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TITLE: Why Does Acute Postwhiplash Injury Pain Transform into Chronic Pain? Multimodal Assessment of Risk Factors and Predictors of Pain Chronification

PRINCIPAL INVESTIGATOR: Prof. David Yarnitsky

CONTRACTING ORGANIZATION: Technion – Research and Development Foundation (TRDF), Haifa, Israel,

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14. ABSTRACT :
This project aims to find why some acute mTBI patients turn into chronic pain patients, and other do not. We recruited patients shortly after the accident, and assigned them to clinical, psychophysical and psychological assessment, brain MRI, EEG, and genetic tests. We then followed up on the pain levels along one year. All clinical work was done in Israel, analysis was done in cooperation with our research partners in the US, Canada and Australia.
We recruited 462 patients, 249 of them completed the first visit performed within 72 hours after the accident; 197 participants answered the 3 months follow up questions, 213 answered the 6 months, and 210 answered the 12 months follow up questions. 96 participated in the 6 months visit and 86 participated in the 12 months visit, of which 49 were tested in both sessions. 60 patients gave blood samples for RNA sequencing in genetics data collection. Others failed to continue with the protocol despite their initial consent and are dropouts. At the present time we have analyses of the specific lines in parallel, as presented below. We will continue to analyze the amalgamated data from all line in near future.

1. Psychophysical and psychological assessment at baseline: We have shown that the somatosensory changes are independent of the psychological state of the patients; despite normal psychological profile, the mTBI patients demonstrate pro-nociceptive pattern of psychophysical responses already at hyper-acute post-traumatic stage. Further, using mediation analysis we found that the relationship between headache and pain-catastrophizing is mediated by the individual trait of pain sensitivity.th latter could explain the high variability of acute mTBI headaches.
2. Psychophysical and psychological parameters in prediction of chronic pain: Chronic post-traumatic pain occurrence is predicted by acute head pain, low socioeconomic status and higher activity of central pain facilitatory pathways as reflected by enhanced summation of experimental pain perception. Post-mTBI pain chronification at 6 month after the accident is associated with pro-nociceptive pattern of pain modulation at the hyper-acute post-mTBI; patients that had less efficient ability of endogenous inhibition of the perception of brief noxious heat stimuli, had higher chronic post-mTBI pain at 6 month.
3. EEG in prediction of chronic pain: Baseline EEG activity predicts incidence and intensity of chronic post-traumatic pain. More specifically, higher EEG resting-state alpha power is significantly associated with chronic headache and neck pain. Moreover, based on the parameters of intra-cortical connectivity, the patients that developed chronic pain had higher baseline synchronization between the activities of pain-processing brain areas.
4. MRI in explaining acute and chronic post mTBI pain: In a functional analysis of connectivity at baseline we found a negative connectivity between nucleus accumbens and the somatosensory area in the recovery group, that did not exist in the chronified group. Further, PAG was also in connectivity with the somatosensory area in the recovery group only. In a separate analysis that explored the structural state at baseline and in relation to the acute pain we found that stronger white matter tracts within the sensorimotor, thalamic-cortical, and default-mode systems predicted 20% of the variance in pain severity within 72 hours of the injury.
5. The role of genetics in pain chronification: While our patients' number did not allow for GWAS analysis, we did obtain baseline and follow up samples for mRNA dynamics analysis, in line with updated developments in the field. We found these dynamics to differ between the recovery and choronification groups. The extracted alleles at gene loci that were expressed, imputed the genetic data, and tested for association between SNP rs4903580 and pain chronification by the time of the second visit. The study results suggest the T allele to be protective for pain chronification, albeit in a non-significant fashion possibly due to sample size.

15. SUBJECT TERMS-
Mild traumatic brain injury, Pain perception, Pain modulation, fMRI, EEG, Chronic pain, Acute pain, Whiplash injury

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1. INTRODUCTION:

The study aims to explore why acute pain turns, in some patients, into chronic pain, and to develop tools for prediction of this transition. We use mild traumatic brain injury as our work model, to study which of the factors measured in the acute whiplash pain phase, influence the chronification of head and neck pain in these patients**.** Our objective is to construct a specific and sensitive tool, based on a broad assessment of pain modulation parameters obtained during acute pain, which allows understanding of the underlying mechanisms relevant for prediction of the transition to the chronic phase. This is a prospective, non-intervening, longitudinal study. Participants with mild traumatic brain injury are recruited when visiting the Rambam Health Care Campus ER immediately after the injury. Psychophysical, neurophysiological, psychological, imaging and genetic data are being collected within 72 hours. Patients are being followed up for one year.

2. KEYWORDS:

Mild Traumatic brain injury, Pain perception, Pain modulation, fMRI, EEG, Chronic pain, Acute pain, Whiplash injury

3. OVERALL PROJECT SUMMARY:

Tasks outlined in the approved SOW :

The study was first approved by Local IRB and director of the institution ("form 7") on 11/Oct/2015, and approved by the HRPO on 7/Mar/2016. Continuing review report was submitted and approved by the local IRB and HRPO every year.

Tasks 1 and 2: Patients recruitment and experimental performance:

We started recruiting immediately after HRPO first approval on March 2016.
First subject was recruited on 31/Mar/2016.

Up to date, we recruited 462 patients, 249 of them participated in the first visit performed within 72 hours after the accident, all patients gave blood sample for DNA sequencing, and additional 60 patients gave samples for RNA analysis, done at baseline and at follow up, the latter is a late addition to the protocol). 197 participants answered the 3 months follow up questions, 213 answered the 6 months, and 210 answered the 12 months follow up questions. 96 participated in the 6 months visit and 86 participated in the 12 months visit, of which 49 participants were tested in both sessions. The rest failed to continue with the protocol despite giving their consent and are dropouts.

In order to further potentiate the consenting, recruitment and follow-up testing, we hired several research assistants and two physicians, which are responsible for recruitments and follow up.

Quad chart was updated again and changed according to the extension and is attached as an appendix A.

Regarding the experimental performance:
249 Blood samples were transferred to the genomic lab, and DNA was extracted. They have processed 250 DNA samples using SNP chips according to the manufacture protocol.

BeadChips were then scanned. SNP QC for all samples was excellent with call rate above 99% of SNPs genotype. Data was shared with sub investigator Dr. Luda Diatchenko and her team. According to the bio-informaticists that looked through all the data, it is a good quality data, and will be analyzed when the group of samples will be higher.
After discussions with the Canadian team, and based on new findings published during our study period, we agreed that addition of RNa dynamics will be of value to aour cause. We were able to collect 60 RNA tubes during visit1, and 36 tubes during visit at 6 months.

MRI scans were saved and backed up, as well as shared with sub investigator Prof. Vania Apkarian at Northwestern University, for analyzing and processing. The MRI team has been putting the data through the pre-processing pipeline, with the aim of cleaning up the data quality and identifying any missing scans. The results from Prof. Apkarian's group analysis were submitted for publication in PLOS biology.Dr. Noam Bosak at our lab analyzes MRI scans too, and will soon publish her results.

Task 3. Patients follow-up:
3a. We collected data on clinical pain and analgesics consumption once a month, using a smart-phone application or personal phone-based follow-up along first post-recruitment year. All the participants were requested to follow our pain scale application and report their pain rates during the first year following the accident. Those who could not use the smart phone application, answered our pain questions on personal phone calls. 197 participants answered the 3 months follow up questions, 213 answered the 6 Months, and 210 answered the 12 months questions.

3b. Visits 6 and 12 months: 96 participated in the 6 months visit and 86 participated in the 12 months visit, of which 49 participants were tested in both sessions.
3c. No additional visits were done at patient's demand in our special dedicated hospital clinic.

Task 4. Interim data analyses:
4a. Data analyzing was done mainly during the recent year, while continuing collecting data. It is noted that by looking at cumulating follow up data, it seems that number of chronic pain patients exceeds the expected 20%. Since our initial recruiting numbers plan was based on a minimum of 20% chronic pain sufferers out of all our patients, we might be able to reach solid conclusion based on lower numbers of overall recruitees.

Analysis of QST and Selected Questionnaires, as well as analysis of Resting State EEG and pain-evoked potentials has been performed for most of the data collected.
Analysis of pain progression for the first year post injury has been performed for patients who have reached one year post injury. In addition preliminary analysis has been done on clinical data collected during the 6 months follow up visits.

The preliminary results, abstracts to conferences and accepted papers are detailed below in sections 4, 5 and 7.

4b. Ongoing review of quality of the imaging data is performed by the team at Northwestern University, USA.

4c. Ongoing review of psychophysical and neurophysiological data is performed by our team at the Technion as well as the sub investigator at University of Haifa, Israel.

4d. Consultation regarding the psychological data is done by the team at Griffith University, Brisbane, Australia.

The study team had two face to face meetings: in 17th World Congress on Pain in 2018, USA, and during the 11th congress of the European Pain Federation, EFIC, Spain, in 2019, before COVID19. The team discussed the progress of the MRI data analyses, RNA collection and analyzing, as well as RNA data from McGill University.

4. KEY RESEARCH ACCOMPLISHMENTS:

The clinical characteristics of acute and chronic mTBI

a. Psychophysical-psychological dichotomy in very early acute mTBI pain: A prospective study. Kuperman et al., 2018, NEUROLOGY

Chronic stage of the whiplash-associated disorder (cWAD) is associated with central somatosensory pro-nociceptivity as demonstrated by local and widespread hyperalgesia to experimental pain stimuli, inefficient conditioned pain modulation (CPM) and enhanced temporal summation (TS) of pain. Whether these changes are a physiological direct consequence of the trauma, or due to psychological factors, such as the higher presence of anxiety, depression, and psychological distress, observed among cWAD, is still debated, some even posit feigning as their source.

Like cWAD, patients in the acute post-injury stage also show somatosensory changes, sometimes as early as 7 days post-injury. While researchers have noted immediate psychological symptoms, like elevated levels of distress within one-month post-whiplash, others suggested a delayed appearance, such as elevated levels of anxiety and depression only among patients at least 2 years post-injury.

The present study prospectively explored somatosensory and psychological presentation in very early acute mTBI (<72h post-accident), a time-frame not yet explored.

Our finding pointed on a dichotomy between the somatosensory and psychological changes at the very acute post-injury stage. The post-mTBI patients demonstrated a pro-nociceptive pattern of pain processing as compared with the control subjects, with several significant correlations between clinical pain and psychophysical measures. In contrast, no significant differences from healthy controls in most pain-related psychological variables and said psychological findings were observed.

In the context of the ongoing debate on the pathophysiological nature of the post-mTBI syndrome, our findings support its 'physical' basis, free of mental influence, at least in the short time window after the injury. The lack of significant psychological differences in the same time-frame suggests that mental changes may take longer to develop, lending support to the assertion that the pathophysiology of the clinical pain reported post-mTBI is mostly organic, free of mental influences. Finding a dichotomy between the somatosensory and psychological changes in this time window would provide support to the organic basis of the somatosensory hypersensitivity and pain syndrome in the context of very early acute mTBI.

b. Explaining very early acute mild traumatic brain injury after motor vehicle collision pain variability: additive value of pain sensitivity questionnaire. Kuperman et al., 2020; PAIN Reports

Chronic pain is a common post-collision consequence. Wherein, a clearer understanding of acute pain can help stem the acute-to-chronic pain transition. However, the variability of acute pain is only partially explained by psychophysical pain characteristics as measured by quantitative sensory testing. The Pain Sensitivity Questionnaire (PSQ) may reflect inherent psychocognitive representations of patient's sensitivity and thus may reveal less-explored pain dimensions. In the vein of the biopsychosocial approach, we assumed that PSQ holds additive value in explaining head and neck pain reports in very early acute-stage mild traumatic brain injury (mTBI) after collision, above the use of psychophysical assessment.
We found that the Pain Sensitivity Questionnaire scores were significantly correlated with acute clinical, psychophysical, and pain-related psychological measures. Regression model ($R2 = 0.241$, $P < 0.001$) showed that, together, age, sex, high PSQ, enhanced temporal summation, and less-efficient conditioned pain modulation explained head and neck pain variance. This model demonstrated that the strongest contribution to degree of post-injury pain was independently explained by PSQ (ß = 0.32) and then pressure pain threshold-conditioned pain modulation (ß = −0.25) (see Table 1).

Table 1

Outcome measure	Model by model fit	Term	Estimate	*P*	Std beta
Area of injury pain at baseline (<72 h)	$r = 0.49$	Intercept	44.51	<0.001	0
	$P < 0.001$	Age	−0.19	0.257	0.10
		Sex	3.14	0.122	0.14
		mTS	0.03	0.854	0.02
		PPT-CPM	−6.75	0.005	−0.25
		Pain sensitivity questionnaire (PSQ)—total score	4.57	<0.001	0.32

CPM, conditioned pain modulation; mechanical TS; PPT, pressure pain threshold; PSQ, Pain Sensitivity Questionnaire; TS, temporal summation.

Appraisal of cognitive daily-pain representations, by way of memory and imagination, provides an additional important dispositional facet to explain the variability in the acute mTBI postcollision clinical pain experience, above assessing nociceptive responsiveness to experimentally induced pain.

c. Head- and neck-related symptoms post-motor vehicle collision (MVC): Separate entities or two-sides of the same coin? Kuperman et al., 2021; INJURY

Although post-motor vehicle collision (MVC) pain and symptoms are largely convergent among those with mild traumatic brain injury (mTBI) and whiplash associated disorder (WAD), and patients oftentimes report initial neck and head complaints, the clinical picture of mTBI and WAD has been primarily studied as separate conditions which may result in an incomplete clinical picture. As such, we explored the role of pain and post-traumatic psychological features in explaining both head and neck-related symptom variability in a cohort of post-collision patients. This is with the goal of disentangling if contributory factors are uniquely related to each diagnosis, or are shared between the two. While post-collision research continues to mount, a single comprehensive model has yet to have reached consensus.

We hypothesized that this obscurity may be partially attributable to the inevitable separation which occurs when whiplash and mTBI are addressed as separate post-collision conditions; where the overlapping acute and chronic symptom presentation would suggest that similar factors influence the patient's clinical manifestation. As such, this study was conducted to explore the role of clinical pain and post-traumatic psychological features in explaining both NDI and PCS variability in a cohort of patients 6 months post- MVC. This is with the goal of disentangling if the aforementioned contributory factors are uniquely related to each diagnosis, or are shared between the two.

In order to determine which factors were unique to whiplash or mTBI, and which ones were overlapping for both post-collision head and neck related symptoms, mean head pain, mean neck pain, female gender, number of post-collision painful body areas, PTSD, and depression were included in both models. This regression analysis showed that neck-related disability, as measured by the NDI, could be explained sufficiently by measures of clinical pain –neck pain and the number of painful body areas, and less so by depression (which can be linked with musculoskeletal complaints) (See Table 1).

Table 1
Initial regression model for the Neck Disability Index.

Outcome Measure	Model by Model Fit	Term	Estimate	Standard Error	P-value	Standardized Beta
NDI	$r = 0.84$	Intercept	0.036	0.021		
		Mean Neck Pain	0.0025	0.00070	**<0.001**	0.42
		Mean Head Pain	−0.00094	0.00063	0.143	−0.15
		No. Painful Body Areas	0.037	0.013	**0.0060**	0.31
		PTSD Total Score	0.0036	0.0020	0.075	0.21
		Gender (F)	−0.0078	0.012	0.520	−0.050
		HADS-Depression	0.0096	0.0044	**0.032**	0.24

Whereas, in contrast, post-mTBI symptoms requires both clinical pain reports-number of painful body areas and psychological factors- both the level of self-reported depression and PTSD symptoms, with the strongest influence provided by PTSD (ß= 0.37, $p < 0.001$). Female sex also influenced head-related, but not neck-related symptoms (see Table 2).

Table 2
Initial Regression Model for the Rivermead Post-Concussion Scale.

Outcome Measure	Model by Model Fit	Term	Estimate	Standard Error	P-value	Standardized Beta
Rivermead Post-Concussion Scale (PCS)	$r = 0.87$	Intercept	−2.19	1.47		
		Mean Neck Pain	0.060	0.049	0.22	0.13
		Mean Head Pain	−0.079	0.044	0.080	−0.17
		No. Painful Body Areas	3.17	0.91	**0.0010**	0.35
		PTSD Total Score	0.50	0.14	**<0.001**	0.37
		Gender (F)	2.47	0.85	**0.0049**	0.19
		HADS-Depression	0.98	0.30	**0.0021**	0.31

With the understanding that pain and disability are highly correlated, and as such the presence of chronic pain may confound the analysis of post-collision symptoms, regression models were performed a second time, with the area of direct injury pain (head for RPQ, and neck for NDI) removed. The results of these model reinforced the previous findings, where neck disability can be explained solely by the number of post-collision affected areas, and head- related symptoms can be explained by both physical and psychological factors (see Tables 3 and 4).

Table 3
Secondary Regression model for the Neck Disability Index.

Outcome Measure	Model by Model Fit	Term	Estimate	Standard Error	P-value	Standardized Beta
NDI	$r = 0.80$	Intercept	0.040	0.023		
		Mean Head Pain	$7.3561\ e^{-5}$	0.00062	0.91	0.012
		No. Painful Body Areas	0.061	0.012	**<0.001**	0.52
		PTSD Total Score	0.0040	0.0022	0.070	0.23
		Gender (F)	−0.0027	0.013	0.840	−0.016
		HADS-Depression	0.0081	0.0048	0.095	0.20

Table 4
Secondary Regression for the Rivermead Post-Concussion Scale.

Outcome Measure	Model by Model Fit	Term	Estimate	Standard Error	P-value	Standardized Beta
Rivermead Post-Concussion Scale (PCS)	$r = 0.86$	Intercept	−2.20	1.50		
		Mean Neck Pain	0.021	0.044	0.628	0.046
		No. Painful Body Areas	2.86	0.91	**0.0026**	0.31
		PTSD Total Score	0.48	0.14	**0.0013**	0.36
		Gender (F)	2.50	0.86	**0.0053**	0.19
		HADS-Depression	0.97	0.31	**0.0028**	0.31

It seems that while mechanisms of neck- and head-related symptoms in post-collision patients do share a common explanatory feature, of residual body pain, they are not entirely overlapping. In that, psychological factors influence post-concussion syndrome symptoms, but not post-whiplash neck disability.

