

Hippocampal functional connectivity after whiplash injury is linked to the development of chronic pain

Received: 11 August 2023

Accepted: 11 September 2024

Published online: 24 October 2024

Check for updates

Paulo Branco[1,2], Noam Bosak[3,4], Andrew D. Vigotsky[2,5,6], Yelena Granovsky[3,4], David Yarnitsky[3,4] & A. Vania Apkarian[1,2,5]

Brain-centric theories propose that chronic pain is driven and maintained by maladaptive negative emotional learning, with the hippocampus playing a crucial role in the transition from acute to chronic pain. However, little is known about what triggers this maladaptive learning in the first place, especially in the early acute stages following injury. We imaged 110 patients within days of whiplash and mild traumatic brain injury and tested whether hippocampal adaptations impart risk for chronic pain one year later. Patients who went on to develop chronic pain one year later showed increased connectivity between the hippocampus and its posterior network, as well as increased network connectivity across posterior hippocampal network nodes and the amygdala bilaterally. This connectivity was linked to anxiety and increased with time lapse from injury to brain scans. Our findings link rapid hippocampal network reorganization with the development of chronic pain.

Chronic pain remains a major source of disability and suffering worldwide, carrying large health and societal costs. While the underlying mechanisms of chronic pain are not yet fully understood, the brain's emotional-learning circuits have been hypothesized to play an important role in the development and persistence of chronic pain[1–3], arguably by controlling the (motivational, salient) value of incoming nociceptive inputs, driving conscious pain perception and further impacting behavior selection through positive and negative reinforcement[1]. Within this circuitry, the hippocampus plays a key role in encoding, consolidating, and retrieving emotionally salient experiences[4], including pain[5,6], and its functional and structural properties have been linked with pain and pain-related behavior: in the spared-nerve-injury rodent model for chronic pain, blocking hippocampal neurogenesis halts or delays the development of mechanical allodynia[7], and direct activation of hippocampal neurons can revert mechanical allodynia and induce affect-motivated place preference[8]. Human studies have also shown that hippocampal morphology mediates the exaggeration of pain intensity recall in chronic pain patients[9] and, most important, that hippocampus volume and functional connectivity predict which patients transition from subacute to chronic back pain[10–12]. Yet underlying mechanisms remain poorly understood, partly because learning-induced plastic changes in hippocampal circuitry are known to occur within hours to days[13], and this critical time window has not yet been studied regarding the transition to chronic pain. As a consequence, it remains uncertain whether abnormal hippocampal properties represent pre-existing risk factors or, instead, (mal)adaptive changes that develop over time after injury—and in the latter scenario, what underlies these processes, and at what timescales do they occur?

Within this context, patients presenting with whiplash and mild traumatic brain injury (mTBI) following a motor vehicle crash provide

[1]Department of Anesthesiology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. [2]Center for Translational Pain Research, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. [3]Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel. [4]Department of Neurology, Rambam Health Care Campus, Haifa, Israel. [5]Department of Neuroscience, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. [6]Departments of Biomedical Engineering and Statistics, Northwestern University, Evanston, IL, USA.
e-mail: a-apkarian@northwestern.edu

a unique opportunity to study early and rapid plasticity within emotional-learning circuitry: their pain onset is well defined, patients can be scanned within hours of the accident without brain damage confounds, and up to 50% continue to experience pain one year after injury[14]. Moreover, such accidents usually result in heightened anxiety and stress[15], which are risk factors for developing chronic pain[6] and are known to impact learning, with further consequences for hippocampal function and structure[16,17]. In this study, we set to investigate if rapid hippocampal plasticity is associated with the transition from acute to chronic pain. To do so, we collected structural and resting-state magnetic resonance imaging (MRI) scans in mTBI and whiplash patients within five days of a motor vehicle accident. These patients were then followed up for up to one year after the injury, to test if structural and functional hippocampal properties at the early acute pain stage can dissociate between patients who develop chronic pain versus those who are pain-free one year later.

Results

We recruited 249 mTBI and whiplash patients, of which 110 patients completed structural and resting-state scans, had usable data after quality control, met clinical criteria for both whiplash injury and mTBI, had no post-traumatic memory loss, and completed the longitudinal pain ratings at the one-year endpoint (Methods). Fifty-eight patients reported clinically significant persistent pain one year after injury (53%, numerical rating scale (NRS) ≥ 30/100; we label these chronic pain), and 52 patients were pain-free (47%, NRS = 0; Fig. 1a). After the accident and at the time of their brain scan, patients whose pain persisted one year later reported higher acute pain ($t = 2.02$, $P = 0.046$; Fig. 1a) and marginally higher depression ($t = 1.88$, $P = 0.064$) compared with patients who recovered; otherwise, the two groups reported similar post-injury psychological characteristics, including anxiety ($t = -0.6$, $P = 0.55$), perceived stress ($t = 0.04$, $P = 0.96$), and pain catastrophizing ($t = -0.88$, $P = 0.38$).

To study hippocampal functional connectivity and given its known functional segregation along its longitudinal axis, we parcellated the region into its head, body, and tail[4]. We performed seed-based correlation analyses between hippocampal regions of interest (ROIs) from the left and right hippocampi and voxels within the extended hippocampal network (Fig. 1b). In the days following the accident, patients whose pain persisted showed higher functional connectivity than did patients who fully recovered between the left hippocampus body and the posterior cingulate, the left angular gyrus, and the right inferior parietal lobule (Fig. 1c). These results were mirrored for the right hippocampus body—higher connectivity to the posterior cingulate and right inferior parietal lobule (Fig. 1d). Similar findings were observed for the left tail of the hippocampus, with differences in connectivity to the right inferior parietal lobule and right angular gyrus (Fig. 1e). A multivariate non-parametric combination analysis was also performed including all six ROIs, and while the results recapitulated the main findings, only the right inferior parietal lobule cluster survived multiple comparisons correction (Supplementary Fig. 1). Hippocampal connectivity in chronic pain patients was also substantially higher than meta-analytic connectivity estimates from 273 healthy participants, for both the left and right hippocampal bodies (but not as evident for the hippocampal tail; Fig. 1c–e).

Considering the fast timescales for plasticity within hippocampal networks[13,18], we studied the time dependence of hippocampal functional connectivity. We modeled hippocampal connectivity as a function of the group (chronic versus recovered) and hours since the accident. For each of the three aforementioned ROIs, there was a significant group-by-time interaction (left body: $\beta = 0.004$, $t = 2.83$, $P = 0.006$; right body: $\beta = 0.003$, $t = 2.48$, $P = 0.015$; left tail: $\beta = 0.003$, $t = 2.56$, $P = 0.012$). Patients who developed chronic pain showed higher hippocampal functional connectivity to the posterior hippocampal network. This connectivity increased with the time lag from the accident to the brain scan (Fig. 2a–c). We also grouped the data on the basis of chronological days (1, 2 or 3 days after injury) to account for sleep; all three ROIs showed high discriminability between groups at later days after the accident, with areas under the receiving operator characteristic curve (AUCs) > 0.59, 0.66, and 0.85 when patients were scanned 1, 2, and 3 days after injury, respectively (Fig. 2a–c bottom panel).

Hippocampal volumes were also examined given previous data linking lower hippocampal volumes with increased risk for the transition from subacute to chronic back pain[10]. Hippocampal volumes, for the whole structure and head, body, and tail ROIs, were quantified using FreeSurfer hippocampal segmentation[19]. No appreciable differences in hippocampal volumes were found between groups (whole structure, or head, body or tail separately, all Ps > 0.24; Supplementary Fig. 2a and Supplementary Table 1), even when including group-by-time interactions (all Ps > 0.74; Supplementary Fig. 2b and Supplementary Table 2).