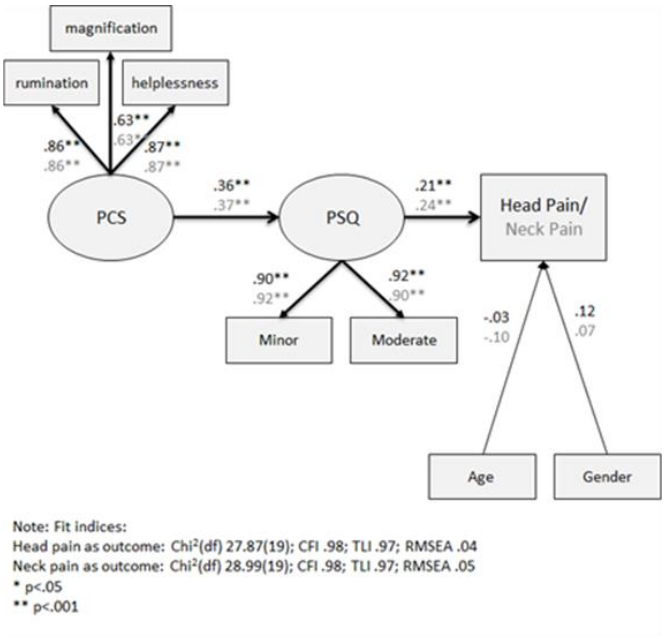
<u>D. Dispositional and situational personal features and acute post-collision head and neck pain: Double mediation of pain catastrophizing and pain sensitivity (Granot et al., 2021 PLOS ONE, in press).</u>

Pain variability can be partially attributed to psycho-cognitive features involved in its processing. However, accumulating research suggests that simple linear correlation between situational and dispositional factors may not be sufficiently explanatory, with some positing a

role for mediating influences. In addition, acute pain processing studies generally focus on a post-operative model with less attention provided to post-traumatic injury. As such, we investigated a more comprehensive pain processing model that included direct and indirect associations between acute pain intensity in the head and neck, pain catastrophizing (using pain catastrophizing scale (PCS)), and pain sensitivity (using the pain sensitivity questionnaire (PSQ)), among patients with an mTBI post-motor vehicle collision. The effect of personality traits (using Ten Items Personality Inventory (TIPI)) and emotional status (using Hospital Anxiety and Depression Scale (HADS) and Perceived Stress Scale (PSS)) on that model was examined as well. To this end, three Structural Equation Modeling (SEM) analyses were conducted.

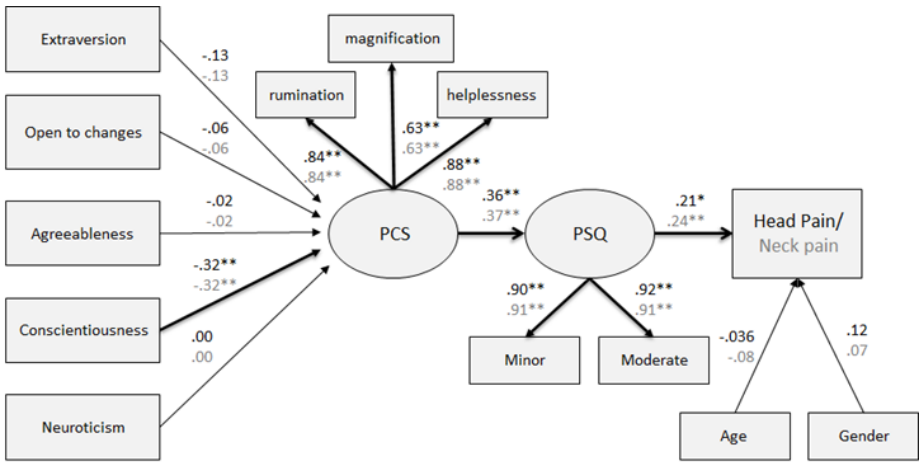
The SEM analyses revealed several mediations. Specifically, pain catastrophizing was significantly associated with pain sensitivity for head and neck pain models (γ=.36 and γ=.37, respectively; $p<0.001$). In turn, pain sensitivity was significantly related to acute head and neck pain: β=.21, $p<0.05$ and β=.24, $p<0.001$, respectively (Figure 1).

Figure 1



The second SEM analysis model included the five personality traits: extraversion, agreeableness, conscientiousness, neuroticism and open for changes, as independent variables that are both directly associated with pain catastrophizing and also correlated with each other (Figure 2). Only the personality trait of conscientiousness was significantly negatively related to pain catastrophizing γ= -.32 ($p<0.001$) in both models.

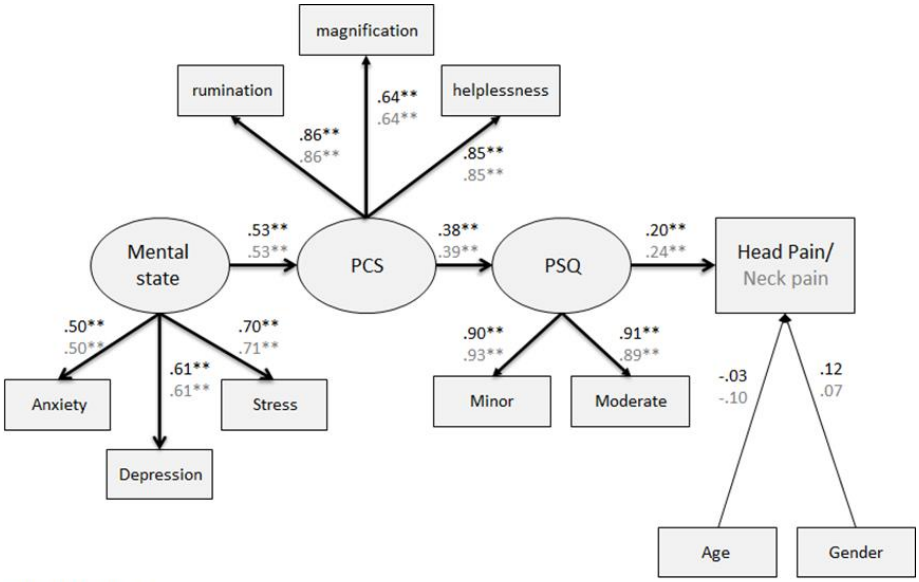
Figure 2



Note: Fit indices:
Head pain as outcome $Chi^2(df)$ 75.27(54); CFI .97; TLI .94; RMSEA .04
Neck pain as outcome $Chi^2(df)$ 77.28(54); CFI .96; TLI .94; RMSEA .04
* p<.05
** p<.001

The third SEM analysis model included an independent latent variable of emotional status, as measured by stress (PSS), anxiety, and depression (HADS), which was directly associated with pain catastrophizing (Figure 3).

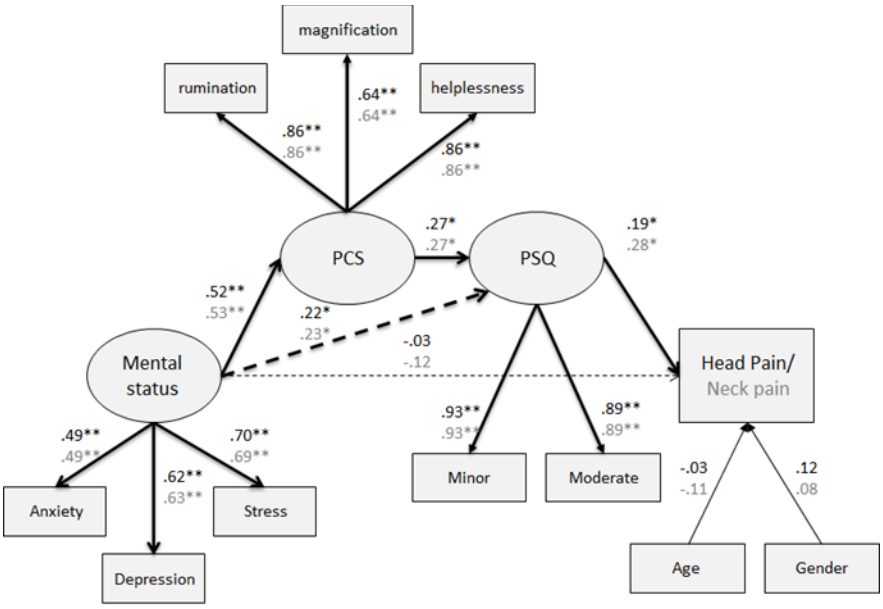
Figure 3



Note: Fit indices:
Head pain as outcome $Chi^2(df)$ 78.45(42); CFI .95; TLI .92; RMSEA .06
Neck pain as outcome $Chi^2(df)$ 80.29(42); CFI .95; TLI .92; RMSEA .06
* p<.05
** p<.001

Two new direct paths were entered into alternative models (Figure 4). The first path was a direct path between emotional status and pain sensitivity and the second path was a direct path between emotional status and acute head or neck pain intensity ratings. While the first path was significant for both models (γ=.22, p<0.05 for head pain model and γ=.23, p<0.05 for neck pain model), the second path was not (p=.730 and p=.161, respectively).
Thus, the alternative model with these additional direct paths is the preferred one for neck pain intensity model.

Figure 4



Note: Fit indices:
Head pain as outcome Chi^2(df) 74.40(40); CFI .95; TLI .92; RMSEA .06
Neck pain as outcome Chi^2(df) 74.05(40); CFI .95; TLI .92; RMSEA .06
* p<.05
** p<.001

In summary, the third SEM analysis suggests just like pain catastrophizing and personality traits, emotional status had no direct association with acute head and neck pain intensity rating. However, unlike conscientiousness, which was not directly linked to pain sensitivity, higher emotional status was significantly associated with higher pain sensitivity. Thus, the relationship between a heightened emotional status and high acute head and neck pain was partially mediated by high levels of pain catastrophizing and fully mediated by high pain sensitivity.

In conclusion, these results suggest that during the acute post-traumatic phase, pain sensitivity intermediates between emotional states and personality traits, partially via elevated pain catastrophizing thoughts.

Psychophysics

PMP as obtained by QST at baseline in prediction of chronification of post mTBI pain

This section focuses on the role of experimental-induced pain (QST) in prediction of the risk for development of chronic pain following mTBI as well as to reveal the factors that contribute to the transition from acute to chronic pain and better address the relationships between clinical and experimental pain as obtained at the acute and chronic phases. The main question was which psychophysical factors construct the individual pain modulation profile (PMP) that characterize the vulnerability for chronification.

Among all measured QST parameters, most measures are correlated with the intensity of acute clinical pain. Moreover, several psychophysical measures were also correlated with the intensity of the chronic pain, as well as the transition to chronicity at 6 or 12 months. Therefore, this report will focus mainly on QST parameters that were consistently relevant for head and neck pain predictions.

The emerged picture shows that both less-efficient CPM (i.e., descending inhibition) and enhanced temporal summation (TS) (i.e., ascending facilitation) represent the advanced psychophysical measures that characterize patients at greater risk. In addition, few static QST measures also contribute to identifying those individuals who may develop chronic pain. As obtained at the acute phase, this pro-nociceptive PMP can serve as valuable biomarkers to early personalized medical intervention.

Outcome measures:

The primary outcome measure was set as the maximal pain intensity obtained for either head or neck pain as reported by patients at three time points: baseline (BL) (up to 72 hrs. post MVC), 6 months, and 12-month post MVC. In addition, pain intensity ratings for neck and head (separately) were also analyzed as secondary outcome measures. For clinical purposes, we also defined participants as painful (30-100NPS) or non-painful (0-29NPS). This classification serves as an additional outcome measure. This was performed for each of the 3-time point assessments that allow us to explore the transition from pain-free to chronic pain in the follow-up assessments.

An important observation was that each of the above measures distinctively represents pain experience. Thus, to attain more information about the mechanisms that shape each of these pain measures, we analyzed them separately.

Table1: presents the differences between the number of patients who were defined as painful or painless at the head or neck and maximal pain as observed at baseline.

	Head NPS	**Neck NPS**	**Maximal NPS**
Painful	162	172	192
Pain-free	61	51	31
Total	223	223	223

The assessed QST measures were: heat, cold, electrical, and pressure pain thresholds; pain ratings for contact-heat and cold water hand immersion; the temperature that induced pain intensity of 50 at 0-100 NPS (pain-50). In addition, pain ratings for the 1st and 10th pinprick and

electrical stimulations as static measures. These measures allow us to calculate the dynamic QST measures: electrical-TS, mechanical-TS, pressure pain PPT-CPM, and heat-CPM. Tests were obtained at the nonpainful area, remote from the site of injury (forearm) as well as the area of injury (neck).

Given that the distribution of clinical pain intensity was not normal, we performed a non-parametric statistical test.

Do QST measurements reflect the intensity of acute pain at BL 0-72h?

Most of the QST parameters were correlated with patients' clinical acute mTBI pain rating (P >0.0001 - 0.039). Generally, the pronociceptive pain profile was correlated with enhanced clinical pain as reported at the neck, head, and the maximal pain ratings. This observation supports our previous report (Kuperman et al.; Neurology,2018). However, head pain ratings were more highly associated with QST measures than neck pain.

Table 2: Spearman's correlations between QST and clinical pain at BL.

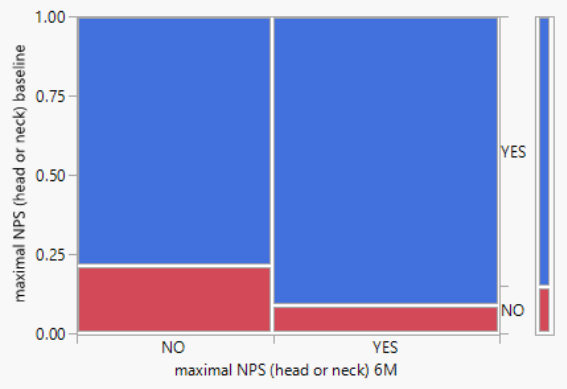
		Acute Head NPS	Acute Neck NPS	Acute Max NPS
HPT	r	-.152	-.212	-.220
	p	0.025	0.002	0.001
CPT	r	.151	.149	.183
	p	0.026	0.028	0.007
Mechanical 1st	r	.166	.012	.112
	p	0.013	0.861	0.095
Mechanical 10th	r	.232	.068	.180
	p	0.000	0.311	0.007
Mechanical TS	r	.128	.056	.092
	p	0.057	0.402	0.173
1st Pulse VAS	r	.185	.165	.173
	p	0.006	0.015	0.011
10th Pulse VAS	r	.280	.235	.281
	p	0.000	0.000	0.000
Electrical TS	r	.175	.146	.196
	p	0.01	0.032	0.004
Pain50- temp	r	-.299	-.291	-.372
	p	0.000	0.000	0.000
Pre-PPT avg.	r	-.237	-.226	-.304
	p	0.000	0.001	0.000
PPT Conditioned avg.	r	-.262	-.277	-.356
	p	0.000	0.000	0.000
PPT-CPM	r	-.174	-.218	-.252
	p	0.01	0.001	.000
Cold Water pain	r	.224	.265	.264

	p	0.001	0.000	0.000

Is pain-free or painful state is stable along follow-up?

Chi-square tests showed that most patients remained stable (those who were pain-free at BL remain pain-free at 6 and 12 months and those who were painful at base line, worsen or remained painful.). However, some patients **shifted** from one group to the other.

BL and 6 months (6M)

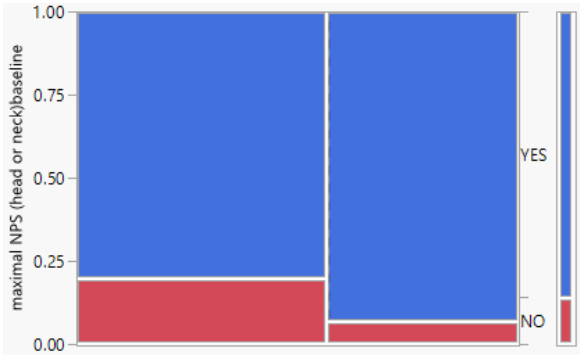


H_OR_N_MAX_BL_pain_yes/no

H_OR_N_MAX_6M_pain_yes/no	NO	YES	Total
Count			
Total %			
Col %			
Row %			
NO	19	69	88
	10.05	36.51	46.56
	67.86	42.86	
	21.59	78.41	
YES	9	92	101
	4.76	48.68	53.44
	32.14	57.14	
	8.91	91.09	
Total	28	161	189
	14.81	85.19	

Among 189 patients who were assessed for clinical pain at baseline and at 6 months, 19/88 (21.6%) who were pain-free (below 30) at baseline remained pain-free also at 6 months. 92/161 patients (57.14%) who were defined as **painful** at baseline remained painful at 12M. However, 9 out of 28 (32.1%) of the pain-free at baseline reported being painful at 12month. Importantly, majority of patients who were painful at baseline remained painful after 6 months (57.14%), but 69 out of 161 (42.9%) of the painful **became pain-free** at 6 months (Chi= 6.054, p = 0.0139).

BL and 12 months (12M)



H_OR_N_MAX_BL_pain_yes/no

H_OR_N_MAX_12M_pain_yes/no	NO	YES	Total
Count			
Total %			
Col %			
Row %			
NO	22	90	112
	11.11	45.45	56.57
	78.57	52.94	
	19.64	80.36	
YES	6	80	86
	3.03	40.40	43.43
	21.43	47.06	
	6.98	93.02	
Total	28	170	198
	14.14	85.86	

Among 198 patients who were assessed for clinical pain at both BL and 12M, 22/112 (19.6%) who were pain-free at BL remained pain-free at 12 months. As for the painful group, 80/170 patients (47%) who were defined as painful at BL remained painful at 12M. However, 6/86 (7%) patients of the pain-free at BL reported being painful at 12M. In addition, 90/170 (53%) of the painful at BL became pain-free at 12M (Chi- 6.884, p = 0.008).

These results mainly show that the presence of acute clinical pain predicts chronic pain, and the absence of pain at baseline indicates low or no-clinical pain at the chronic phase. Although this is not the case for all patients, it encouraged us to explore further which QST parameters predict the transition and are associated with being in the painful or pain-free groups.

QST measurements that predict the intensity of chronic mTBI pain

Table 3: Spearman correlations between QST at baseline and clinical pain at 6M.

		Head 6M	**Neck 6M**	**Max 6M**
Pain50 temp	r	-.156	-.164	-.155
	P	0.031	0.023	0.031
Cold Water pain	r	0.1	.162	.150
	P	0.170	0.026	0.04
Heat Pain-CPM 1	r	0.094	.152	.161
	P	0.198	0.037	0.027
Heat Pain-CPM 2	r	0.057	.144	.155
	P	0.436	0.049	0.033

Some of the QST measurements was correlated with magnitude of pain intensity as reported for the head, neck, or maximal pain. Interestingly, at the same time point, head and neck pain ratings seem to represent a **distinctive pain experience**.

Table 4: Spearman correlations between QST at baseline and clinical pain at 12M

		Head 12M	**Neck 12M**	**Max 12M**
Mechanical TS	r	.144	.166	.145
	P	0.041	0.018	0.04
Pain50 temp	r	-.160	-.167	-.176
	P	0.024	0.018	0.013
Pre-PPT avg.	r	-.143	-0.127	-0.129
	P	0.044	0.074	0.069
Heat Pain3	r	-.165	-0.05	-0.072
	P	0.02	0.486	0.311
PPT Conditioned avg.	r	-.153	-0.127	-.147
	P	0.031	0.073	0.039
Cold Water pain	r	.150	.192	.184
	P	0.035	0.007	0.009

PPT-CPM, Pain-50 temperature, and cold-water pain ratings as obtained at baseline seem to be the most consistent QST measures associated with clinical pain intensity at both 6 and 12M.

Can baseline QST distinguish between painful and pain-free groups at the chronic phase?

Group comparison revealed no group differences for **Head** NPS as obtained at 6M. Nonetheless, comparing **Neck** NPS or maximal pain at 6M revealed several significant differences in QST measures and emphasize the difference between the meaning of Head and Neck pain experience.

Table 5: t-test between Neck painful and pain-free groups by their baseline QST

	Neck 6M>30	**Mean**	**Std. Deviation**	**p**
Mechanical TS	**Painful**	12.08	16.23	
	pain-free	7.89	14.32	0.061
10th Pulse VAS	**Painful**	61.43	23.25	
	pain-free	51.62	27.78	0.009
Electrical TS	**Painful**	26.71	19.24	
	pain-free	20.69	19.23	0.032
Pain50 temp	**Painful**	45.36	2.96	
	pain-free	46.19	2.58	0.041
Cold Water pain	**Painful**	76.49	24.83	
	pain-free	67.16	31.08	0.027
HeatPain-CPM 1	**Painful**	-5.1	22.44	
	pain-free	-11.83	24.49	0.053
HeatPain-CPM 2	**Painful**	-5.2	20.93	
	pain-free	-10.58	20.08	0.066

Table 6: t-test between painful and pain-free participants (maximal pain) by their baseline QST

	Head or Neck Maximal 6M>30	**Mean**	**Std. Deviation**	**p**
Pain50 temp	**painful**	45.47	2.91	
	pain-free	46.23	2.60	0.060
Heat Pain 1 Conditioned	**Painful**	49.52	22.32	
	pain-free	40.51	25.01	0.010
Cold Water pain	**Painful**	76.54	24.60	
	pain-free	65.33	32.03	0.008
Heat Pain-CPM 1	**Painful**	-4.76	22.92	
	pain-free	-13.49	24.03	0.012
Heat Pain-CPM 2	**Painful**	-4.76	20.39	

	pain-free	-11.91	20.26	0.017

Comparison for clinical pain measures assessed at 12M :

Table 7: t-test between painful and pain-free participants (maximal pain) by their baseline QST

	Head 12M>30	Mean	Std. Deviation	P
Mechanical TS	**painful**	13.36	15.61	
	pain-free	9.18	16.09	0.074
Pain 50 temp	**Painful**	45.12	2.74	
	pain-free	45.90	3.01	0.067
Heat Pain3	**Painful**	39.03	27.53	
	pain-free	48.31	25.93	0.02
PPT Conditioned avg.	**Painful**	3.52	1.91	
	pain-free	4.17	2.29	0.042
Cold Water pain	**Painful**	78.92	22.67	
	pain-free	67.45	31.34	0.005
PPT-CPM	**Painful**	0.17	0.749	
	pain-free	0.43	0.93	0.043

Table 8: t-test between neck painful and pain-free groups by their baseline QST

	Neck12M>30	Mean	Std. Deviation	P
Mechanical 10th	**painful**	23.33	24.08	
	pain-free	17.20	19.25	0.069
Mechanical TS	**Painful**	14.91	17.07	
	pain-free	8.53	15.03	0.007
PPT Conditioned avg.	**painful**	3.43	1.79	
	pain-free	4.19	2.31	0.018
Cold Water pain	**Painful**	78.84	25.59	
	pain-free	67.50	29.91	0.008
PPT-CPM	**Painful**	0.17	0.69	
	pain-free	0.43	0.95	0.051

Table 9: t-test between painful and pain-free participants (maximal pain) by their baseline QST

	Head or Neck Maximal 12M>30	Mean	Std. Deviation	P
Mechanical TS	**painful**	13.52	16.48	
	pain-free	8.59	15.37	0.032
PPT Conditioned avg.	**painful**	3.54	1.87	
	pain-free	4.23	2.34	0.028
Cold Water pain	**painful**	77.87	24.93	
	pain-free	66.42	30.89	0.005
PPT-CPM	**painful**	0.17	0.73	
	pain-free	0.47	0.94	0.014

Generally, painful patients showed a pro-nociceptive pain profile already at the acute phase, and thus it seems that psychophysical evaluation is relevant to predict cornification.

Models for predicting the risk of chronic mTBI pain

In order to further explore the predictive role of QST and construct a **predictive model** for chronicity, logistic regressions were performed to define variables that increase or decrease the risk of being in the painful group at 6M and 12M.

Prediction of chronic pain at 6M: Tables 10 and 11 show the prediction model Neck and Maximal pain. No significant model for Head pain was found.

Table 10 – prediction of being in the Neck chronic pain group at 6M

Effect	p	Estimate	95% Confidence Limits
Pain 50 temp	0.0005	0.846	0.753-0.951
Heat Pain CPM 1	0.0376	1.015	1.001-1.029

Table 11 – prediction of being in the Maximal chronic pain group at 6M

Effect	p	Estimate	95% Confidence Limits
Pain50 temp	0.0171	0.87	0.774 - 0.975
Heat Pain1Conditioned	0.0045	1.02	1.006 -1.033

Patients who were more sensitive to heat stimulation (had lower pain-50 temperature) are at higher risk for chronicity for Neck and Maximal pain at 6M. Further, for Neck pain, less-

efficient CPM indicates risk of developing chronicity, and Maximal heat pain reports under conditioning stimuli (cold water) predict chronicity.

Prediction of chronic pain at 12M: Tables 12 -14 show the prediction models for chronicity. Notably, at follow-up at 12M, the models are more consistent for both Head and Neck pain.

Table 12 – **prediction of being in the** head **chronic pain group at 12M**

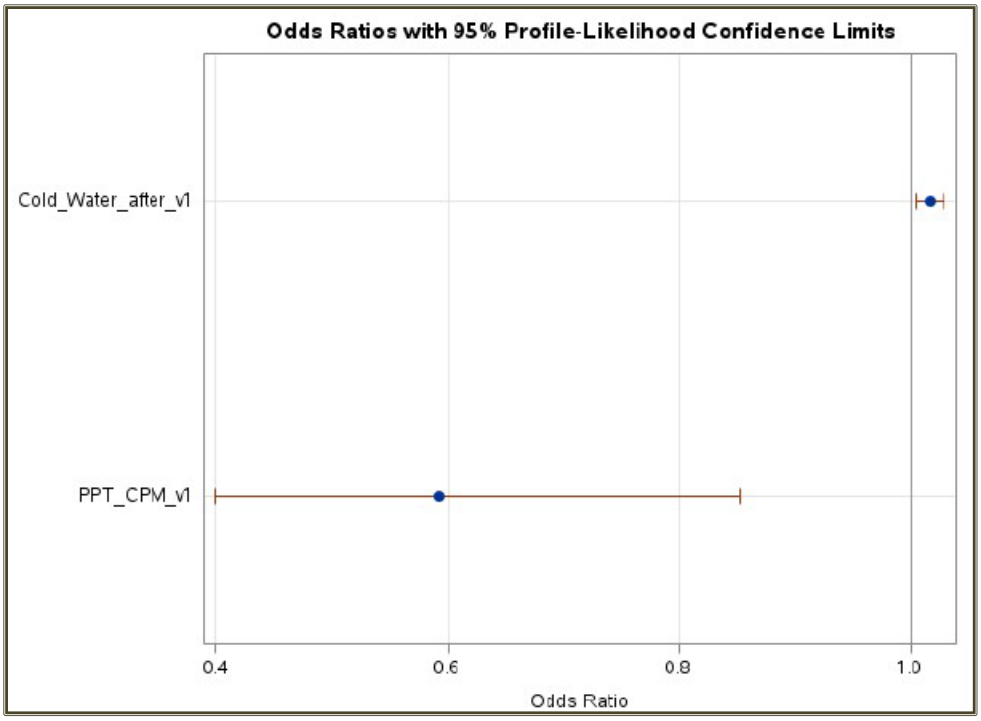
Effect	**p**	**Estimate**	**95% Confidence Limits**
CPT	0.005	0.940	0.9-0.982
Mechanical TS	0.029	1.023	1.002-1.044
Cold Water pain	0.004	1.018	1.006-1.031
PPT CPM	0.018	0.634	0.435-0.925

Table 13 – prediction of being in the neck **chronic pain group at 12M**

Effect	**p**	**Estimate**	**95% Confidence Limits**
Mechanical TS	0.0181	1.024	1.004-1.044
Cold Water pain	0.0128	1.016	1.003-1.028
PPT CPM v1	0.0291	0.644	0.434-0.956

Table 14– **prediction of being in the Maximal chronic pain** group at 12M

Effect	**p**	**Estimate**	**95% Confidence Limits**
Cold Water pain	0.0035	1.017	1.006-1.029
PPT CPM	0.0067	0.593	0.4060.865



The finding of the logistic regression shows that patients who demonstrated more pro-nociceptive pain profile as observed by less-efficient ppt-CPM (which serves as the main predictor) and enhanced pain sensitivity during the acute mTBI phase had a higher risk for chronicity at 12M. Nevertheless, it should be noted that the observations for 6 or 12 M are not similar as well as the predictors for Neck, Head, and Maximal pain measures are not the same. This necessitates further exploration about the meaning of the follow-up time and the assessed body area.

Prediction of chronic pain intensity

Linear regression models to predict the intensity of pain at 6 M

Variable	Estimate	Standard Error	Type II SS	F Value	p
Intercept	95.06	38.16	4485.8	6.21	0.0137
10th Pulse VAS	0.17	0.08	3181.8	4.4	0.0373
Pain 50temp	-1.67	0.79	3247.1	4.49	0.0354
Heat Pain CPM MES 1	0.16	0.08	2611.2	3.61	0.059

Prediction of chronic pain intensity

The following model predicts the intensity of **Maximal pain at 6M**.

Table 15- Linear regression models to predict the maximal pain intensity at 6 M

	Parameter Estimate	Standard Error	P
Electrical TS	0.1703	0.1108	0.1262
Pain 50 temperature	-1.9652	0.847	**0.0214**
Cold Water pain	0.1232	0.0806	0.1281
Heat Pain CPM 1	0.2399	0.0951	**0.0125**

The P value for that model is P=0.0019, R-Square=0.092. Although not all parameters are significant, they contribute to the overall prediction. In this model, the significant parameters are both static and dynamic psychophysical parameters.

Prediction of Neck pain intensity at 6M:
The regression model show that pro-nociceptive pain modulation profile as obtained at the acute phase following injury by enhanced pain facilitation (TS) as well as less-efficient descending inhibition pathways (CPM) and higher pain sensitivity expressed by the static QST measures are associated with augmented Neck pain ratings at 6 months.

Table 16- Linear regression models to predict the **Neck pain intensity at 6 M**

Variable	Parameter Estimate	Standard Error	p
HPT	1.6926	0.557	**0.0028**
Mechanical TS	0.4036	0.126	**0.0016**
EPT 30	2.8918	1.384	**0.0382**
Pain 50 temp	1.2428	0.823	0.1328
Heat Pain1	0.2274	0.103	**0.0284**
Cold water pain	0.1279	0.072	**0.0757**
Heat Pain1 Conditioned	0.2020	0.101	**0.0473**
Heat Pain2 Conditioned	-0.4264	0.097	**<.0001**
Heat Pain CPM MES 3	0.1593	0.080	**0.0494**

The P value for that model is P<.0001, R-Square=0.225.

Prediction of Maximal pain intensity at 12M:
Notably, the general picture shows similar findings such that pro-nociceptive pain functioning is associated with enhanced chronic pain. However, the measures that predict pain intensity are not that same to the QST measures that predict pain intensity ratings at 6M. This may suggest that

the experience of chronic pain altered along time. Furthermore, the specific outcome measure also plays significant role in pain experience and its prediction.

Table 17- Linear regression models to predict the Maximal pain intensity at 12 M

Variable	Parameter Estimate	Standard Error	p
CPT	-0.7503	0.3016	0.0138
Mechanical TS	0.3858	0.1469	0.0094
Heat Pain1 Conditioned	0.3377	0.1307	0.0106
Heat Pain2 Conditioned	-0.3565	0.1207	0.0036
Cold Water pain	0.2428	0.0837	0.0042
PPT CPM	-6.9361	2.6759	0.0103

The P value for that model is P<.0001, R-Square=0.1513.

Summary:
The regression model supports the notion that early evaluation of patient's pain functioning by QST allows to define which parameters are associated with the transition into pain chronicity. The regression models, both the logistic and the linear suggest that pro-nociceptive pain modulation profile as obtained at the acute phase following injury by enhanced pain facilitation (TS) as well as less-efficient descending inhibition pathways (CPM) as dynamic measures and higher pain sensitivity expressed by the static QST measures are associated with the development and manifestation of chronic mTBI pain.

Structural and functional MRI

Functional MRI analysis:

The connectivity between nucleus accumbens and periaqueductal gray matter to injury-specific areas in the somatomotor system at the early-acute phase in the prediction of pain chronification; ***Analysis lead by Dr Noam Bosek at Technion; paper in preparation***

Background and Aims: The functional connectivity (FC) pattern of nucleus accumbens (NAc), that is hypothesized to serve as the limbic-motor interface, to other components of the meso-cortico-limbic reward system (RS), was previously suggested as predictor for the conversion of sub-acute to chronic pain (1). The periaqueductal gray matter (PAG) activation and connectivity were repeatedly shown to correlate with descending pain modulation system (DPMS) features and specifically predict individual placebo analgesia magnitude, both affected by acute experimental and chronic pain states (2). We aimed to test whether the connectivity of those two major hubs of pain-related processing at the acute state could predict future conversion to chronic pain.

Methods: We obtained the left NAc (lNAc) and PAG whole-brain FC maps of the participants suffering from mild traumatic pain injury and fulfilling the criteria for whiplash-associated disorder diagnosis, using resting-state fMRI scans acquired during the early-acute phase (within 72-hours from injury). They were further classified into chronic pain and recovery groups based on their pain ratings regarding the area of injury (AOI), i.e., which includes both head and neck, after 12 months.

Results: The recovery group (N=60) presented a clear negative correlation between lNAC and bilateral primary somatomotor cortices (PSC), consisting of pre/post-central gyri (pre/post-CG, comprised mainly of M1/S1 area, respectively); figure 1, A), while strikingly no such significant pattern was detected in the future chronic pain group (N=45; figure 1 B). A similar finding was observed regarding PAG connectivity (figure 2, A-B). According to the classical Penfield homunculus, the cortical representation of the AOI is complicated, as it is inconsistent across the PSC such that the closest structures somatotopically in the pre-CG and post-CG are the upper limb and the trunk, respectively. Given that and based on recently published somatosensory stimulation-based cortical mapping (3), we could conclude that the major significant clusters of the between-group comparison maps (Figure 1 C, Figure 2 C), overlap primarily with PSC areas corresponding to the AOI. Specifically, lNAC comparison map overlaps most strongly with the left representation of the upper limb in the pre-CG, while the parallel map of PAG overlaps with the bilateral trunk representation in the post-CG.

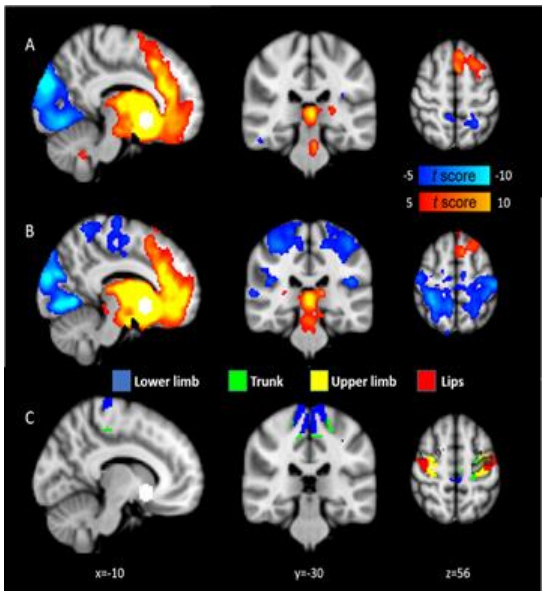


Figure 1 – Group differences in the spatial signature of lNAC negative correlation at the early-acute phase. Statistical parametric maps for lNAC region of interest, positive correlation areas in warm shades and negative in blue shades: one-sample t-tests ($p<0.05$, FWE-corrected) of chronic pain group (A) and recovery group (B). Two-sample t-test ($p<0.0001$, cluster-level FWE-corrected, adjusted for age, gender, movement and baseline pain rating) of the comparison chronic pain > recovery marked in transparent grayscale (C), projected on top of somatotopic masks of the pre-CG based on Saadon-Grossman et al. (3) according to the color coding system detailed in the figure.

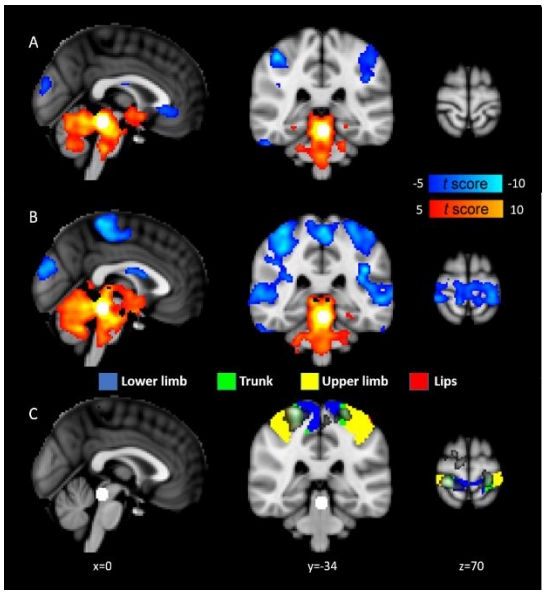


Figure 2 – Group differences in the spatial signature of PAG negative correlation at the early-acute phase. Statistical parametric maps for PAG region of interest, positive correlation areas in warm shades and negative in blue shades: one-sample t-tests ($p<0.05$, FWE-corrected) of chronic pain group (A) and recovery group (B). Two-sample t-test ($p<0.0001$, cluster-level FWE-corrected, adjusted for age, gender, movement and baseline pain rating) of the comparison chronic pain > recovery marked in transparent grayscale (C), projected on top of somatotopic masks of the post-CG based on Saadon-Grossman et al. (3) according to the color coding system detailed in the figure.

Conclusions: Our findings imply an injury-specific pattern of interaction of the DPMS and RS with the somatomotor system. Moreover, the differential signature on pre/post-CG areas matches the presumed nature of interplay between the relevant systems. This marks potential biomarkers for the prediction of acute to chronic pain conversion, and may also shed light on the underlying central nervous system (CNS) mechanism leading to it. In order to further examine whether this pattern represents an a-priori individual feature, we intend to explore the volumetric parameters and the structural connectivity (by means of diffusion tensor imaging MRI) of relevant brain areas.

Further aims and preliminary results: These results were included in a manuscript soon to be submitted. Moreover, we are in the process of investigating the long-term CNS process using repeated MRI scans performed on a sub-population of participants from both groups, 6-12 months following injury. A preliminary finding among the individuals who indeed developed chronic pain (N=19), is an enhancement in the connectivity between the relevant areas of representation of the AOI that were demonstrated in the prior analyses, namely upper limb in pre-CG and trunk in the post-CG, in the chronic pain phase when compared with the acute pain phase (figure 3). The parallel analysis involving the individuals who recovered (N=27), results in no significant differences (not shown in figure).