To relate these findings to psychological features, we examined whether anxiety over the past week and perceived stress over the past 4 weeks can explain hippocampal connectivity differences. These variables were singled out given their known relationship with hippocampal function[17,20] and chronic pain[6]. We found significant three-way interactions between group, time, and anxiety for both the left and right hippocampal body ROIs ($t = 2.20$, $P = 0.031$; $t = 2.35$, $p = 0.021$, respectively), but not for the tail of the right hippocampus ($t = 0$, $P = 0.99$). For patients who developed chronic pain 1 year later, the higher reported anxiety and greater duration since the accident corresponded with higher hippocampal functional connectivity (Fig. 2d–f). For pain-free patients at 1 year, there were associations with neither elapsed time nor anxiety. No significant associations were found for perceived stress (Supplementary Table 3). In addition, baseline pain, whiplash severity, and depression did show significant main effects, and did not interact with group in explaining hippocampal connectivity (Supplementary Tables 4–6).

For completeness, we also explored group differences for all pairwise connections between hippocampal network ROIs using network-based statistics (NBS)[21]. The posterior hippocampal network showed large-scale upregulated connectivity for patients who developed chronic pain compared with those pain-free at 1 year (NBS, $P = .049$; Fig. 3a). Besides recapitulating the main hippocampus results (Fig. 3b), differences in connectivity between groups were also observed for connectivity from the amygdala to the posterior cingulate, the precuneus, the angular gyrus, and the inferior parietal cortex (bilaterally; Fig. 3c), as well as connectivity between the left and right inferior parietal lobules (Fig. 3d).

Discussion

In this study, we scanned whiplash and mTBI patients within days of injury and followed them longitudinally over 1 year to investigate early acute changes following injury that may impart risk for chronic pain. Our findings can be summarized in three key points. First, we found that patients who develop chronic pain 1 year after injury show increased connectivity between the hippocampus and its posterior network, as well as increased network connectivity across posterior hippocampal network nodes and bilateral amygdala. Second, we show that this increased connectivity is time sensitive, highlighting maladaptive plastic responses occurring within days of injury. Third, we show that these plastic changes are additionally modulated by anxiety, highlighting a potential pathological state that may be moderating these brain changes.

Previous studies have suggested that chronic pain is in part driven and maintained by maladaptive negative emotional-learning mechanisms[1,3], resulting in several behavioral consequences, including pain overgeneralization and fear avoidance[2,22]. The hippocampus has been suggested to play a key role in these maladaptive processes[1,23,24] due to its role in emotional learning[4,25] and given that its structural and functional

https://doi.org/10.1038/s44220-024-00329-8

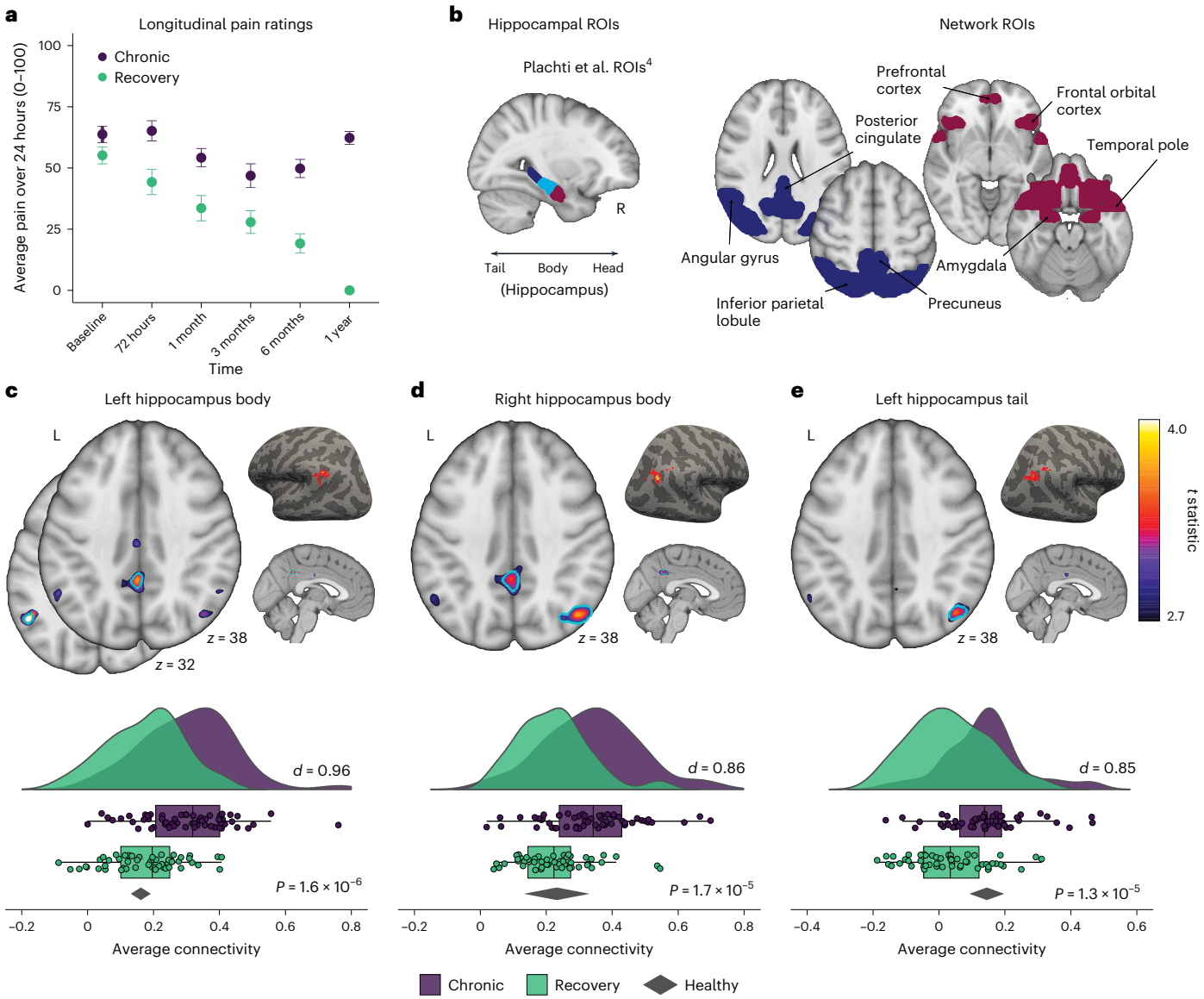

Fig. 1 | Hippocampal functional connectivity within days after injury was associated with pain chronicity 1 year later. a, One-hundred ten patients were scanned after a motor vehicle accident and their pain followed over 1 year: 58 patients reported high pain after a year (NRS ≥ 30 on a 0–100 scale; purple) and 52 were pain-free (NRS = 0, green). Error bars denote the standard error of the mean. **b**, Hippocampal head, body, and tail seeds were defined from the hippocampal parcellation of ref. 4, and target hippocampal network ROIs were defined using the Harvard–Oxford Cortical Atlas. **c**–**e**, Seed-based correlations (univariate linear regressions) were calculated between the hippocampus's head, body, and tail (seeds) to voxels within hippocampal networks, contrasting chronic and recovery groups while controlling for age, sex, and average framewise displacement. The middle (left and right) and posterior hippocampus exhibited upregulated connectivity to the posterior hippocampal network in the chronic pain group (purple) compared with the patients who recovered (green). Connectivity estimates (Fisher's *r*-to-*z* values) are reported for each data point adjusted for sex, age, and average head motion. Effect sizes (Cohen's *d*) and *P* values for the group effects are reported. Box plots denote the interquartile range (IQR) and the median, and whiskers are 1.5× IQR. Diamonds show meta-analytic estimates and prediction intervals (95% prediction interval) derived from 10 datasets with 273 healthy participants, taken to reflect normative connectivity values. Maps were thresholded at threshold-free cluster enhancement (TFCE) $P < 0.001$ uncorrected for illustration; blue outlines denote TFCE clusters with family-wise error correction for multiple comparisons at $P < 0.05$. L, left; R, right.

properties are associated with the transition to chronic pain[1,12]. Our findings lend support to this thesis by showing that connectivity within the posterior hippocampal network, acutely after injury, is associated with the development of chronic pain. The posterior hippocampal network is crucial for memory processes[25,26], and increases in connectivity within this network have been observed during successful learning and memory consolidation[27]. There is also evidence that this network plastically changes within hours as new engrams are formed[13,18], and noninvasive stimulation of the angular gyrus and inferior parietal lobule, indirectly targeting the body of the hippocampus, has been shown to causally enhance learning and recall[25]. Indeed, this increased hippocampal connectivity—in fact also considerably higher than meta-analytical connectivity estimates from healthy individuals—together with the time-dependent increase in hippocampal connectivity hours after injury in patients who go on to develop chronic pain seems to suggest that this circuit is becoming abnormally upregulated after injury, possibly reflecting a maladaptive learning process that is imparting risk for chronic pain. This interpretation, while speculative, also aligns with preclinical findings showing that blocking neurogenesis in rodents, and hence interfering with learning after a peripheral injury, can perturb

https://doi.org/10.1038/s44220-024-00329-8

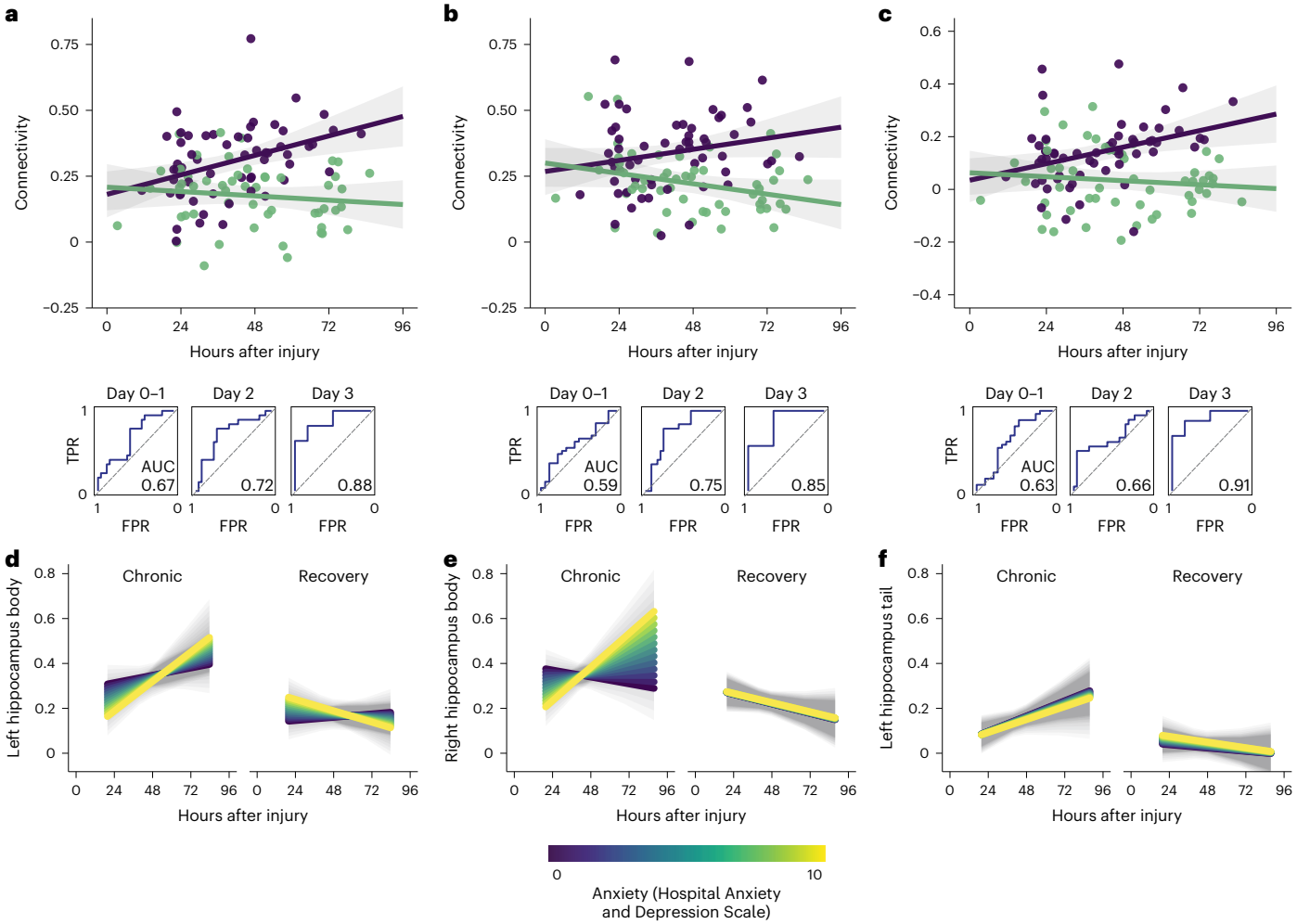

Fig. 2 | Hippocampal functional connectivity increased with the time lag from the accident to the brain scan and with anxiety for participants who develop chronic pain. a–**c**, Hippocampal connectivity was positively associated with time elapsed since injury for patients who developed chronic pain (purple), but not for patients who recovered (green), as indicated by a linear regression with a group-by-time interaction (adjusted for age, sex, and average framewise displacement); this was observed for the left (**a**) and right (**b**) hippocampus body and left hippocampus tail (**c**), with grey areas denoting the 95% confidence intervals. AUCs discriminating patients with chronic pain from patients who were pain-free at 1 year are reported below each ROI, grouping patients by chronologic days instead of elapsed hours (days zero and one, day two, and day three). For all ROIs, hippocampal connectivity successfully discriminated patients who recovered from those who developed chronic pain at day two and, better, at day three. FPR, false positive rate; TPR, true positive rate. **d**–**f**, Adding anxiety (Hospital Anxiety and Depression Scale) to the linear regression model additionally revealed the role of anxiety in hippocampal functional connectivity, with three-way interactions (group by time by anxiety) found for the left (**d**) and right (**e**) hippocampus body; the higher anxiety and more time elapsed since injury, the higher the hippocampal functional connectivity, but only for patients who developed chronic pain. This result was not observed for the left hippocampus tail (**f**).

the development of chronic pain[7]. Our post hoc network analyses also revealed a substantial participation of the amygdala in this maladaptive response. The amygdala has been linked to both emotional learning and the transition to chronic pain[1,10] and is well known to participate in emotional and aversive processing, as well as fear conditioning[28]. Reciprocal connections between hippocampus and amygdala have also been shown to play a key role in the formation of emotional and aversive memories[29,30]. In our network analyses, we did not find an association between hippocampus and amygdala connectivity and long-term pain outcomes, yet the amygdala's participation suggests an engagement of emotional-learning circuitry. The specific role of the amygdala in the transition to chronic pain in this context remains to be studied in the future.