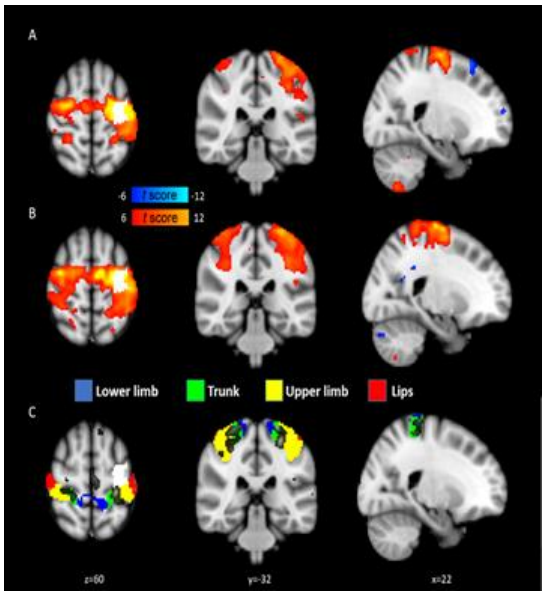


Figure 3 – Longitudinal differences in the connectivity within the somatotomor system among individuals who developed chronic pain. Statistical parametric maps for the left pre-CG somtotopic mask of the upper limb as a region of interest. Positive correlation areas in warm shades and negative in blue shades: one-sample t-tests ($p<0.0001$, FDR-corrected) of chronic pain group at the acute pain phase (A) and the chronic pain phase (6-12m following injury) (B). Paired t-test ($p<0.05$, FDR-corrected) for the comparison between the phases, the presented contrast is chronic pain > acute pain phase (C).

1) Baliki, M. N., Petre, B., Torbey, S., Herrmann, K. M., Huang, L., Schnitzer, T. J., ... & Apkarian, A. V. (2012). Corticostriatal functional connectivity predicts transition to chronic back pain. *Nature neuroscience*, *15*(8), 1117-1119.
2) Linnman, C., Moulton, E. A., Barmettler, G., Becerra, L., & Borsook, D. (2012). Neuroimaging of the periaqueductal gray: state of the field. *Neuroimage*, *60*(1), 505-522.
3) Saadon-Grosman, N., Loewenstein, Y., & Arzy, S. (2020). The 'creatures' of the human cortical somatosensory system. *Brain Communications*, *2*(1), fcaa003.

Structural MRI analysis:

Structural brain connectivity predicts acute pain after mild traumatic brain injury (Lead by Dr Apkarian's group; paper under review at PLOS biology).

Mild traumatic brain injury, mTBI, is a leading cause of disability worldwide, with acute pain manifesting as one of its most debilitating symptoms. Understanding acute post-injury pain is important since it is a strong predictor of long-term outcomes. Early acute pain after mTBI is, however, poorly understood. The etiology of pain does not map well onto injury-related imaging findings and is only partially explained by psychological and psychophysical pain characteristics. In this study, we used MRI to study brain functional and structural properties of mTBI patients after a motorized vehicle accident and probed for properties that can determine or predispose patients to experience pain after injury. We imaged the brains of 172 patients with mTBI, following a motorized vehicle collision and used a machine learning approach to extract white matter structural and resting state fMRI functional connectivity measures to predict acute pain.

Stronger white matter tracts within the sensorimotor, thalamic-cortical, and default-mode systems predicted 20% of the variance in pain severity within 72 hours of the injury (figure 4).

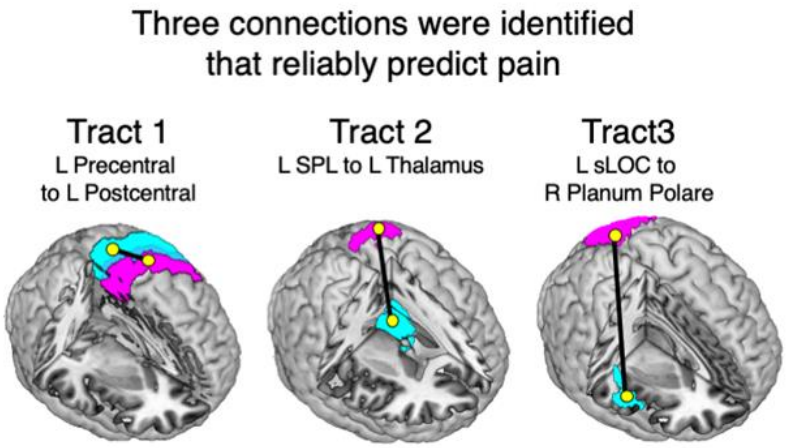


Figure 4. Somatotopic organization of structural connectivity within the sensorimotor cortex and the thalamocortical system reveal topographically appropriate linkages related to acute mTBI pain. Within the sensorimotor homunculi, the most predictive connectivity is between the face and arm ROIs. Connectivity between the precentral arm and postcentral trunk (site of injury, i.e. head/neck representation in the motor and sensory homunculi, respectively) also show significant correlation with observed pain. For the thalamus, the strongest predictor was the VPL nuclei (top panel), together with an anterior/medial region. The VPL nuclei was also the nucleus with strongest absolute connectivity to the parietal cortex.

This result generalized in two independent groups: 37 mTBI patients and 13 mTBI patients without whiplash symptoms. White matter measures collected at 6-months after the collision still predicted mTBI pain at that time point (n = 36). These white-matter connections were associated with two nociceptive psychophysical outcomes tested at a remote body site – namely conditioned pain modulation and magnitude of suprathreshold pain –and with pain sensitivity questionnaire scores.

Our validated findings demonstrate a stable white-matter network, the properties of which determine a significant amount of pain experienced after acute injury, pinpointing a circuitry engaged in the transformation and amplification of nociceptive inputs to pain perception.

Genetics

Cohort.
Recruitment All the patients were recruited in the DOD study starting from April 2018 until the end of recruitment phase of DOD, last of our patients recruited in November 2019.
Patients were recruited when visiting the Emergency Room at Rambam HealthCare Campus. Inclusion criteria: diagnosis of mTBI injury in road accident up to 24 hours before ER arrival; direct or indirect head and neck injury, Included participants (18–70 years old) reported head and/or neck pain and fulfilled the criteria for mild TBI (Glasgow Coma Scale [GCS] score ≥ 13

upon arrival with no subsequent decline and a transient brain function alteration reported without consciousness loss or shorter than 30 minutes). Exclusion criteria included Hebrew illiteracy, pregnancy, traumatic brain findings on CT if performed, other major bodily injuries at the present accident, prior chronic head/neck pain requiring regular treatment, head or neck injury in the past year, and convulsive, neurodegenerative and psychotic spectrum disorders. added to those general DOD criteria, was our RNAseq collection in which: Two blood samples collected at time 0, which is 0-72 hours from the accident, and a second sample collected 6-12 month after the accident. Patients with one sample only were excluded; patients whos samples has a technical failure such as incorrect collection, inappropriate storage or low quality of extracted RNA were wxcluded as well.
Starting with a total of 60 patients that gave the first sample, 21 droped from the study, hence did not return to give the second sample, and 3 were excluded due to technical issues with RNA collection or extraction. Leaving us with a sample of 36 patients.

The collection process – whole blood samples were collected with PAXgene® Blood RNA Tube, according to formal PAXgene brochure: first venipuncture and drainage with "discard tube", followed by 8-10 tube inversions immediately after collection, Then storage at upright position for 2-72 hours in room temperature, and only then tubes were transferred to long doration storage at -20 celcius degrees.

Sequencing - NA extraction and QC Total RNA was extracted from blood using the Qiacube (Qiagen) with the PAXgene® Blood RNA Kit. Quality measurements for total RNA were performed using TapeStation (Agilent). RNAseq libraries (NEBNext UltraII Directional RNA Library Prep Kit for Illumina, cat no. E7760) were produced. All libraries were mixed into a single tube with equal molarity. The RNAseq data was generated on Illumina NextSeq500, 75 cycles, highoutput mode (Illumina, cat no. 20024906). Quality control was assessed using Fastqc (v0.11.5). 75 bp single-end reads were aligned to a human reference genome (Human_GRCh38.92.dna.primary_assembly. fa from ENSEMBL) and annotation file (Homo_sapiens.GRCh38.92.gtf from ENSEMBL) using STAR aligner (v2.6.0a). The number of reads per gene was counted using Htseq-count (v0.9.1).

A statistical analysis was preformed using DESeq2 R package (version 1.25.0) (Genome Biology 2014 15:550). The number of reads per gene was extracted into CountWithSymbol.csv and NormalizedCountWithSymbol.csv files for raw counts and normalized counts, respectively. For downstream analysis the globin gene was removed from the analysis (ENSG00000244734) since the samples were originated from blood.

The persistent (P) pain group was defined as those still experiencing chronic pain at the time of the second collection, while the resolved (R) pain group as those whose pain resolved by the time of the second collection. The final cohort was comprised of 36 subjects: 22 males (M) and 14 females (F), of which 23 in the P pain group (15 M, 8 F) while 13 (7 M, 6F) in the R group. A logistic regression between chronification status at second visit with either sex (P=0.50), age (P=0.13) or sex-times-age interaction (P=0.55) failed to find any significant associations.
RNA-Seq. The RNA-Seq data was comprised of a total of 72 samples derived from 36 individuals that volunteered blood samples at two time points (Table S1).

Table S1

sample	timepoint	input_nb	input_len	map_unique	map_unique_(	map_len	map_multiple	map_multiple_	too_many_pct	too_short_pct
TBIKS146THE	2	48,037,714	83	17,315,255	36.05%	78.69	12,219,089	25.44%	0.28%	38.16%
TBIZD324DVT	1	31,247,115	83	22,625,597	72.41%	82.98	8,377,827	26.81%	0.31%	0.32%
TBIVRVVT1SH	1	30,828,830	83	23,332,874	75.69%	82.91	7,287,636	23.64%	0.25%	0.25%
TBIVRVVT1SH	2	32,359,449	83	24,653,792	76.19%	82.96	7,491,798	23.15%	0.30%	0.21%
TBIZD324DVT	2	32,083,500	83	24,945,708	77.75%	82.98	6,929,876	21.60%	0.25%	0.20%
TBIGL071GKP	2	41,526,064	83	32,305,427	77.80%	83.01	8,903,866	21.44%	0.26%	0.30%
TBIDU3120XP	1	39,978,926	83	31,269,749	78.22%	82.63	7,449,627	18.63%	0.37%	2.59%
TBIPP464EXI	1	28,301,989	83	22,356,940	78.99%	83.01	5,760,184	20.35%	0.28%	0.20%
TBIXV677QMT	1	29,207,470	83	23,395,197	80.10%	83.00	5,630,108	19.28%	0.23%	0.23%
TBIPP464EXI	2	27,641,192	83	22,261,852	80.54%	83.00	5,214,234	18.86%	0.25%	0.20%
TBIPE394UZW	1	25,646,714	83	20,664,857	80.58%	83.02	4,821,171	18.80%	0.27%	0.21%
TBIBN613PFH	1	51,436,575	83	41,566,969	80.81%	83.07	9,553,579	18.57%	0.28%	0.17%
TBIQK553XAY	2	43,177,428	83	35,096,143	81.28%	82.79	7,335,490	16.99%	0.42%	1.10%
TBITP910DK6	1	46,092,512	83	37,479,831	81.31%	83.05	8,333,280	18.08%	0.27%	0.15%
TBIQP464JXU	1	27,964,804	83	22,739,107	81.31%	83.01	5,021,726	17.96%	0.36%	0.21%
TBIHG332WMH	1	37,623,448	83	30,607,898	81.35%	83.00	6,690,913	17.78%	0.33%	0.34%
TBIWF938XQG	1	46,255,907	83	37,667,922	81.43%	82.97	8,168,413	17.66%	0.33%	0.41%
TBIPE394UZW	2	31,135,691	83	25,396,988	81.57%	82.99	5,533,726	17.77%	0.30%	0.20%
TBIEH659BQL	2	47,224,177	83	38,751,137	82.06%	82.97	7,792,773	16.50%	0.67%	0.58%
TBISE740DEW	2	39,757,662	83	32,672,395	82.18%	82.75	6,343,111	15.95%	0.52%	1.10%
TBIRZ897BD6	2	50,664,560	83	41,644,218	82.20%	83.03	8,692,455	17.16%	0.26%	0.21%
TBIUM616OTJ	1	30,604,162	83	25,200,349	82.34%	83.01	5,217,517	17.05%	0.26%	0.17%
TBIGN829KJU	1	28,397,466	83	23,451,348	82.58%	82.98	4,755,675	16.75%	0.24%	0.26%
TBIAC246QUR	2	44,179,785	83	36,513,334	82.65%	82.95	7,226,846	16.36%	0.28%	0.49%
TBIGN829KJU	2	27,896,803	83	23,171,608	83.06%	82.98	4,529,863	16.24%	0.25%	0.23%
TBIVK682WHK	2	49,487,905	83	41,229,675	83.31%	82.91	7,872,450	15.91%	0.26%	0.32%
TBIXV677QMT	2	31,418,098	83	26,198,258	83.39%	83.00	5,026,546	16.00%	0.24%	0.19%
TBIBM870YQM	1	43,422,926	83	36,395,567	83.82%	82.96	6,436,113	14.82%	0.40%	0.73%
TBIUM616OTJ	2	32,783,813	83	27,483,548	83.83%	83.00	5,093,552	15.54%	0.26%	0.18%
TBIWF938XQG	2	48,210,658	83	40,520,060	84.05%	82.93	6,957,650	14.43%	0.30%	1.00%
TBIGZ833NQG	1	36,557,935	83	30,867,657	84.43%	83.00	5,360,452	14.66%	0.40%	0.30%
TBITP910DK6	2	51,300,869	83	43,354,644	84.51%	83.04	7,617,259	14.85%	0.26%	0.18%
TBIRZ897BD6	1	52,828,748	83	44,648,977	84.52%	83.05	7,827,936	14.82%	0.31%	0.15%
TBIQP464JXU	2	29,940,648	83	25,388,030	84.79%	82.96	4,310,692	14.40%	0.32%	0.32%
TBIVK682WHK	1	45,450,572	83	38,684,838	85.11%	82.93	6,141,894	13.51%	0.28%	0.87%
TBIBM870YQM	2	46,557,559	83	39,704,802	85.28%	82.93	6,267,444	13.46%	0.27%	0.75%
TBIKS146THE	1	27,525,908	83	23,549,768	85.55%	82.86	3,475,222	12.63%	0.53%	1.02%
TBITD0617FIW	1	46,534,257	83	39,903,280	85.75%	82.98	6,257,380	13.45%	0.30%	0.33%
TBIQK553XAY	1	44,717,330	83	38,346,969	85.75%	82.89	5,742,756	12.84%	0.53%	0.61%
TBIEQ001WMH	2	44,681,207	83	38,396,232	85.93%	82.96	5,783,841	12.94%	0.41%	0.48%
TBIVB826RXB	1	46,742,431	83	40,233,212	86.07%	82.89	6,094,780	13.04%	0.35%	0.29%
TBIGZ833NQG	2	50,727,394	83	43,672,697	86.09%	82.93	6,580,385	12.97%	0.32%	0.40%
TBIQD654BMT	2	51,155,020	83	44,041,931	86.10%	82.93	6,651,177	13.00%	0.32%	0.36%
TBIW2700JJM	1	49,309,587	83	42,556,552	86.30%	82.88	6,269,405	12.71%	0.35%	0.39%
TBIHG332WMH	2	42,527,445	83	36,906,702	86.78%	82.99	5,280,873	12.42%	0.31%	0.28%
TBIMZ234CRQ	2	44,912,914	83	38,980,282	86.79%	83.03	5,458,271	12.15%	0.64%	0.23%
TBIFB126SRS	2	45,100,576	83	39,307,590	87.16%	82.91	5,403,400	11.98%	0.28%	0.34%
TBIEH659BQL	1	44,874,690	83	39,151,553	87.25%	82.97	5,251,712	11.70%	0.50%	0.35%
TBIM719RSW	1	43,327,504	83	37,884,190	87.44%	82.72	4,048,193	9.34%	0.34%	2.48%
TBIDW009LOV	2	36,272,714	83	31,740,418	87.50%	83.01	4,111,816	11.34%	0.28%	0.62%
TBIW2700JJM	2	50,626,122	83	44,489,312	87.88%	82.91	5,720,936	11.30%	0.33%	0.28%
TBIUP693XYW	2	45,552,500	83	40,044,614	87.91%	83.01	5,134,249	11.27%	0.34%	0.28%
TBIDW009LOV	1	46,264,558	83	40,725,350	88.03%	82.93	5,070,910	10.96%	0.34%	0.47%
TBIDU3120XP	2	43,961,153	83	38,707,114	88.05%	82.94	4,904,297	11.16%	0.25%	0.33%
TBIEQ001WMH	1	46,664,582	83	41,128,192	88.14%	82.98	5,004,463	10.72%	0.34%	0.53%
TBIMZ234CRQ	1	40,603,695	83	35,873,186	88.35%	82.81	3,744,946	9.22%	0.45%	1.53%
TBIM719RSW	2	51,189,516	83	45,250,634	88.40%	82.87	5,459,802	10.67%	0.34%	0.33%
TBIRL771TG1	2	50,095,023	83	44,325,292	88.48%	83.04	5,436,003	10.85%	0.27%	0.19%
TBIGL071GKP	1	39,686,113	83	35,395,242	89.19%	82.89	3,821,614	9.63%	0.39%	0.50%
TBIFB126SRS	1	42,812,675	83	38,292,247	89.44%	82.84	4,142,527	9.68%	0.34%	0.32%
TBIFB701LYP	2	54,175,447	83	48,560,359	89.64%	83.02	5,149,971	9.51%	0.29%	0.34%
TBIUP693XYW	1	45,811,126	83	41,287,825	90.13%	82.99	4,070,735	8.89%	0.31%	0.44%
TBIAC246QUR	1	39,516,784	83	35,678,170	90.29%	83.00	3,495,579	8.85%	0.30%	0.37%
TBITD0617FIW	2	49,566,303	83	45,040,078	90.87%	82.99	3,872,315	7.81%	0.28%	0.77%
TBIFB701LYP	1	54,775,938	83	49,902,954	91.10%	83.02	4,384,656	8.00%	0.33%	0.29%
	average:	42,004,144		35,453,599	84.10%	82.90	5,869,941	14.36%	0.33%	0.99%
	stddev:	7,991,218		7,963,387	6.98%	0.51	1,615,751	4.28%	0.09%	4.46%
	min:	25,646,714		17,315,255	36.05%	78.69	3,475,222	7.81%	0.23%	0.15%
	max:	54,775,938		49,902,954	91.10%	83.07	12,219,089	26.81%	0.67%	38.16%

Sequencing reads were 83 nucleotides long, single-ended. All samples were retained for further analyses following quality controls. The average number of reads per sample was 42 million (standard deviation ±8), of which 84% (±7%) mapped uniquely on the human genome, while only 14% (±4%) were multi-mapped. Reads discarded for 'too many hits' (>10 hits) or 'too short' (<2/3 of the length of the read was mapped) are all below 1%. One sample (TBIKS146THE, 2nd time point) displayed an unusually high 'too short' un-mapping rate at 38%, but the sample's transcriptome did not show up as an outlier in the PCA plot. Another sample (TBIQK553XAY, 2nd time point) featured a transcriptome Z-score of 3.16, but its mapping statistics did not feature any peculiar deviations. The deep-sequencing reads were mapped using STAR version 2.7.3a, with gene expression quantitation derived using the "--quantMode GeneCounts" option (*1*). Reads were mapped on human genome version GRCh38, with Ensembl gene annotations version 103 (*2*). Differential expression of genes detected using DESeq2 version 1.26.0 (*3*), with age and sex as co-variables, on genes that displayed at least an average of 10 counts. Differential expression of pathways detected using fgsea version 1.12 (github.com/ctlab/fgsea). Gene expression in tpM units estimated using TPMCalculator version 0.0.3 (*4*). Blood cell type fractions were estimated using CIBERSORT version 1.04 (*5*).

Genetics. From the transcriptome data at the first visit, we first extracted the allele content at transcribed regions to sketch the genotyping backbone of each individual, then we imputed the backbone data to obtain a denser/fuller genetic data. The conversion of BAM to VCF data was done by a publicly available script (https://www.biorxiv.org/content/10.1101/684993v1; https://github.com/inab/RDConnect_RNASeq/), itself using the GATK toolkit (https://doi.org/10.1101/201178) (*6*). Imputation of the VCF data was done using SHAPEIT for pre-phasing (*7*), followed by IMPUTE2 for imputing (*8*), with the 1000 Genomes Project Phase 3 reference panel. The imputation score statistic at SNP rs4903580 was "1.0", meaning "very high certainty".

Pathways. Pathway analyses were conducted using the R package 'fgsea' (https://github.com/ctlab/fgsea; https://github.com/ctlab/fgsea), while pathway ontologies taken from Gene Ontology (kindly provided here in a convenient format: http://download.baderlab.org/EM_Genesets/December_11_2020/Human/symbol/) (*9*). Considered pathways were comprised of one to one thousand genes, favouring specificity of function. Pathways were summarized using REVIGO (http://revigo.irb.hr/) (*10*).

RESULTS

Differential expression of genes

The study designed allowed to perform four comparisons (Fig. 1); two that compared pain groups at first (t_1; Fig. 1A) and second (t_2; Fig. 1B) collections, and two that compared changes between collections, separately in both the P (Fig. 1C) and R (Fig. 1D) pain groups. Despite hundreds of genes co-regulated, only one gene reached transcriptome-wide statistical significance at the FDR 10% level (Fig. 1B). That gene was Sterile Alpha Motif Domain Containing 15 (*SAMD15*), down-regulated in the P pain group compared to R's at the second collection time point. Not much is known about the gene's function, but is it reported that SNPs in that gene were found strongly associated in a GWAS of myeloid white cell counts in individuals of European ancestry (*11*) (https://www.genecards.org/cgi-bin/carddisp.pl?gene=SAMD15; https://www.ebi.ac.uk/gwas/variants/rs4903580; rs4903580; $P=3x10^{-18}$). There, the risk allele T at 46% frequency confers about 2% SD unit increase in cell counts.

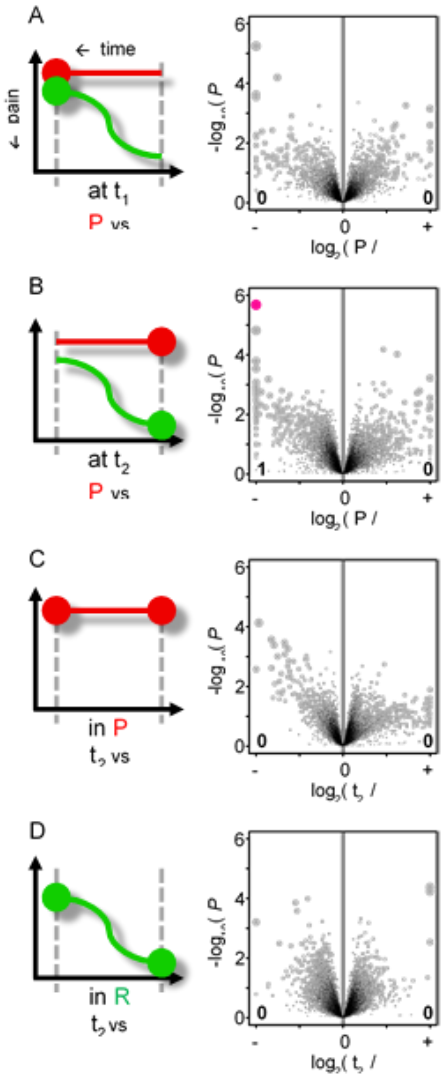


Figure 1. Differential Expression of Genes in Study Design Contrasts. Study design pictograms (left panels) are juxtaposed to volcano plots (right panels). The pictogram highlights the two contrasted conditions (large dots), situated in time (X axis) and on the pain scale (Y axis, arbitrary units). The volcano plot shows statistical significance (y axis) as a function of fold change (x axis); each dot is a gene. Genes that would end up outside of the plot are squeezed inside. Vertical grey line indicates null fold change. Genes reaching statistical significance at the FDR 10% level are highlighted in pink. Numbers in bold indicate counts of significantly differentially expressed genes that are down-regulated (lower left corner) or up-regulated (lower right corner). **(A, B)** Contrast between patients with persistent (P) and resolved

We then looked at *SAMD15* in all four contrasts to build a better picture of the dynamics of the expression of the gene (Table S2). At t_1, *SAMD15* is lowly expressed, and showed no significant difference between the pain groups (P=0.2). However, with time, *SAMD15*'s expression increases in the R pain group (log_2FC=+3.1, P=$6x10^{-5}$), while stays constant in the P group (P=0.9), hence the significant difference observed at t_2.

Table S2

fixed	variable	HGNC.symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	numerator	denominator
P	t2_vs_t1	SAMD15	10.07	0.03	0.16	0.16	8.73E-01	10.17	9.98
R	t2_vs_t1	SAMD15	112.88	3.09	0.77	4.02	5.79E-05	213.09	12.67
t1	P_vs_R	SAMD15	10.53	-0.27	0.21	-1.27	2.05E-01	9.75	11.91
t2	P vs R	SAMD15	84.68	-3.13	0.66	-4.74	2.10E-06	10.70	215.58

Differential expression of blood cell type fractions

We next explored whether if blood cell type fractions could be different in any of the four comparisons. Fractions for each sample were estimated using CIBERSORT, which uses

signature genes to deconvolute the RNA-Seq data into estimates of pure immune cell types (*5*). No significant differences were found in any of the four contrasts.

Relationship between rs4903580 and chronic pain

From the transcriptomics data, we extracted the alleles at gene loci that were expressed, imputed the genetic data, then performed association test between SNP rs4903580 and pain chronification by the time of the second visit. The T allele was already strongly associated with increased neutrophil counts (*11*). Here, we found the T allele to be protective for pain chronification, albeit in a non-significant fashion possibly due to sample size (OR=0.60, P=0.19). Using the large cohort of the UK Biobank, the T allele of the SNP rs4903580 was found significantly protective for chronic pain for the back (FDR=0.5%) and knee (FDR=8%), and also for the total number of pain sites (FDR=4%).

We then investigated if SNP rs4903580 could be an expression quantitative trait locus (eQTL) via the GTEx portal (*12*). There, the SNP was found strongly associated with the expression level of gene *SAMD15* in multiple tissues, including testis ($P=1x10^{-33}$), thyroid ($P=2x10^{-14}$), and whole blood ($P=1x10^{-10}$), with increased expression with T allele dosage. Furthermore, the multi-tissue posterior probability for eQTL effect (the m-value) was 1.0, indicating overwhelming evidence for a true eQTL effect of the SNP (since >0.9), with consistent and significant effect across multiple tissues (*13*). Finally, SNP rs4903580 was also found strongly associated with the expression levels of as many as eight different genes (up to $P=8x10^{-5}$), hinting at a possible pleiotropic effect of the SNP. We failed to find both the SNP rs4903580 and the SAMD15 gene (along with FAM15A and C14orf174 deprecated alias gene symbols) in an eQTL study of human neutrophils (*14*), nor in a highly-powered eQTL study of human blood (*15*).

Differential expression of pathways

To make sense of all of the differentially expressed genes data, we performed pathway analyses in study design contrasts that followed transcriptional changes in the R and P pain groups over time (Fig. 2). In each pain group, we found several hundreds of significantly differentially expressed pathways when comparing t_2 versus t_1; a total of 1362 pathways in the P group while 1215 in R. However, each pain group displayed pathway fold changes in the opposite manner: in the persistent pain group, about 11x more significantly differentially expressed pathways were activated at 6 months (Fig. 2A), whereas in the resolved pain group, about 5x more significantly differentially expressed pathways were down-regulated over 6 months observation period (Fig. 2B). Concisely, many pathways were found activated over time in the persistent pain group, while de-activated over time in the resolved pain group, and overall differential pathways were significantly anti-correlated.

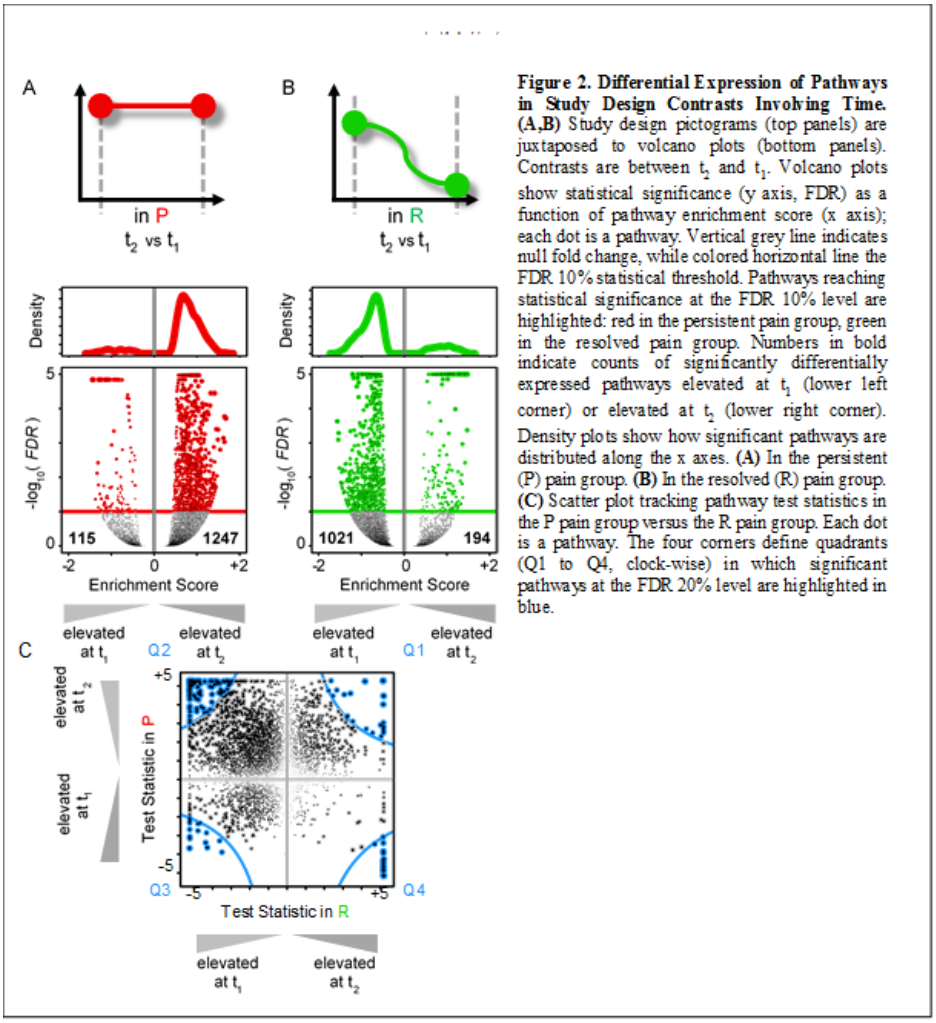
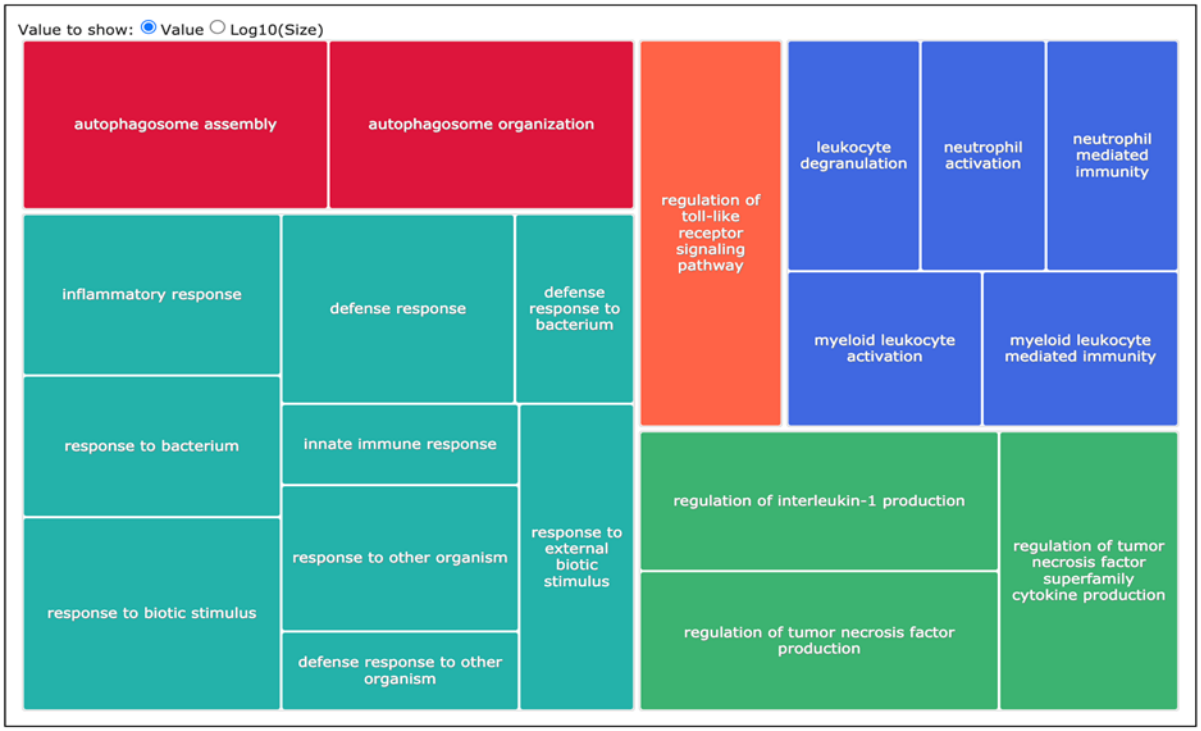


Figure 2. Differential Expression of Pathways in Study Design Contrasts Involving Time. **(A,B)** Study design pictograms (top panels) are juxtaposed to volcano plots (bottom panels). Contrasts are between t_2 and t_1. Volcano plots show statistical significance (y axis, FDR) as a function of pathway enrichment score (x axis); each dot is a pathway. Vertical grey line indicates null fold change, while colored horizontal line the FDR 10% statistical threshold. Pathways reaching statistical significance at the FDR 10% level are highlighted: red in the persistent pain group, green in the resolved pain group. Numbers in bold indicate counts of significantly differentially expressed pathways elevated at t_1 (lower left corner) or elevated at t_2 (lower right corner). Density plots show how significant pathways are distributed along the x axes. **(A)** In the persistent (P) pain group. **(B)** In the resolved (R) pain group. **(C)** Scatter plot tracking pathway test statistics in the P pain group versus the R pain group. Each dot is a pathway. The four corners define quadrants (Q1 to Q4, clock-wise) in which significant pathways at the FDR 20% level are highlighted in blue.

We then wanted to contrast determine which pathways were activated when in each pain groups. To do so, a scatter plot tracked time differential expression of each pathway, in each pain group (Fig. 2C). This way, a pathway's position in the plot indicated if the pathway was activated earlier (at t_1) or later (at t_2), and that for both pain groups. Pathways deemed interesting were those close to each quadrant's corners, displaying large (in magnitude) test statistics in both pain groups. The sign of a pathway's test statistic is the same as its enrichment score, and so the time information direction is preserved. Furthermore, the magnitude of the test statistic is proportional to its statistical significance, where lower P-values inflate test statistics. At the first visit (Fig. 2C, quadrant Q3), both pain groups displayed elevated pathways for "autophagosome assembly", "leukocyte degranulation", "regulation of interleukin-1", "regulation of toll-like receptor signaling pathway", and "inflammatory response" (Table S3A).

Table S3A

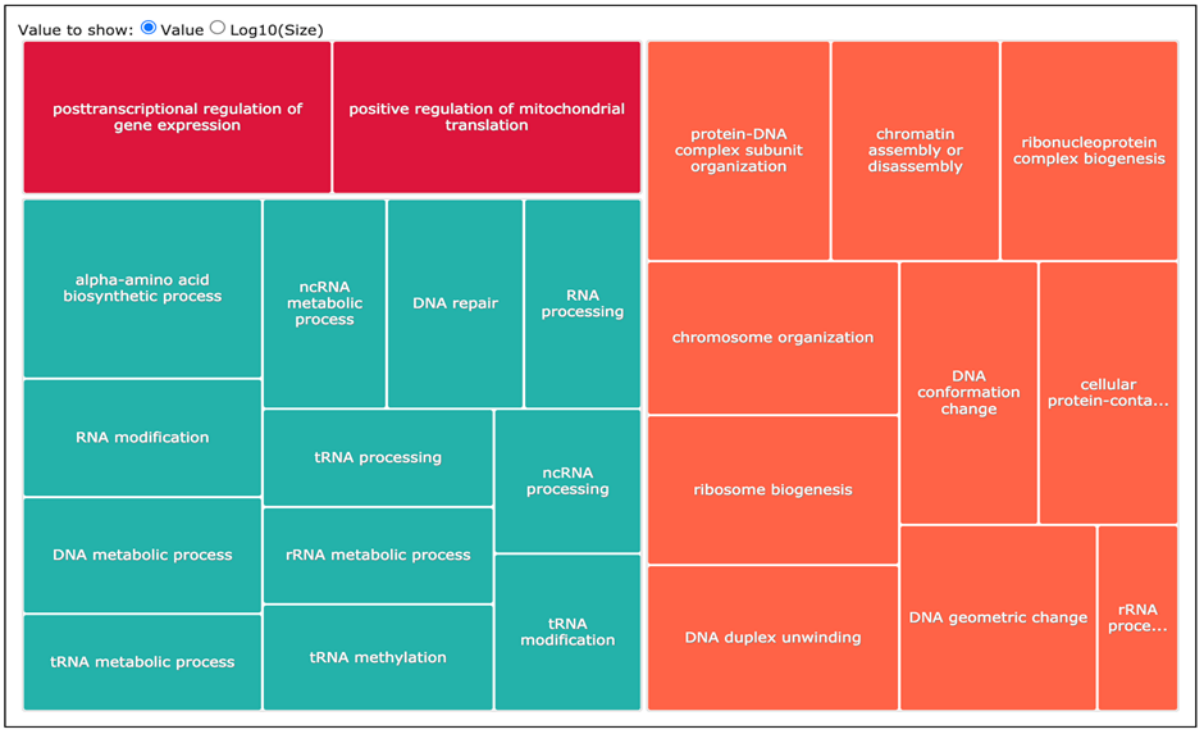
TermID	Name	Frequency	Uniqueness	Dispensability	Representative
GO:0000045	"autophagoso	0.34772855	0.92184563	0	null
GO:1905037	"autophagoso	0.37016265	0.92183108	0.85282355	"autophagosome assembly"
GO:0006954	"inflammatory	2.75939428	0.73123963	0	null
GO:0009617	"response to b	3.98766125	0.64097002	0.11514987	"inflammatory response"
GO:0009607	"response to b	8.52495794	0.87587865	0.13800076	"inflammatory response"
GO:0006952	"defense resp	7.91362872	0.77658849	0.41979189	"inflammatory response"
GO:0042742	"defense resp	1.92933259	0.39686929	0.65378989	"inflammatory response"
GO:0045087	"innate immur	4.71676949	0.33844128	0.76922564	"inflammatory response"
GO:0051707	"response to c	7.85754347	0.60974695	0.83713182	"inflammatory response"
GO:0098542	"defense resp	5.74312956	0.32958664	0.86764817	"inflammatory response"
GO:0043207	"response to e	7.86315199	0.61865229	0.88555737	"inflammatory response"
GO:0034121	"regulation of	0.42063937	0.99274858	0	null
GO:0043299	"leukocyte deg	2.84352215	0.54282782	0.00405073	null
GO:0042119	"neutrophil ac	2.79865395	0.51179662	0.42907654	"leukocyte degranulation"
GO:0002446	"neutrophil me	2.7874369	0.53999943	0.78181255	"leukocyte degranulation"
GO:0002274	"myeloid leuko	3.3034212	0.53452868	0.80140444	"leukocyte degranulation"
GO:0002444	"myeloid leuko	2.9220415	0.53775468	0.84938898	"leukocyte degranulation"
GO:0032652	"regulation of	0.62254627	0.88746436	0.02601453	null
GO:0032680	"regulation of	0.88053842	0.88541937	0.62321112	"regulation of interleukin-1 production"
GO:1903555	"regulation of	0.91418957	0.88520633	0.64861083	"regulation of interleukin-1 production"



At the second visit (Fig. 2C, Q1), both pain groups now displayed elevated pathways for "posttranscriptional regulation of gene expression", "alpha-amino acid biosynthetic process" and "protein-DNA complex subunit organization" (Table S3B).