The hippocampus is also remarkably sensitive to stress and anxiety given its link with the hypothalamic–pituitary–adrenal axis[20]. Rodent studies show that hippocampal neurogenesis and other physiological properties are altered after stress-inducing events[31], including traumatic brain injury[32], and hippocampal volume and evoked responses to experimental pain stimuli have been shown to be associated with basal cortisol levels in chronic pain patients[6]. Further, stress and anxiety are also well known to impact learning and hippocampal functioning in humans[16,17,33], and in fact, connectivity increases between hippocampus and the posterior hippocampal network after a traumatic event predicts future post-traumatic stress disorder[34,35], a condition also tightly linked with emotional learning. It is also known that anxiety and other related cognitive processes, including fear avoidance, kinesiophobia, and pain catastrophizing, are risk factors for the development of chronic pain in several pain conditions[36], and their resolution through, for example, pain reprocessing therapy, shows promise for the treatment of chronic pain[37]. In our study, we found no significant associations between hippocampal functional connectivity and the perceived stress scale that measures stress symptoms over 4 weeks—likely too large of a timescale to capture the acute stress response caused by the accident; however, we found that hippocampal connectivity in chronic pain patients is influenced by both the time elapsed since injury and anxiety, which instead measures a shorter 1 week period.

https://doi.org/10.1038/s44220-024-00329-8

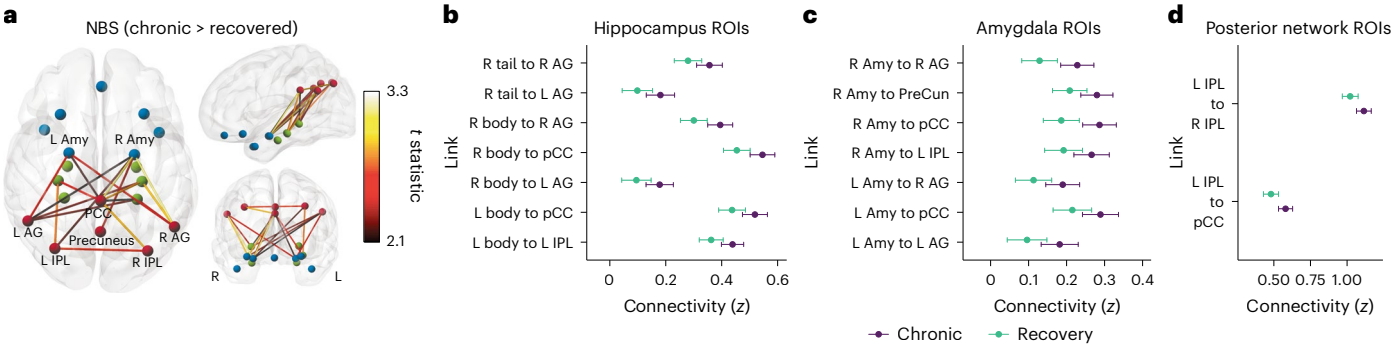

Fig. 3 | NBS also showed upregulated connectivity for the amygdala and several posterior hippocampal network regions in patients who developed chronic pain. a, NBS showed widespread increased connectivity across the hippocampus, the amygdala, and multiple posterior hippocampal network nodes (but not anterior) in patients who developed chronic pain 1 year later compared with those who recovered ($N = 110$, permutation test, $P = 0.047$ after 5,000 permutations, testing for network extent with link threshold set at Cohen's $d > 0.2$ one-tailed, and controlling for age, sex, and average framewise displacement). **b**–**d**, Hippocampal connectivity was higher for several links in the hippocampus as reported in the seed-based correlational analyses (**b**) but also for the amygdala (**c**) and between the left and right inferior parietal lobules (IPLs), and between the left IPL and the posterior cingulate cortex (pCC) (**d**). Purple represents patients who developed chronic pain. Green represents patients who fully recovered. Mean connectivity estimates (Fisher *r*-to-*z* values) are reported as dots, with corresponding 95% confidence intervals, adjusted for age, sex, and average framewise displacement. AG, angular gyrus; PreCun, precuneus cortex; Amy, amygdala.

Thus, anxiety may be moderating this maladaptive process at this early post-accident stage, and it seems that this response is likely more tied to the immediate, transient, and post-injury state-anxiety rather than to more persistent predisposing traits. Importantly, anxiety alone did not differentiate the two groups, perhaps suggesting an indirect effect, at least at this early acute stage. In the future, it will be important to associate plastic events with more direct and time-locked physiological measures of stress to further shed light on this association.

Our results also show a dissociation between the anterior and posterior hippocampal networks: while the latter is associated with pain outcomes, the former is not. Canonically, the anterior hippocampal is more frequently associated with emotional processing while the posterior network is associated with declarative and autobiographical memory[4,38–42]. In the context of pain, however, there is evidence supporting the involvement of both systems. Connectivity from the hippocampus to both anterior and posterior hippocampal networks has been shown to predict the transition from subacute to chronic back pain, as well as show plastic changes over the course of this transition[12]. A previous study also showed that exaggerated memories of pain in chronic pain patients relate to structural properties of the posterior hippocampus[9], and indeed, in the spared-nerve-injury rat model, stimulation of the dorsal (posterior in humans) hippocampus leads to pain analgesia, while stimulation of the anterior hippocampus yields no effects[8]. It is also worth noting that the angular gyrus, the inferior parietal lobule, and the precuneus have also been linked previously with the persistence of pain 3 months after mild traumatic brain injury through decreased functional connectivity to the periaqueductal grey area (PAG), which was interpreted as reflecting diminished top-down regulation of pain perception through descending modulation[43]. If our results are indeed taken as reflecting maladaptive learning, it makes intuitive sense that changes in this system may indeed further impact descending modulation systems as a way to guide adaptive behavior[1,44]. In our previous work in this same dataset, we performed a comparable PAG analysis and could not find similar results, yet PAG was indeed also predictive of pain outcomes 1 year later through increased connectivity to the head/neck region within the postcentral gyrus[45]. The intricate interplay between emotional processing, learning, and memory and descending modulation circuitry remains unclear at present and should be the object of future study.

Finally, the temporal specificity for hippocampal responses evidenced here, together with the lack of any volumetric differences at this early acute stage, also supports the view that hippocampal plastic changes develop over time following injury and are not predisposing factors as previously speculated[10]. These findings further highlight a potential critical time window for the transition to chronic pain, providing a window of opportunity for its treatment, management, and prevention[46]. Alternatively, the lack of volumetric differences found in this study, in contrast to previous findings in subacute back pain[10], could point out that different pain conditions may have different timescales for plasticity, or differing neural mechanisms.

Our study comes with limitations. The whiplash and mTBI clinical model used here might introduce biases such as undetectable brain lesions, concussion effects, variations in treatment access, post-injury treatments, and other factors that could affect outcomes. We also did not systematically collect medication status throughout the study, which could have impacted pain ratings at follow-up. Our analysis covered six ROIs, and when performing a multivariate analysis with all ROIs and adjusting for multiple comparisons across both voxels and ROIs, only the right inferior parietal lobule cluster survived adjustment for multiple comparisons, with the two other clusters not surpassing the adjusted significance threshold. The voxel size used in this study might have influenced functional connectivity estimates and consequently the sensitivity of the analysis, particularly for the tail of the hippocampus (the smallest of the three ROIs). Further, even if the time from injury to data collection was determined mostly by scanner availability, we cannot rule out that additional unknown factors (for example, access to health care and socioeconomical status) could have also played a role in our findings. Further studies employing a within-subject design are needed to control for possible inter-individual differences. A considerable number of participants opted out of scanning (29%), and some were lost to follow-up (11%). While pain outcomes were similar for patients who underwent scanning versus those who did not ($P = 0.30$), this could potentially introduce some bias in our studied sample. Finally, in our sample, we analyzed only a homogeneous group of patients, given inclusion and exclusion criteria. Our findings replicate in 23 individuals who were left out of the main analyses (Supplementary Fig. 3), but stronger validation approaches are needed to assess the generalizability of these findings.

In summary, our study identified a time-dependent maladaptive hippocampal response that places patients at risk for chronic pain after acute injury. Our findings provide a plausible mechanism for theories claiming that maladaptive emotional learning is central to the pathophysiology of chronic pain. Future studies are required to test this hypothesis conclusively by studying other brain properties,

assessing longitudinal changes in this system, and explicitly studying memory consolidation and behavior consequences in these patients.