Table S3B

TermID	Name	Frequency	Uniqueness	Dispensabilit	Representative
GO:0010608	"posttranscriptional regulation of gene expression"	3.37633202	0.98444193	0	null
GO:0070131	"positive regulation of mitochondrial translation"	0.08412787	0.98444193	0.17961914	"posttranscriptional regulation of gene expre
GO:1901607	"alpha-amino acid biosynthetic process"	0.37016265	0.87511931	0	null
GO:0009451	"RNA modification"	0.95344924	0.57614862	0.11489994	"alpha-amino acid biosynthetic process"
GO:0006259	"DNA metabolic process"	4.30734717	0.56792749	0.47205084	"alpha-amino acid biosynthetic process"
GO:0006399	"tRNA metabolic process"	1.03196859	0.47359432	0.48141199	"alpha-amino acid biosynthetic process"
GO:0034660	"ncRNA metabolic process"	2.89399888	0.52934279	0.55042564	"alpha-amino acid biosynthetic process"
GO:0006281	"DNA repair"	2.93886708	0.58451418	0.55169486	"alpha-amino acid biosynthetic process"
GO:0006396	"RNA processing"	5.15984296	0.50168875	0.68263524	"alpha-amino acid biosynthetic process"
GO:0008033	"tRNA processing"	0.73471677	0.46103577	0.72519417	"alpha-amino acid biosynthetic process"
GO:0016072	"rRNA metabolic process"	1.50869321	0.45642897	0.78470711	"alpha-amino acid biosynthetic process"
GO:0030488	"tRNA methylation"	0.23555805	0.50180575	0.82567204	"alpha-amino acid biosynthetic process"
GO:0034470	"ncRNA processing"	2.41727426	0.43391448	0.86907833	"alpha-amino acid biosynthetic process"
GO:0006400	"tRNA modification"	0.5159843	0.47187922	0.88926799	"alpha-amino acid biosynthetic process"
GO:0071824	"protein-DNA complex subunit organization"	1.64329781	0.82242985	0.00384801	null
GO:0006333	"chromatin assembly or disassembly"	0.95344924	0.7572033	0.17660484	"protein-DNA complex subunit organization"
GO:0022613	"ribonucleoprotein complex biogenesis"	2.56870443	0.79738348	0.1889343	"protein-DNA complex subunit organization"
GO:0051276	"chromosome organization"	5.93381941	0.78798691	0.34828499	"protein-DNA complex subunit organization"
GO:0042254	"ribosome biogenesis"	1.83398766	0.76704563	0.41362551	"protein-DNA complex subunit organization"
GO:0032508	"DNA duplex unwinding"	0.5832866	0.74764931	0.54238602	"protein-DNA complex subunit organization"
GO:0071103	"DNA conformation change"	1.73864274	0.74410748	0.61043776	"protein-DNA complex subunit organization"
GO:0034622	"cellular protein-containing complex assembly"	4.82333146	0.74836313	0.6172752	"protein-DNA complex subunit organization"
GO:0032392	"DNA geometric change"	0.61132922	0.74673121	0.7775424	"protein-DNA complex subunit organization"
GO:0006364	"rRNA processing"	1.45821649	0.30681267	0.86735564	"protein-DNA complex subunit organization"



Other pathways displayed significant but asynchronous time activation between the P and R pain groups (Fig. 2C, quadrants Q2 and Q4). Pathways activated earlier in the persistent pain group but later in the resolved group were (Fig. 2C, Q4): “viral gene expression”, “cellular amide metabolic process”, and “protein targeting” (Table S3D). However, by far the largest numbers of pathways were in quadrant Q2: pathways activated earlier in the resolved pain group but later in the persistent pain group abound (Fig. 2C, Q2).

Of those, we found “wound healing” and “coagulation”, “cell junction assembly”, "striated muscle

cell differentiation", but also “central nervous system development” as well as “regulation of neuron differentiation” and "neuron projection development" (Table S3C). The latter probably present the most interesting category of pathways because they may contribute to underlined molecular pathophysiology of the development of musculoskeletal pain after a stressful event.

Table S3D

TermID	Name	Frequency	Uniqueness	Dispensabilit\	Representative
GO:0019080	"viral gene expression"	0.95905777	0.94534764	0	null
GO:0019083	"viral transcription"	0.64498037	0.94534764	0.74817289	"viral gene expression"
GO:0043603	"cellular amide metabolic process"	4.51486259	0.66739666	0	null
GO:1901361	"organic cyclic compound catabolic process"	2.63039821	0.66394579	0.12469446	"cellular amide metabolic process"
GO:0043604	"amide biosynthetic process"	2.9220415	0.56122835	0.40823258	"cellular amide metabolic process"
GO:0046700	"heterocycle catabolic process"	2.35558048	0.65734293	0.51476923	"cellular amide metabolic process"
GO:0019439	"aromatic compound catabolic process"	2.44531688	0.65603427	0.55275378	"cellular amide metabolic process"
GO:0044270	"cellular nitrogen compound catabolic process"	2.38923163	0.56625767	0.5538069	"cellular amide metabolic process"
GO:0034655	"nucleobase-containing compound catabolic process"	2.07515423	0.48784932	0.55727111	"cellular amide metabolic process"
GO:0006412	"translation"	2.10880538	0.54839152	0.80031378	"cellular amide metabolic process"
GO:0002181	"cytoplasmic translation"	0.67863152	0.59128452	0.83689304	"cellular amide metabolic process"
GO:0006518	"peptide metabolic process"	2.9893438	0.55941879	0.83996381	"cellular amide metabolic process"
GO:0006413	"translational initiation"	0.78519349	0.58605761	0.85091347	"cellular amide metabolic process"
GO:0000956	"nuclear-transcribed mRNA catabolic process"	1.09366237	0.50019991	0.87240756	"cellular amide metabolic process"
GO:0006401	"RNA catabolic process"	1.36848009	0.50193863	0.89635465	"cellular amide metabolic process"
GO:0043043	"peptide biosynthetic process"	2.26023556	0.55236504	0.89678846	"cellular amide metabolic process"
GO:0006605	"protein targeting"	1.77790241	0.63057818	0.00524085	null
GO:0072599	"establishment of protein localization to endoplasmic reticulum"	0.64498037	0.61691546	0.51982928	"protein targeting"
GO:0072657	"protein localization to membrane"	3.00056085	0.59813434	0.61343688	"protein targeting"
GO:0070972	"protein localization to endoplasmic reticulum"	0.78519349	0.65361727	0.62307027	"protein targeting"
GO:0072594	"establishment of protein localization to organelle"	2.17610768	0.6188976	0.71041686	"protein targeting"
GO:0006612	"protein targeting to membrane"	1.00953449	0.58574989	0.80641787	"protein targeting"
GO:0006613	"cotranslational protein targeting to membrane"	0.55524397	0.59926034	0.81486336	"protein targeting"
GO:0090150	"establishment of protein localization to membrane"	1.72742569	0.60955237	0.86367721	"protein targeting"

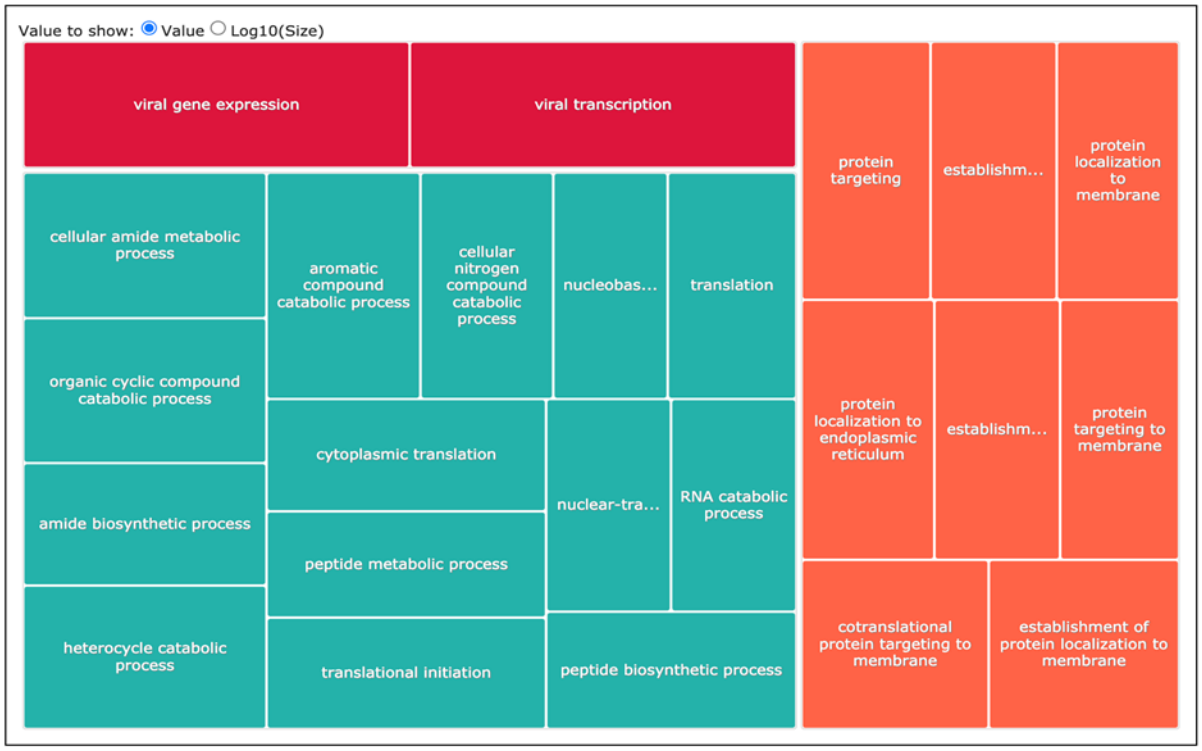
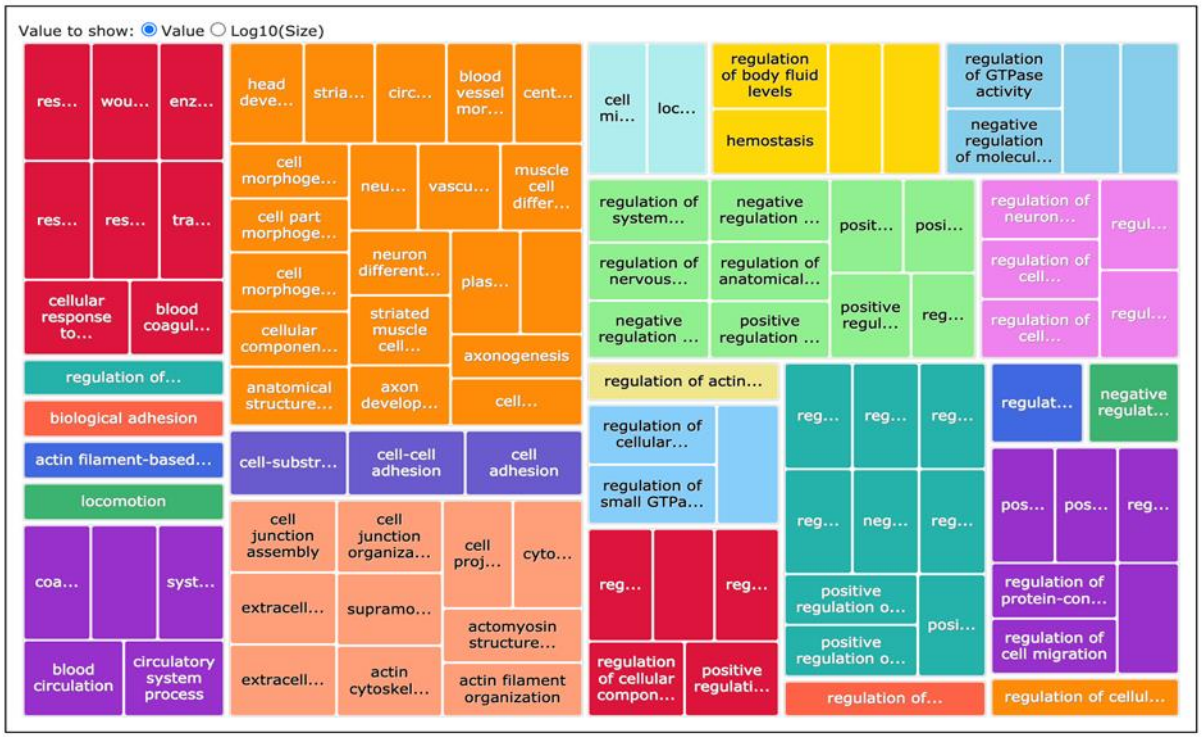


Table S3C

TermID	Name	Frequency	Uniqueness	Dispensabilit\	Representative
GO:0009725	"response to hormone"	4.55973079	0.97293899	0	null
GO:0042060	"wound healing"	2.46775098	0.96788397	0.11552899	"response to hormone"
GO:0007167	"enzyme linked receptor protein signaling pathway"	4.02131239	0.93134188	0.12448134	"response to hormone"
GO:0009719	"response to endogenous stimulus"	7.93045429	0.98350711	0.13951692	"response to hormone"
GO:0009611	"response to wounding"	3.00056085	0.97697099	0.3568082	"response to hormone"
GO:0007169	"transmembrane receptor protein tyrosine kinase signaling pathv	2.89399888	0.93411782	0.50110881	"response to hormone"
GO:0071495	"cellular response to endogenous stimulus"	6.41615255	0.97222326	0.79991856	"response to hormone"
GO:0007596	"blood coagulation"	1.65451486	0.86263938	0.87169837	"response to hormone"
GO:0010810	"regulation of cell-substrate adhesion"	1.18339877	0.96138853	0	null
GO:0022610	"biological adhesion"	5.31127314	1	0	null
GO:0030029	"actin filament-based process"	3.32024678	0.99647421	0	null
GO:0040011	"locomotion"	7.43129557	1	0	null
GO:0050817	"coagulation"	1.66573191	0.95070321	0	null
GO:0003012	"muscle system process"	1.60964666	0.93915073	0.14256831	"coagulation"
GO:0003008	"system process"	11.463825	0.93535725	0.1872725	"coagulation"
GO:0008015	"blood circulation"	2.15367358	0.93708288	0.48139427	"coagulation"
GO:0003013	"circulatory system process"	2.66404936	0.93549771	0.51389216	"coagulation"
GO:0060322	"head development"	4.40269209	0.89437597	0	null
GO:0051146	"striated muscle cell differentiation"	1.04879417	0.85320186	0.22247677	"head development"
GO:0072359	"circulatory system development"	4.85137409	0.86088201	0.27789354	"head development"
GO:0048514	"blood vessel morphogenesis"	2.26584408	0.81286498	0.31847843	"head development"
GO:0007417	"central nervous system development"	5.51318003	0.82709345	0.36628266	"head development"
GO:0000904	"cell morphogenesis involved in differentiation"	3.04542905	0.78470346	0.51481334	"head development"
GO:0032990	"cell part morphogenesis"	2.8771733	0.76770787	0.53228078	"head development"
GO:0000902	"cell morphogenesis"	3.89231632	0.84616608	0.5561544	"head development"
GO:0032989	"cellular component morphogenesis"	3.35389792	0.78823943	0.56423404	"head development"
GO:0048646	"anatomical structure formation involved in morphogenesis"	4.87941671	0.84147623	0.59799118	"head development"
GO:0031175	"neuron projection development"	3.76332025	0.71483139	0.64363277	"head development"
GO:0001944	"vasculature development"	2.8323051	0.85422724	0.78329255	"head development"
GO:0042692	"muscle cell differentiation"	1.36848009	0.85372645	0.80165225	"head development"
GO:0030182	"neuron differentiation"	5.69826136	0.78390144	0.80817911	"head development"
GO:0055002	"striated muscle cell development"	0.32529445	0.84715562	0.82303232	"head development"
GO:0061564	"axon development"	2.30510376	0.7297551	0.83113825	"head development"
GO:0120036	"plasma membrane bounded cell projection organization"	6.46102075	0.86180838	0.83691476	"head development"
GO:0048666	"neuron development"	4.63264162	0.77371107	0.85068987	"head development"
GO:0007409	"axonogenesis"	2.10319686	0.6954905	0.88559999	"head development"
GO:0048667	"cell morphogenesis involved in neuron differentiation"	2.46214246	0.74679574	0.89486214	"head development"
GO:0031589	"cell-substrate adhesion"	1.03196859	0.97188188	0.00468168	null
GO:0098609	"cell-cell adhesion"	2.86595625	0.96942938	0.6861003	"cell-substrate adhesion"
GO:0007155	"cell adhesion"	5.27201346	0.96818598	0.85586564	"cell-substrate adhesion"
GO:0034329	"cell junction assembly"	1.53673584	0.93171307	0.0049276	null
GO:0030198	"extracellular matrix organization"	2.13123948	0.92887662	0.19283317	"cell junction assembly"
GO:0043062	"extracellular structure organization"	2.13684801	0.92885291	0.20109533	"cell junction assembly"
GO:0034330	"cell junction organization"	2.7650028	0.92645254	0.2081377	"cell junction assembly"
GO:0097435	"supramolecular fiber organization"	3.30902973	0.92468689	0.22114729	"cell junction assembly"
GO:0030036	"actin cytoskeleton organization"	2.90521593	0.90203118	0.22272192	"cell junction assembly"
GO:0030030	"cell projection organization"	6.7190129	0.91688864	0.25329082	"cell junction assembly"
GO:0007010	"cytoskeleton organization"	6.80314077	0.90891425	0.41825542	"cell junction assembly"
GO:0031032	"actomyosin structure organization"	0.6057207	0.91652193	0.76159815	"cell junction assembly"
GO:0007015	"actin filament organization"	1.48625911	0.90843818	0.84983348	"cell junction assembly"
GO:0016477	"cell migration"	5.35614133	0.97546981	0.00589924	null
GO:0051674	"localization of cell"	6.1357263	0.98676223	0.26716847	"cell migration"
GO:0050878	"regulation of body fluid levels"	2.7650028	0.94494442	0.02636133	null
GO:0007599	"hemostasis"	1.67134044	0.93833664	0.30724534	"regulation of body fluid levels"
GO:0032535	"regulation of cellular component size"	2.14806506	0.87759716	0.31762404	"regulation of body fluid levels"
GO:0090066	"regulation of anatomical structure size"	2.86034773	0.94471907	0.33035931	"regulation of body fluid levels"
GO:0043087	"regulation of GTPase activity"	1.92933259	0.94465976	0.02807109	null
GO:0044092	"negative regulation of molecular function"	6.30959058	0.9434722	0.43323085	"regulation of GTPase activity"
GO:0051336	"regulation of hydrolase activity"	5.94503646	0.94110355	0.52051612	"regulation of GTPase activity"
GO:0043547	"positive regulation of GTPase activity"	1.43017386	0.94639517	0.64787997	"regulation of GTPase activity"