Methods

Participants

Participants included in this study were recruited at the Rambam Health Care Campus emergency department presenting with whiplash and mild traumatic brain injury at a maximum of 24 hours after a motor vehicle collision. Participants signed an informed consent in agreement with the Declaration of Helsinki. The study was approved by the institutional review board of Rambam Health Care Campus (ref. 0601-14). Participants received compensation for their time. Inclusion criteria were direct or indirect head and neck injury, a Glasgow Coma Scale score of 13–15, and no subsequent decline. Exclusion criteria were any imaging-based traumatic brain findings when available, more than 30 minutes of unconsciousness, other major bodily injuries, chronic head/neck pain before the injury, injuries in the head/neck area within 1 year before the current injury, and neurological diseases compromising the interpretation of brain function (for example, neurodegenerative diseases). The full inclusion and exclusion pipeline can be found in Supplementary Fig. 4. Two hundred forty-nine patients were initially recruited, of which 74 were excluded from the study: 64 did not perform an MRI (claustrophobia or not willing to participate), 8 did not perform a resting-state functional MRI (fMRI) scan (patients unwilling to continue with the scan after structural imaging), 1 patient had an incidental brain finding, and 1 was diagnosed with a major psychiatric condition. Of the remaining 175 participants, 23 patients did not reach the end of the study: 19 participants were lost to follow-up at the 1 year period, and 4 patients were involved in another motor vehicle collision.

Of the participants who completed the study, we removed an additional 3 patients who presented with post-traumatic amnesia, 11 patients who did not meet clinical criteria for whiplash associated disorder, 7 patients who did not meet criteria for mild traumatic brain injury, and 3 patients whose baseline clinical data, including pain ratings, could not be collected. Three additional patients had less than 5 minutes of usable data (see the following). For transparency, and since these patients were still scanned at baseline and completed the study, we instead examined their data post hoc: we fitted a logistic regression model with hippocampal features in the main sample and used this model to predict which patients develop chronic pain in this extra unseen sample. This model achieved good classification accuracy even in this heterogeneous sample (AUC = 0.77, 95% confidence interval: 0.55–1.00); however, given the exploratory nature of these results, they are not reported in the main manuscript and can be seen in Supplementary Fig. 3.

The sample reaching the end of the study consisted of 125 patients (mean age 36.7 years ± 11.5 (s.d.), range 18–67 years, 53% male). At the 1 year endpoint, 58 participants reported clinically significant pain (NRS ≥ 30/100), 52 reported no pain (NRS = 0), and 15 reported minimal pain (0 < NRS < 30; Supplementary Fig. 4). To heighten the contrast between samples and for interpretability, we analyzed only patients who fully recovered versus those with clinically significant pain (we excluded patients with pain outcomes between 1 and 29), leaving our final sample with 110 patients. This was decided a priori. Since patients with chronic low-intensity pain were excluded from the main analyses, for transparency, we reran the analyses by including all participants and modeling pain continuously to avoid arbitrary thresholding and found similar results for all analyses (Supplementary Fig. 5).

Study design and procedures

After the emergency room visit, patients were informed about the study and signed informed consent. Patients were then scheduled for a baseline visit within 5 days of the injury (mean 1.7 days, range = 0–5), where they performed an MRI scan (structural and resting-state protocols). The time between the injury and scanning was determined by scanner and subject availability and was not confounded by clinical status or injury severity. Participants were asked to refrain from taking analgesic medication for 24 hours before the data collection. Patients also performed a series of clinical, psychological, and psychophysical tests, which are explained in detail in a previous publication[47]. Briefly, they completed a battery of psychological questionnaires measuring anxiety and depression (Hospital Anxiety and Depression Scale), pain catastrophizing (Pain Catastrophizing Scale), pain sensitivity (Pain Sensitivity Questionnaire), and perceived stress (Perceived Stress Scale), performed electroencephalography and quantitative sensory testing, and provided blood samples for genetic analyses (a detailed list of collected measures is provided in the Supplementary Information). Some of these data are featured in other publications examining psychophysical and psychological predictors of early acute pain[48] and examining the predictive ability of the ventral striatum and descending modulatory circuitry for chronic pain[45]. Patients were then followed for 1 year, when they were either contacted by phone or asked to return to the lab for a follow-up visit. They reported their pain at 72 hours after their baseline visit and every month thereafter for up to a year. Most pain ratings were obtained at 12 months, which was the primary outcome of the study. They were asked to rate their average pain over the previous 24 hours, separately for their neck and head, using an numeric rating scale (0–100; 0: no pain, 100: worst imaginable pain); their actual pain was determined to be the highest of the two.

Scanner parameters

All images were collected on a 3T (MR 750, SIGNA 20, GE Medical Systems) scanner with a 16-channel head coil. High-resolution T1-weighted images were collected with an IR-FSPGR-BRAVO sequence with the following parameters: field of view (FOV) = 256 × 256 mm², flip angle = 12°, slice thickness = 1 mm, in-plane pixel size 1 × 1 mm², axial slices = 172. Resting-state fMRI images were collected with an echoplanar imaging sequence with the following parameters: repetition time = 2,000 ms; echo time = 30 s; FOV = 220 × 220 mm²; flip angle = 75°; slice thickness = 3.4 mm; in-plane pixel size 3.44 × 3.44 mm²; and axial slices = 43, for a total duration of 10 minutes. The first 8 seconds of acquisition in each run were excluded due to T1 equilibration effects. Participants were instructed to stay as still as possible, close their eyes, not fall asleep, and think of nothing in particular.

Data preprocessing

Data were preprocessed with standard methods, using fMRIPrep 20.2.4[49], and step-by-step detailed methods can be consulted in the Supplementary Information. Briefly, T1 data were skull-stripped and segmented using FAST[50] and registered to MNI (Montreal Neurological Institute) space using nonlinear transformation with ANTs[51]. Functional imaging was motion-corrected and slice-time corrected. Several confounding time series were extracted from functional data: framewise displacement, a derivative of the root mean squared over voxels (DVARS), head-motion parameters, including their temporal derivatives and quadratic terms for each, and signals within the cerebral spinal fluid (CSF) and white matter (WM). To better estimate noise components from CSF and WM, component-based noise correction (CompCor) was also performed[52]; here we used the anatomical variant, aCompCor, which runs voxelwise principal component analyses to extract orthogonal components related to signal within CSF and WM ROIs. Functional data were normalized to MNI space before any additional preprocessing steps. Freesurfer hippocampal segmentations were extracted using Freesurfer 7.1.1 hippocampal and amygdala segmentations[19] from the Freesurfer data generated as part of default fMRIPrep pipeline. The ENIGMA hippocampal segmentation quality control pipeline[53] was implemented to verify the successful execution of all segmentations (Supplementary Information).

To de-noise the data, first, we regressed out motion and noise parameters from the data, namely, the six head-motion parameters,

their squared parameters, and the temporal derivatives of both (also known as the Friston 24 expansion), to which we added the framewise displacement, the average signal from the WM and CSF, as well as the first ten principal components aCompCorr generated by fMRIPrep. These parameters were regressed out of the data in a single step through multivariable regression, using the python tool Denoiser 1.0.1 (https://github.com/arielletambini/denoiser). Data were filtered using a band-pass filter from 0.01 to 0.1 Hz. Then high-motion timepoints and their adjacent volumes (the timepoint before and the four points after the identified motion outliers, as recommended by ref. 54 were also removed if they exceeded one of two criteria: timepoints with a framewise displacement larger than 0.5 or DVARS larger than 2.3. Participants with less than 5 minutes of remaining data were excluded from further analyses ($n = 3$). Average framewise displacement was used as a covariate of no interest for all analyses. Finally, the data were spatially smoothed using a 5 mm full-width-at-half-maximum Gaussian kernel.