ACKNOWLEDGEMENTS

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LEGENDS

Table S1. Deep-sequencing reads quality control and statistics. (A) Genomic mapping statistics from the STAR aligner. Columns are: A) sample, the anonymized individual ID; B) timepoint, indicating first (1) or second (2) blood collection times; C) input_nb, the total number of reads produced by the sequencing machine and input for genomic mapping; D) input_len, the number of nucleotides sequenced in each read; E) map_unique, the number of reads that uniquely mapped to the human genome; F) map_unique_pct, same as previous but expressed as a percentage; G) map_len, the average number of nucleotides mapped; H) map_multiple, the number of reads that mapped to multiple loci (≥2); I) map_multiple_pct, same as previous but expressed as a percentage; J) too_many_pct, percent of reads discarded because mapped to too many loci (>10); K) too_short_pct, percent of reads discarded because the mapped portion of the read is too short (<2/3 length of the read was mapped); L) other, percent for reads discarded for other reasons.

Table S2. **Results for the *SAMD15* genes, all four contrasts.**

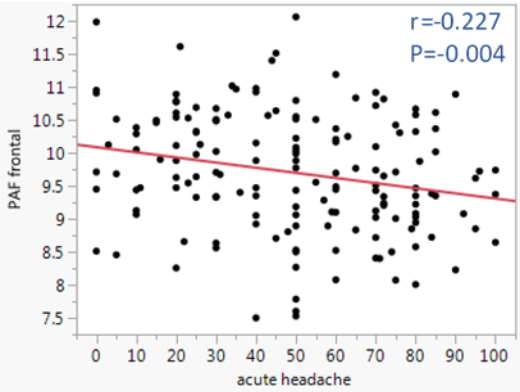
Table S3. Contrast of time-evolution of pathways by pain groups. Pathways that reached statistical significance at the FDR 20% level were highlighted: green for quadrant Q1, red for Q2, blue for Q3, and yellow for Q4. **(A)** reviGO summary results for pathways at the FDR 20% level in quadrant Q3. **(B)** reviGO summary results for pathways at the FDR 20% level in quadrant Q1. **(C)** reviGO summary results for pathways at the FDR 20% level in quadrant Q2. **(D)** reviGO summary results for pathways at the FDR 20% level in quadrant Q4.

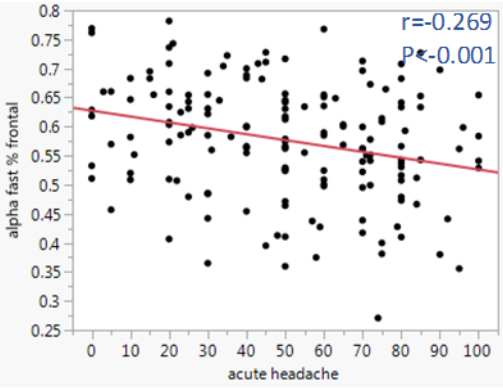
Resting-state EEG – the FFT analyses

At this point, our main analyses focused on resting-state EEG activity.

At baseline, 164 mTBI patients underwent EEG recording; 161 of them provided clinical pain scores and were included in the subsequent data analysis.

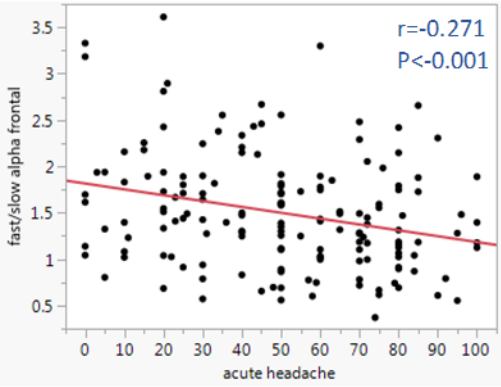
<u>The relationship between the baseline EEG parameters and acute pain</u>
Pearson correlation analysis revealed an association between baseline clinical pain and several EEG measures; higher acute post-traumatic headache scores correlated with slower oscillations at alpha band (slower PAF values; Figure 1 A) and lower relative activity within the fast alpha sub-band (Figure 1B), mainly at the frontal area. These findings are in line with the reported associations of slower and lower alpha activity, and stronger acute experimental pain in healthy subjects.
Figure 1

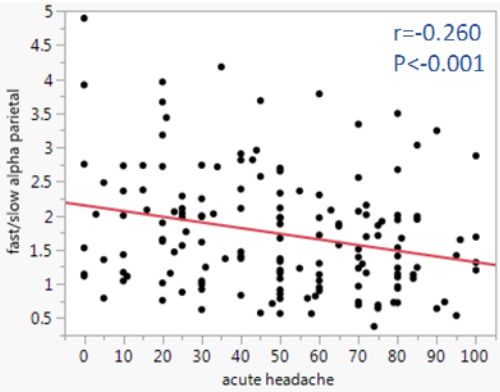




Further, higher headache scores were associated with lower prevalence of fast/slow alpha power, at all the tested regions (Figure 2).

Figure 2

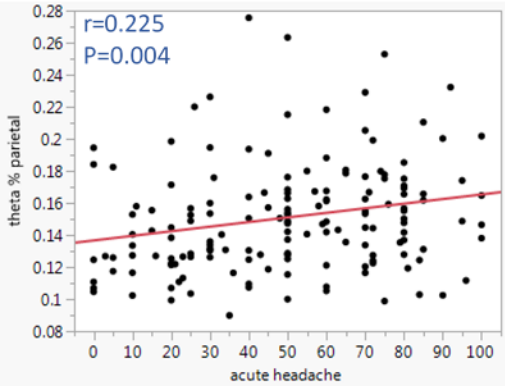




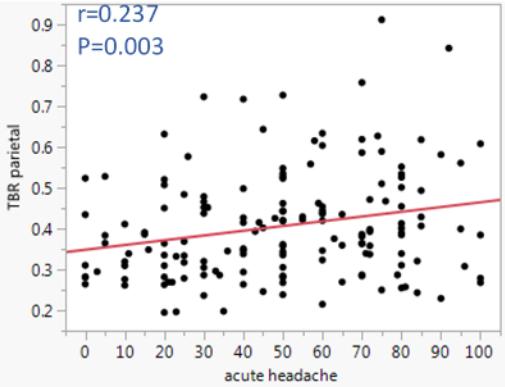
In line with the reported association between acute pain and EEG activity at the theta band, higher pain scores correlated with higher theta activity relative to the whole EEG spectrum, mostly at parietal area (Figure 3A). Moreover, higher acute pain scores related with higher value of theta/beta ratio at centro-parietal areas (Figure 3B), suggesting less efficient attentional control in stressful situation.

Figure 3

A



B



<u>The relationship between baseline EEG parameters and chronic 6m and 12m pain</u>

No significant correlation was found between the baseline (acute phase) EEG and chronic pain. Due to demonstrated relationships between EEG activity and acute post-traumatic pain, and in order to take into account the possible modulating effect of acute pain on the EEG parameters, and, as a result, on EEG-based prediction of chronic pain development, we further divided the patients into 4 groups: (1) the patients who had both acute and chronic pain at some follow up time point (N=76), (2) the patients who had acute pain only (n=63), (3) the patients who had chronic pain with no acute pain (N=7), and (4) the patients that had no acute and no chronic pain (N=13). The definition of having clinically significant pain was based on the NPS≥30 for the maximal score of headache or neck pain.

Thus, as expected, our findings point to an association between acute post-traumatic headache and slowed resting-state EEG activity.

<u>The relationship between baseline EEG parameters and chronic 6m and 12m pain</u>

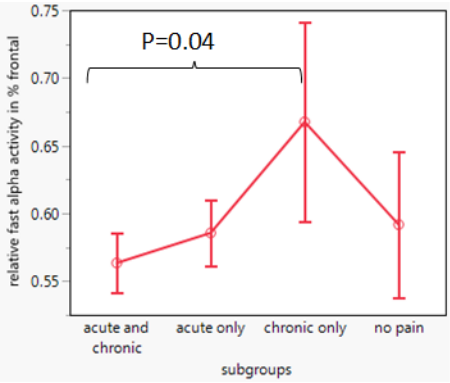
No significant correlation was found between the baseline (acute phase) EEG power at any band, and chronic pain.

Due to demonstrated relationships between *baseline* EEG activity and acute post-traumatic pain, and in order to take into account the possible modulating effect of acute pain on the EEG parameters, and, in an attempt to reach an EEG-based prediction of chronic pain development, we further divided the patients into 4 groups: (1) patients who had both acute and chronic pain at some follow up time point (N=76), (2) patients who had acute pain only (n=63), (3) patients who had chronic pain with no acute pain (N=7), and (4) patients that had no acute and no chronic pain

(N=13). The definition of having clinically significant pain was based on the NPS≥30 for the maximal score of headache or neck pain at the baseline.

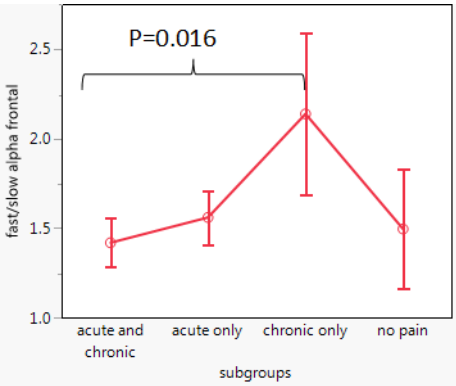
ANOVA indicated a significant group effect for the fast alpha activity (% activity relatively to the whole alpha band), at frontal region. Highest alpha activity was noted for those patients who later developed chronic pain but at the acute post-injury phase were pain-free. It was significantly different from the alpha activity in patients who experienced both acute and chronic post-traumatic pain (Figure 4).

Figure 4



In line, the patients that developed chronic pain without experiencing the acute pain, had higher predominance of fast over slow alpha activity; this value was significantly different from that of the patients who experienced both acute and chronic pain (Figure 5).

Figure 5



So, at this point we can conclude that at the group level, lower alpha activity at the acute post-injury phase is associated with experiencing acute pain, along with the future development of chronic pain.

On the other hand, the 'extreme' alpha activity at the acute phase (*baseline*) in some patients, as well as the predominance of fast alpha oscillations, doesn't protect them from the development of chronic post-traumatic pain. We may therefore suggest that this enhanced *baseline* alpha activity

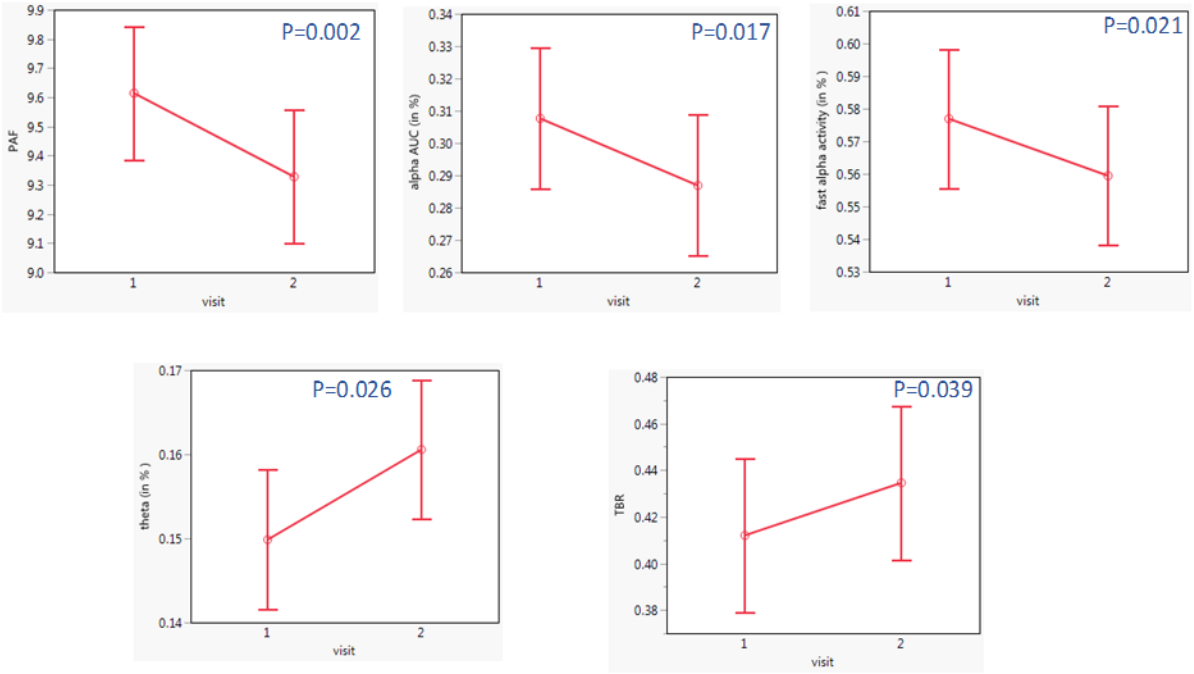
is a pre-existing individual patient characteristic, unrelated to the current mTBI, possibly reflecting disrupted thalamo-cortical brain connections, which are often associated with various neurological states.

The changes in EEG from acute to chronic post-traumatic phases

Thirty-six patients underwent repeated EEG assessment at 6m visit; additional 24 patients underwent EEG recording at 12m after the injury. In order to increase the analyzed N, we combined the EEG data for these two timelines, replacing also the pain scores of 6m with the 12m reports for the relevant subjects. So, the total data sample of two-visits EEG data consisted of 60 patients.

Significant reduction in clinical pain from acute to chronic phases was found for this sub-group of patients (42.1±3.6 (SE) vs. 21.4±3.6, p<0.001, headache; 47.8±3.6 (SE) vs. 23.4±3.6, p<0.001, neck pain; 54.5±3.6 (SE) vs. 28.3±3.6, p<0.001, maximal pain score between headache and neck pain). Despite significant pain reduction, the changes in the EEG activity wrer opposed to the expected directions: slowing PAF, reduced relative (in %) alpha activity, reduction in relative fast alpha activity, reduction in the ratio of fast to slow alpha activity, increase in relative theta activity, and increased TBR, at most of the recording sites; the P values presented for the frontal area (Figure 6):

Figure 6

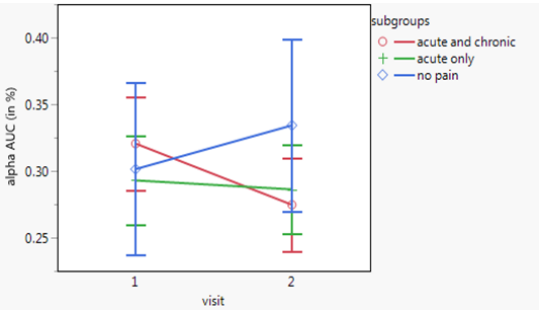


Further, bearing in mind that EEG activity at the acute phase was related to acute pain intensity, and considering that not all the patients had clinically significant acute or chronic pain, we divided this sample into 4 sub-groups as described before, to try to determine whether the changes in the EEG were associated with the presence of acute or chronic pain, or both. Due to very small number of patients that had chronic pain only (N=2), they were excluded from this

analysis. So, the subgroups were as follow: (1) patients who had acute and chronic pain (N=24), (2) patients who had acute pain only (n=27), (3) patients that had no acute and no chronic pain (N=7).

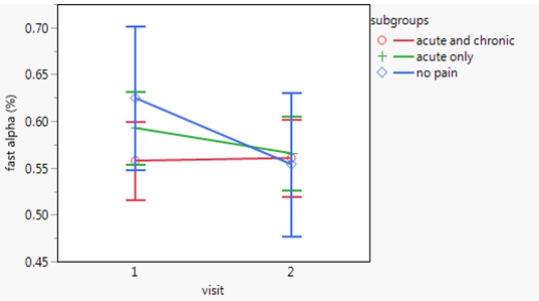
In addition to the reported overall change in some of the EEG parameters, rmANOVA with 'visit' x 'subgroup' interaction revealed significant effects for several EEG parameters; the post-hoc analyses were informative for the overall frontal alpha activity calculated relative to the whole spectrum, and the relative activity in the fast alpha band at occipital area. For the overall alpha, significant EEG differences was associated with reduced alpha activity at chronic phase (visit 2) for the patients who experienced acute pain and also developed chronic post-traumatic pain, as compared with their alpha activity at acute phase (visit 1) (red line, interaction P=0.006; contrast P=0.006), while no significant change in the alpha activity was observed for other subgroups. As can be also seen from the Figure 7, the presence of acute pain did not affect the alpha activity in the patients that later had their pain resolved (green line), suggesting that the acute pain experience has no modulating effect on the EEG activity.

Figure 7



Specifically, for the fast alpha activity (the activity at alpha sub-band 7.5-9.5 Hz) calculated relative to the total alpha band, the changes in the EEG were associated with the group that did not experience pain at any visit (blue line, interaction P=0.02; contrast P=0.04) (Figure 8). The enhanced alpha activity during the acute post-traumatic phase in this subgroup might have a protective effect against both acute pain as well as future pain development. Interestingly, the fast alpha activity during visit 2 (chronic phase) in this subgroup was not different from the other patients, suggesting that the presence of chronic pain doesn't influence the ongoing fast alpha activity.