Seed-based correlation analyses

Seed-based correlation analyses were performed between each hippocampal ROI (head, body, and tail of the hippocampus) and voxels within the hippocampal network. Hippocampal ROIs were defined using a published multimodal parcellation of the hippocampus[4]. We used the parcellation with the lowest granularity (three clusters; compare five- and seven-cluster solutions) to minimize the number of comparisons. Given our focus on the hippocampal circuitry, we opted for a conservative approach and tested only voxels that are part of the hippocampal network. To define a hippocampal network mask, we included only ROIs from the Harvard–Oxford Cortical Atlas that have been shown to be robustly connected to the hippocampus, corresponding to the posterior hippocampal network (angular gyrus, inferior parietal lobule, posterior cingulate cortex, and precuneus) and anterior hippocampal network (amygdala, frontal orbital cortex, temporal poles, and ventromedial prefrontal cortex). As an additional validation step, we also identified voxels across the whole brain that show evidence for functional connectivity to any of the aforementioned ROIs in a data-driven way. This was achieved by identifying voxels that are associated ($r > 0.20$) with the center of gravity of either of the six hippocampal ROIs using the Neurosynth normative resting-state dataset (1,000 healthy participants; neurosynth.org ref. 55) The findings from Neurosynth functional connectivity overlapped considerably with the selected anatomical ROIs (Supplementary Fig. 6). For transparency, whole-brain exploratory analyses are provided in Supplementary Figs. 7–9 but are not reported in the main text.

Statistical comparisons were performed using PALM[56], running non-parametric statistics, using TFCE and correction for multiple comparisons using family-wise error. All connectivity values were transformed with Fisher's r-to-z before any statistical analyses to best approximate normal sampling distributions and stabilize the variance across participants. The two groups were compared, while controlling for age, sex, and motion (framewise displacement). We intentionally did not control for any psychological or clinical variables in our analyses as these could reasonably be expected to relate to functional connectivity. Instead, relevant measures (anxiety, stress, baseline pain, depression, injury severity) were regressed on hippocampal connectivity post hoc to explore the data. For post hoc analyses, summary connectivity measures for each ROI were obtained by averaging the connectivity of each ROI to the corresponding significant clusters to obtain one connectivity value per hippocampal ROI for each participant. To further model all ROIs in a multivariate way, we performed a non-parametric combination analysis using PALM's joint inference approach[56]. We used Tippett's method (option -npcmod Tippett) to correct for multiple comparisons across voxels and modalities (here, each hippocampal ROI).

To facilitate the comparison of our data with normative connectivity values, we extracted hippocampal connectivity measures for healthy controls from ten different studies from the 1000 Functional Connectomes Project[57]. Studies were selected to encompass similar scanning parameters and age ranges to those in our study. Using the preceding pipeline, 273 participants were preprocessed, the connectivity from each summary measure was obtained, the contributing effects of age, sex, and average framewise displacement were adjusted to the main sample's mean through linear regression, and the resulting connectivity estimates were pooled using random-effects meta-analysis, which explicitly takes into account and quantifies the variability across studies/sites (heterogeneity). Prediction intervals for connectivity, which incorporate the heterogeneity variance term, were obtained to define conservative normative hippocampal connectivity across studies. Details about the studies used in this step can be found in the Supplementary Information.

Assessing time dependency and relationships with stress and anxiety

To assess the time dependency of hippocampal functional connectivity and its relationship with the outcome measures, we modeled hippocampal connectivity by the time lapse between the accident and the scanning session in hours, while also controlling for average framewise displacement, age, and sex. For brevity, only the highest-order interaction is reported in the main text, and the full set of model parameters, including main effects and lower-order interactions, are presented in Supplementary Table 7. We also examined how discriminative hippocampal connectivity is when patients are grouped by chronological day—that is, if they were scanned one, two, or three days after the injury, considering the night of sleep, which is known to impact learning and hippocampal plasticity[18,58]. To do so, we calculated the AUC for hippocampal connectivity, discriminating between patients who developed chronic pain from those who recovered. No additional model was fit to the data to prevent overfitting; the AUCs were computed using the raw connectivity values, akin to an effect size estimation.

We further examined whether stress and anxiety are associated with hippocampal connectivity. To do so, each parameter was further added to the preceding time-by-group interaction. Again, only the highest-order interaction is reported in the main text, and remaining details are in Supplementary Table 8. One participant was excluded from these analyses as he was scanned much later than other participants in the group (118 hours after injury, >3 s.d. above the group mean, 43 ± 18 hours).

Network analyses

To perform network analyses, for each participant, we computed the correlation matrices between BOLD time courses of regions within the anterior and posterior hippocampal network ROIs, to which we also added the hippocampus proper (head, body, and tail ROIs). The network topography was compared with 5,000 random permutations to establish statistical significance using network-based statistics[21]. Network topography requires the selection of a threshold that identifies the number of effective suprathreshold links and compares them with the permuted random networks. We used an effect size threshold of Cohen's $d > 0.2$ as common in the literature, effectively examining only links where differences between groups are at least of a 'small' effect size (as per Cohen's classic examples).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

After finishing our planned data analyses, the full imaging data will be provided at our data repository, http://www.openpain.org. Until then, data will be shared on reasonable request.

References

1. Baliki, M. N. & Apkarian, A. V. Nociception, pain, negative moods and behavior selection. *Neuron* **87**, 474–491 (2015).
2. Leeuw, M. et al. The fear-avoidance model of musculoskeletal pain: current state of scientific evidence. *J. Behav. Med.* **30**, 77–94 (2007).
3. Vachon-Presseau, E. et al. The emotional brain as a predictor and amplifier of chronic pain. *J. Dent. Res.* **95**, 605–612 (2016).
4. Plachti, A. et al. Multimodal parcellations and extensive behavioral profiling tackling the hippocampus gradient. *Cereb. Cortex* **29**, 4595–4612 (2019).
5. Wimmer, G. E. & Büchel, C. Reactivation of single-episode pain patterns in the hippocampus and decision making. *J. Neurosci.* **41**, 7894–7908 (2021).
6. Vachon-Presseau, E. et al. The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans. *Brain* **136**, 815–827 (2013).
7. Apkarian, A. V. et al. Role of adult hippocampal neurogenesis in persistent pain. *Pain* **157**, 418–428 (2016).
8. Wei, X. et al. Activation of the dorsal, but not the ventral, hippocampus relieves neuropathic pain in rodents. *Pain* **162**, 2865 (2021).
9. Berger, S. E. et al. Hippocampal morphology mediates biased memories of chronic pain. *Neuroimage* **166**, 86–98 (2018).
10. Vachon-Presseau, E. et al. Corticolimbic anatomical characteristics predetermine risk for chronic pain. *Brain* **139**, 1958–1970 (2016).
11. Reckziegel, D. et al. Sex-specific pharmacotherapy for back pain: a proof-of-concept randomized trial. *Pain Ther.* **10**, 1375–1400 (2021).
12. Mutso, A. A. et al. Reorganization of hippocampal functional connectivity with transition to chronic back pain. *J. Neurophysiol.* **111**, 1065–1076 (2014).
13. Brodt, S. et al. Fast track to the neocortex: a memory engram in the posterior parietal cortex. *Science* **362**, 1045–1048 (2018).
14. Lucas, S., Hoffman, J. M., Bell, K. R. & Dikmen, S. A prospective study of prevalence and characterization of headache following mild traumatic brain injury. *Cephalalgia* **34**, 93–102 (2014).
15. Dai, W. et al. Prevalence of acute stress disorder among road traffic accident survivors: a meta-analysis. *BMC Psychiatry* **18**, 188 (2018).
16. Kim, E. J., Pellman, B. & Kim, J. J. Stress effects on the hippocampus: a critical review. *Learn. Mem.* **22**, 411–416 (2015).
17. Kim, J. J. & Diamond, D. M. The stressed hippocampus, synaptic plasticity and lost memories. *Nat. Rev. Neurosci.* **3**, 453–462 (2002).
18. Himmer, L., Schönauer, M., Heib, D. P. J., Schabus, M. & Gais, S. Rehearsal initiates systems memory consolidation, sleep makes it last. *Sci. Adv.* **5**, eaav1695 (2019).
19. Iglesias, J. E. et al. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: application to adaptive segmentation of in vivo MRI. *Neuroimage* **115**, 117–137 (2015).
20. Mirescu, C. & Gould, E. Stress and adult neurogenesis. *Hippocampus* **16**, 233–238 (2006).
21. Zalesky, A., Fornito, A. & Bullmore, E. T. Network-based statistic: identifying differences in brain networks. *Neuroimage* **53**, 1197–1207 (2010).
22. Vlaeyen, J. W. S. & Linton, S. J. Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. *Pain* **85**, 317–332 (2000).
23. Hashmi, J. A. et al. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain* **136**, 2751–2768 (2013).
24. Reckziegel, D. et al. Hippocampus shape deformation: a potential diagnostic biomarker for chronic back pain in women. *Pain* **162**, 1457–1467 (2021).
25. Wang, J. X. et al. Targeted enhancement of cortical–hippocampal brain networks and associative memory. *Science* **345**, 1054–1057 (2014).
26. Gilmore, A. W., Nelson, S. M. & McDermott, K. B. A parietal memory network revealed by multiple MRI methods. *Trends Cogn. Sci.* **19**, 534–543 (2015).
27. Ranganath, C., Heller, A., Cohen, M. X., Brozinsky, C. J. & Rissman, J. Functional connectivity with the hippocampus during successful memory formation. *Hippocampus* **15**, 997–1005 (2005).
28. Tovote, P., Fadok, J. P. & Lüthi, A. Neuronal circuits for fear and anxiety. *Nat. Rev. Neurosci.* **16**, 317–331 (2015).
29. Costa, M. et al. Aversive memory formation in humans involves an amygdala–hippocampus phase code. *Nat. Commun.* **13**, 6403 (2022).
30. Zhang, H. et al. Awake ripples enhance emotional memory encoding in the human brain. *Nat. Commun.* **15**, 215 (2024).
31. Gould, E. & Tanapat, P. Stress and hippocampal neurogenesis. *Biol. Psychiatry* **46**, 1472–1479 (1999).
32. Girgis, F., Pace, J., Sweet, J. & Miller, J. P. Hippocampal neurophysiologic changes after mild traumatic brain injury and potential neuromodulation treatment approaches. *Front. Syst. Neurosci.* **10**, 8 (2016).
33. Henckens, M. J. A. G., Hermans, E. J., Pu, Z., Joëls, M. & Fernández, G. Stressed memories: how acute stress affects memory formation in humans. *J. Neurosci.* **29**, 10111–10119 (2009).
34. Zhou, Y. et al. Early altered resting-state functional connectivity predicts the severity of post-traumatic stress disorder symptoms in acutely traumatized subjects. *PLoS ONE* **7**, e46833 (2012).
35. Fitzgerald, J. M. et al. Hippocampal resting-state functional connectivity forecasts individual posttraumatic stress disorder symptoms: a data-driven approach. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **7**, 139–149 (2022).
36. Hruschak, V. & Cochran, G. Psychosocial predictors in the transition from acute to chronic pain: a systematic review. *Psychol. Health Med.* **23**, 1151–1167 (2018).
37. Ashar, Y. K. et al. Effect of pain reprocessing therapy vs placebo and usual care for patients with chronic back pain: a randomized clinical trial. *JAMA Psychiatry* **79**, 13–23 (2022).
38. Strange, B. A., Witter, M. P., Lein, E. S. & Moser, E. I. Functional organization of the hippocampal longitudinal axis. *Nat. Rev. Neurosci.* **15**, 655–669 (2014).
39. Poppenk, J., Evensmoen, H. R., Moscovitch, M. & Nadel, L. Long-axis specialization of the human hippocampus. *Trends Cogn. Sci.* **17**, 230–240 (2013).
40. Cooper, R. A. & Ritchey, M. Cortico-hippocampal network connections support the multidimensional quality of episodic memory. *eLife* **8**, e45591 (2019).
41. Ranganath, C. & Ritchey, M. Two cortical systems for memory-guided behaviour. *Nat. Rev. Neurosci.* **13**, 713–726 (2012).
42. Ezama, L., Hernández-Cabrera, J. A., Seoane, S., Pereda, E. & Janssen, N. Functional connectivity of the hippocampus and its subfields in resting-state networks. *Eur. J. Neurosci.* **53**, 3378–3393 (2021).
43. Niu, X. et al. Disruption of periaqueductal grey-default mode network functional connectivity predicts persistent post-traumatic headache in mild traumatic brain injury. *J. Neurol. Neurosurg. Psychiatry* **90**, 326–332 (2019).
44. Seymour, B., Crook, R. J. & Chen, Z. S. Post-injury pain and behaviour: a control theory perspective. *Nat. Rev. Neurosci.* **24**, 378–392 (2023).

45. Bosak, N. et al. Brain connectivity predicts chronic pain in acute mild traumatic brain injury. *Ann. Neurol.* **92**, 819–833 (2022).
46. Price, T. J. et al. Transition to chronic pain: opportunities for novel therapeutics. *Nat. Rev. Neurosci.* **19**, 383–384 (2018).
47. Kuperman, P. et al. Psychophysic–psychological dichotomy in very early acute mTBI pain: a prospective study. *Neurology* **91**, e931–e938 (2018).
48. Branco, P. et al. Structural brain connectivity predicts early acute pain after mild traumatic brain injury. *Pain* **164**, 1312–1320 (2023).
49. Esteban, O. et al. fMRIPrep: a robust preprocessing pipeline for functional MRI. *Nat. Methods* **16**, 111–116 (2019).
50. Smith, S. M. Fast robust automated brain extraction. *Hum. Brain Mapp.* **17**, 143–155 (2002).
51. Avants, B. B. et al. A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* **54**, 2033–2044 (2011).
52. Behzadi, Y., Restom, K., Liau, J. & Liu, T. T. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage* **37**, 90–101 (2007).
53. Sämann, P. G. et al. FreeSurfer-based segmentation of hippocampal subfields: a review of methods and applications, with a novel quality control procedure for ENIGMA studies and other collaborative efforts. *Hum. Brain Mapp.* **43**, 207–233 (2022).
54. Power, J. D. et al. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage* **84**, 320–341 (2014).
55. Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C. & Wager, T. D. Large-scale automated synthesis of human functional neuroimaging data. *Nat. Methods* **8**, 665–670 (2011).
56. Winkler, A. M. et al. Non-parametric combination and related permutation tests for neuroimaging. *Hum. Brain Mapp.* **37**, 1486–1511 (2016).
57. Biswal, B. B. et al. Toward discovery science of human brain function. *Proc. Natl Acad. Sci. USA* **107**, 4734–4739 (2010).
58. Abel, T., Havekes, R., Saletin, J. M. & Walker, M. P. Sleep, plasticity and memory from molecules to whole-brain networks. *Curr. Biol.* **23**, R774–R788 (2013).

Acknowledgments

We thank all members of the Apkarian lab for their feedback on the manuscript. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs under award W81XWH-15-1-0603 to D.Y. and A.V.A. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense. This work was further supported by National Institutes of Health grant P50 DA044121 and grant R01AR074274, both to A.V.A.

Author contributions

D.Y., Y.G., and A.V.A. designed the study, coordinated data collection, and obtained funding. N.B. collected data. P.B. performed imaging analyses. P.B. and A.D.V. performed statistical analyses. P.B. drafted the manuscript. P.B., N.B., A.D.V., and A.V.A. further edited and revised the manuscript. All authors discussed the results, contributed to, and approved the final manuscript.

Competing interests

The authors declare competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s44220-024-00329-8.

Correspondence and requests for materials should be addressed to A. Vania Apkarian.

Peer review information *Nature Mental Health* thanks Jacklynn Fitzgerald and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

nature portfolio

Corresponding author(s): A. Vania Apkarian

Last updated by author(s): Aug 13, 2024

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No particular software was used for data collection. Dcm2Niix was used to convert raw MR images to nifti images.
Data analysis	We used fMRIPrep 20.2.4 to pre-process the data. Denoising and filtering was performed using a python 3.7 custom function, Denoiser 1.0.1: https:// github.com/arielletambini/denoiser. Whole-brain statistical analyses were conducted using PALM https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM. Network analyses were conducted using the Network-bases statistics toolbox (NBS 1.2, Zalesky et al. 2010). Follow-up analyses were conducted using R 4.0.3. Several R packages were employed: ggplot2 for generating graphics, ForestR to calculate meta-analytical prediction intervals, ggeffects to estimate marginal means, performance to assess model assumptions including multicollinearity, homoscedasticity, and normality of residuals.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

After finishing our planned data analyses, the full imaging data will be provided at our data repository, http://www.openpain.org.Until then, data will be shared upon reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Biological sex was documented, and demographics are reported in the manuscript. We strived for an equal representation of males and females (about half, see below). All statistical analyses, including whole-brain and follow-up results are adjusted for sex.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity were not assessed in this study due to limited data from minority samples.
Population characteristics	249 subjects were enrolled in the study, but only 125 completed all aspects of the study (see below). All these patients were diagnosed with whiplash and mild-traumatic brain injury. They had a mean age 36.7 yrs, std = 11.5, range 18 – 67yrs. 53% were male, 47% were female.
Recruitment	Participants included in this study were recruited at the Rambam Health Care Campus emergency department presenting with whiplash and mild traumatic brain injury at a maximum of 24 hours after a motor vehicle collision. After the emergency room visit, patients were informed about the study and signed informed consent. Inclusion criteria were direct or indirect head and neck injury, a Glasgow Coma Scale score of 13 to 15 and no subsequent decline. Exclusion criteria were any imaging-based traumatic brain findings when available, more than 30 minutes of unconsciousness, other major bodily injuries, chronic head/neck pain prior to the injury, injuries in the head/neck area within one year prior to the current injury, and neurological diseases compromising the interpretation of brain function (e.g., neurodegenerative diseases). We don't anticipate any bias in the recruitment of patients. However, we acknowledge a large portion of subjects opted out of scanning, which could reflect some inherent and uncontrolled bias in this sample.
Ethics oversight	Rambam Health Care Campus Institutional Review Board (ref. 0601-14).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size was determined based on previous publication from our group and others (e.g. Baliki et al. 2012, Vachon-Presseau et al. 2016, Löffler et al. 2022).
Data exclusions	Out of the initial 249 patients, 64 did not perform an MRI (claustrophobia or not willing to participate), eight did not perform a resting-state fMRI scan (patients unwilling to continue with the scan after structural imaging), one patient had an incidental brain finding, and one was diagnosed with a major psychiatric condition. Out of the remaining 175 subjects, 23 patients did not reach the end of the study, three patients presented with post-traumatic amnesia, 11 patients did not meet clinical criteria for whiplash associated disorder, seven patients did not meet criteria for mild-traumatic brain injury, and three patients did not have baseline clinical data. After data analyses, three additional patients were excluded since they had less that 5 minutes of usable imaging data.
Replication	Replication was not attempted in this dataset. Data is presented as inferential. We validated the findings from the main sample in 23 heterogeneous patients that were left out of the main analysis due to exclusion criteria.

Randomization	No randomization was necessary in the study - this was an observational study.
Blinding	Blinding was not necessary given this study was observational and pain outcomes are determined 1 year after visit. The data analyst (PB) was not involved in data-collection.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

Magnetic resonance imaging

Experimental design

Design type	Resting-state fMRI
Design specifications	One resting-state scan per subject, with 10 minutes continuous acquisition.
Behavioral performance measures	Study was resting-state, no behavioral parameters were collected.

Acquisition

Imaging type(s)	Resting-state fMRI
Field strength	3T
Sequence & imaging parameters	Resting-state fMRI (rsfMRI) images were collected with an EPI sequence with the following parameters: repetition time = 2000 ms; echo time = 30s; FOV = 220 × 220 mm2; flip angle = 75°; slice thickness = 3.4 mm; in-plane pixel size 3.44 × 3.44 mm2, and axial slices = 43, for a total duration of 10 minutes. The first 8 s of acquisition in each run were excluded due to T1 equilibration effects.
Area of acquisition	Whole-brain scan

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Pre-processing was conducted using fMRIPrep 20.2.4. Denoiser 1.0.1: https://github.com/arielletambini/denoiser was used to remove confounds and band-pass filter the data (0.01 to 0.1 Hz) in one single step.

Normalization	Volume-based spatial normalization to standard space (MNI152NLin6Asym) was performed through nonlinear registration with antsRegistration5 (ANTs 2.3.3), as part of the fMRIPrep pipiline
Normalization template	Data were normalized to the FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model, i.e.., MNI152NLin6Asym template
Noise and artifact removal	To denoise the data, first, we regressed out motion and noise parameters from the data, namely the six head motion parameters, their squared parameters, and the temporal derivatives of both (also known as the Friston 24 expansion), to which we added the framewise displacement, the average signal from the WM and CSF, as well as the first 10 principal components aCompCorr generated by fMRIPrep. These parameters were regressed out of the data in a single step through multivariable regression. Average framewise displacement was used as a covariate of no-interest for all analyses.
Volume censoring	High motion timepoints and their adjacent volumes (the timepoint before, and the four points after the identified motion outliers, as recommended by Power et al.(44)) were also removed if they exceeded one of two criteria: timepoints with a framewise displacement larger than 0.5, or DVARS larger than 2.3. Subjects with less than 5 minutes of remaining data were excluded from analyses (n = 3).

Statistical modeling & inference

Model type and settings	Massive univariate statistics were used for the main analyses, using a general linear model where voxels (y) are predicted by Group (chronic or recovery), and adjusted for Age, Sex and Average Framewise displacement. A post-hoc Network analyses was conducted with links between regions being the dependent variable, instead of voxels.
Effect(s) tested	For the main analyses, specific effects of group were assessed (i.e., the coefficient for Group after accounting for age, sex and average framewise displacement). Post-hoc analyses include an interaction term (2 way) with time after injury, and an interactiont erm (3-way) with time and anxiety. Network analyses use the same model as for the main analyses.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s)	Hippocampal ROIs were defined using a published multimodal parcellation of the hippocampus (Plachti et al. 2019). For the hippocampal network target mask we included regions of interest from the Harvard-Oxford Cortical Atlas that have been shown to be robustly connected to the hippocampus , corresponding to the posterior hippocampal network (angular gyrus, inferior parietal lobule, posterior cingulate cortex, and precuneus) and anterior hippocampal network (amygdala, frontal orbital cortex, temporal poles, and ventromedial prefrontal cortex). All the above regions were used in the Network analyses.
Statistic type for inference (See Eklund et al. 2016)	For the mass univariate and network analyses, non-parametric tests were used, with PALM and NBS, respectively. For the mass univariate analyses, we further used threshold-free cluster enhancement (TFCE) to avoid arbitrarily defining extent or intensity thresholds.
Correction	For mass univariate analyses, correction for multiple comparisons was done with family-wise error (FWE). For network analyses, we used network-based statistics.

Models & analysis

n/a	Involved in the study
☐	☒ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	Pearson correlations were used, and r-values were converted from r to z using Fisher's r-to-z conversion.