Figure 8



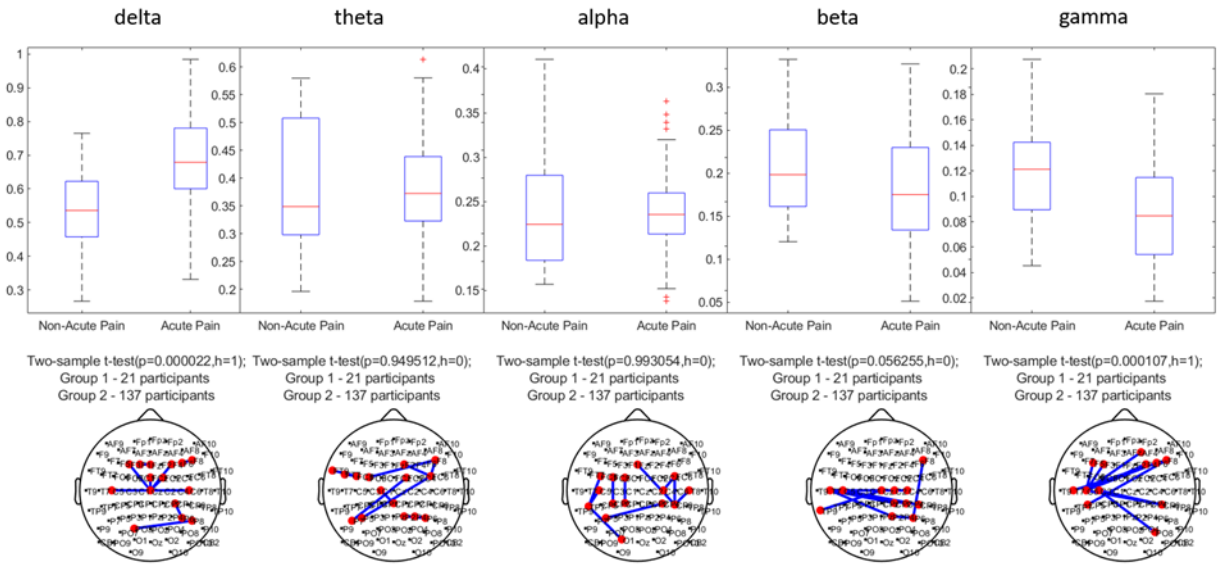
Resting-state EEG – the analysis of intra-cortical functional connectivity

<u>The role of intra-cortical functional connectivity and **sub-acute pain** classification</u>

The division to having or not acute pain (assessed at 72 h after the injury) was based on the maximum score between head and neck pain ratings, where pain under value of 30 was classified as no pain. The averaged between-electrodes functional connectivity was calculated for each frequency band similarly to the method presented in our recent publication, Frid et. al. 2020.

Significant group difference was found for the average connectivity of 10 most discriminative connections, for delta and gamma bands, when the pain patients demonstrate stronger connectivity at delta and weaker connectivity at delta bands (Figure 1).

Figure 1



Further analysis was performed for the delta and gamma bands; the prediction models included demographic, psychological and pain psychophysical parameters that were in correlation with pain (the years of education; pain sensitivity score of the PSQ questioner, the contact heat temperature needed to evoke pain sensation at 50 on the 0-100 numerical pain scale (NPS), the magnitude of pain temporal summation (TS) to suprathreshold electrical stimuli, and the CPM responses (pressure-pain threshold (PPT) conditioned to cold noxious water). Figures 2 and 3 show the results for delta band and gamma bands, for: (a) ROC curve of the average connectivity, (b) ROC curve of the 10 most discriminative electrodes (c) ROC curve of the clinical and psycho-physiological parameters, (d) ROC curve of the clinical and psycho-physiological parameters along with average connectivity, (e) ROC curve of the clinical and psycho-physiological parameters along with the 10 most discriminative electrodes. This order of presentation will be kept for the subsequent ROCs figures in this section.

Figure 2 (delta band connectivity, sub-acute pain classification)

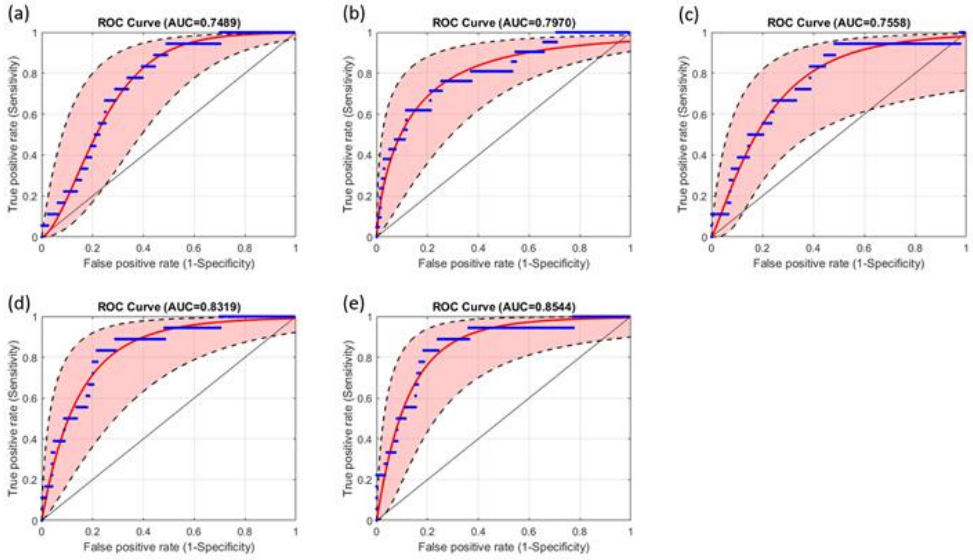
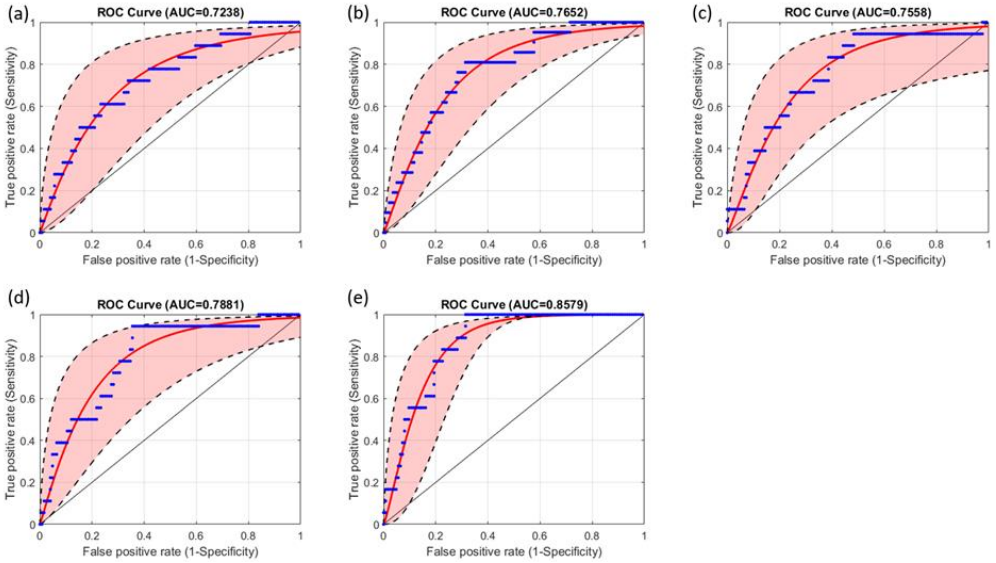


Figure 3 (gamma band connectivity, sub-acute pain classification)

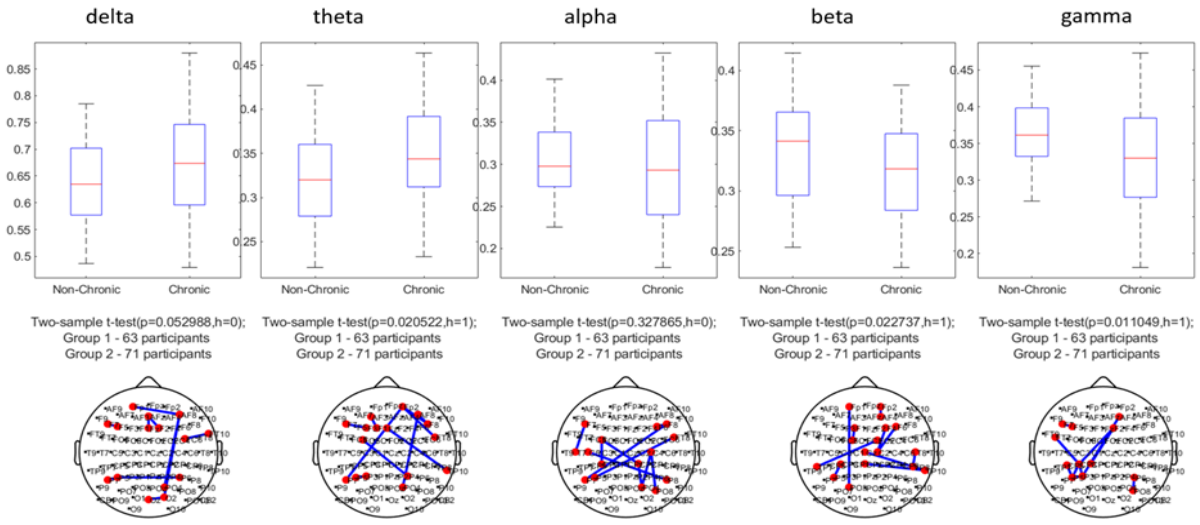


<u>The role of baseline (tested at acute phase) intra-cortical functional connectivity in prediction of **chronic (6m after mTBI) pain**</u>

The analysis was conducted on the longitudinal data, 6 months after the accident. The division to non-chronic and chronic pain was based on the maximum between head and neck ratings, where pain under value of 30 was classified as no chronic pain.

Significant group difference was found for the average connectivity of 10 most discriminative connections, for theta, beta and gamma bands, when the patients that suffered from the post-mTBI pain at 6 m after the injury, were characterized by stronger theta and weaker beta and gamma connectivity of the EEG activity, tested at the acute phase (Figure 4).

Figure 4



Further analysis was performed for theta, beta and gamma bands, and included same parameters as for the analyses of acute phase (Figures 5, 6 and 7, respectively), presenting the ROC curve for each condition:

Figure 5 (theta band connectivity, 6m chronic pain classification)

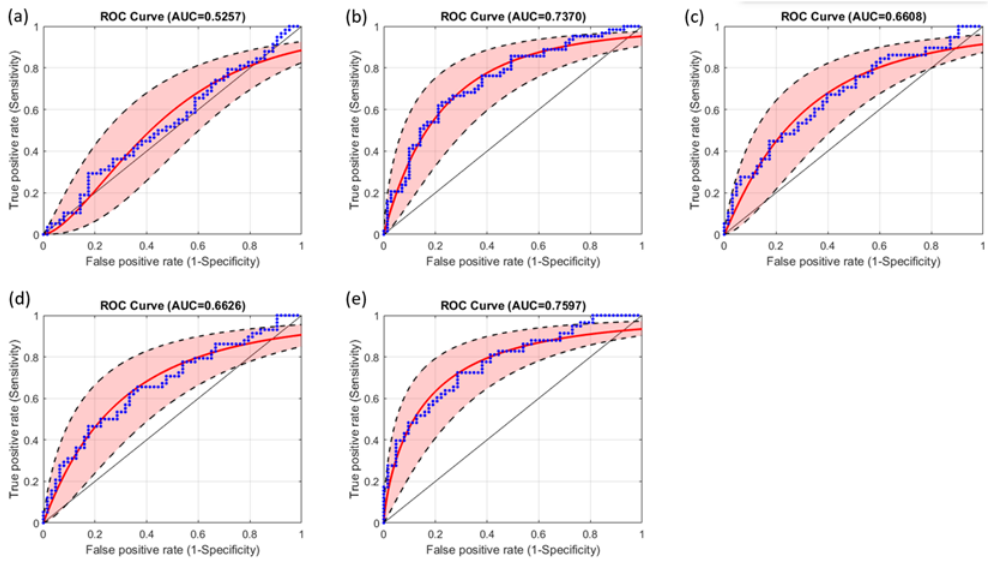


Figure 6 (beta band connectivity, 6m chronic pain classification)

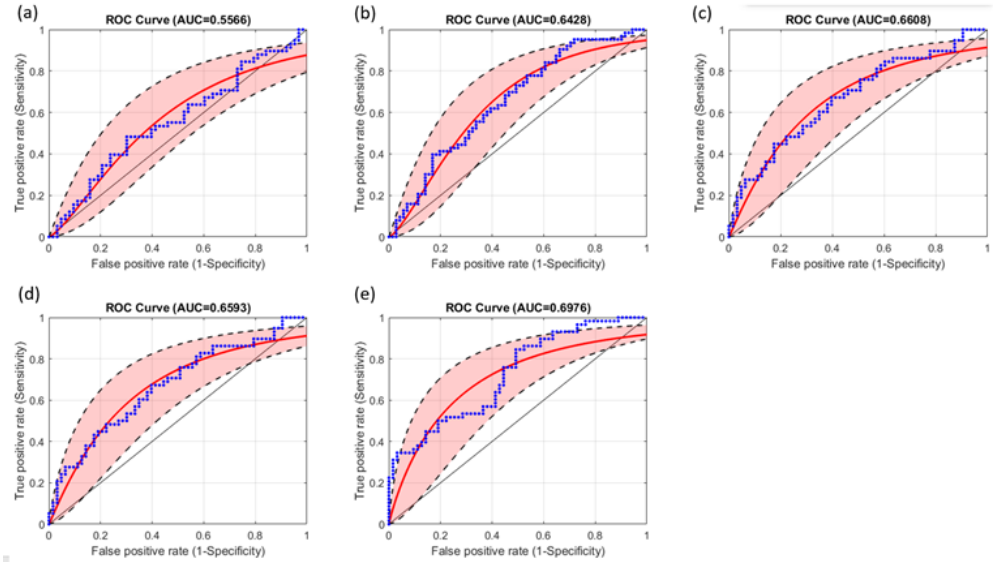
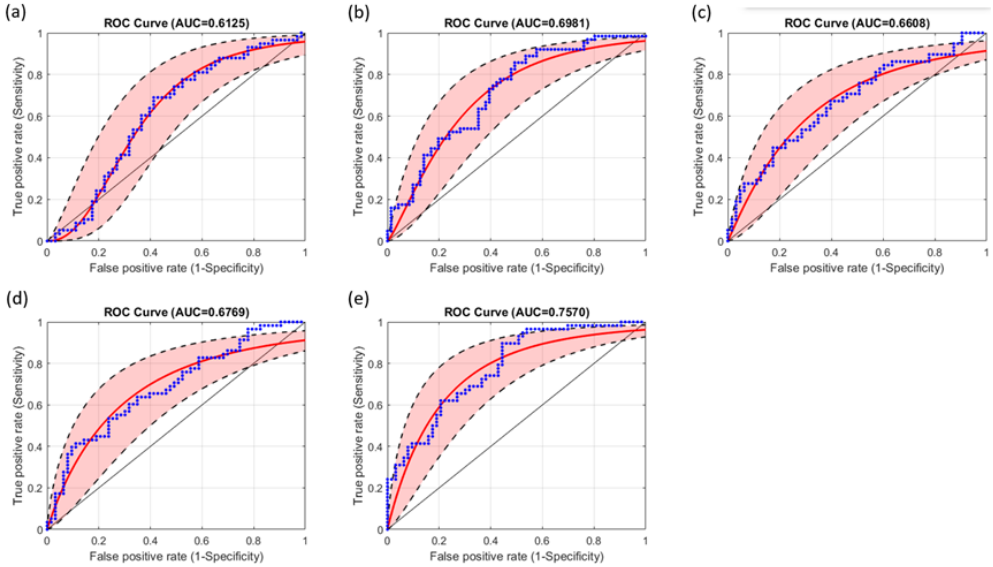


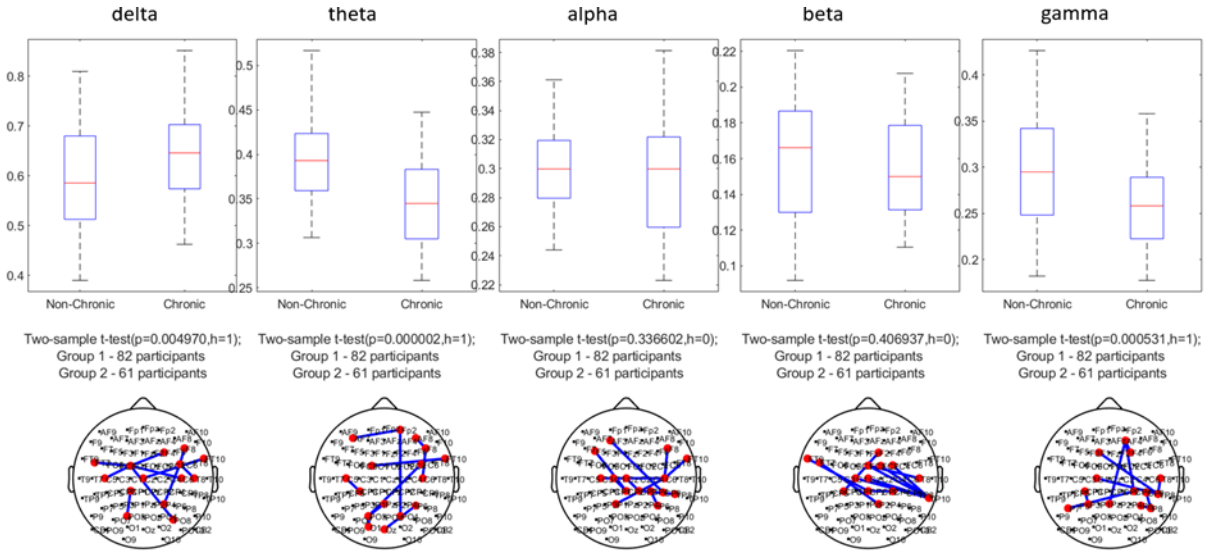
Figure 7 (gamma band connectivity, 6m chronic pain classification)



The role of baseline (tested at acute phase) intra-cortical functional connectivity in prediction of **chronic (12m after mTBI) pain**

The analysis was conducted on the longitudinal data, 12 months after the accident. The division to non-chronic and chronic pain was based on the maximum between head and neck ratings, where pain under value of 30 was classified as no chronic pain.
Significant group difference was found for the average connectivity of 10 most discriminative connections, for delta, theta, and gamma bands, when the patients that suffered from the post-mTBI pain at 6 m after the injury, were characterized by stronger delta and weaker theta and gamma connectivity of the EEG activity, tested at the acute phase (Figure 8).

Figure 8



Further analysis was performed for delta, theta and gamma bands, and included same parameters as for the analyses of acute phase (Figures 9, 10 and 11, respectively), presenting the ROC curve for each condition:

Figure 9 (delta band connectivity, 12m chronic pain classification)

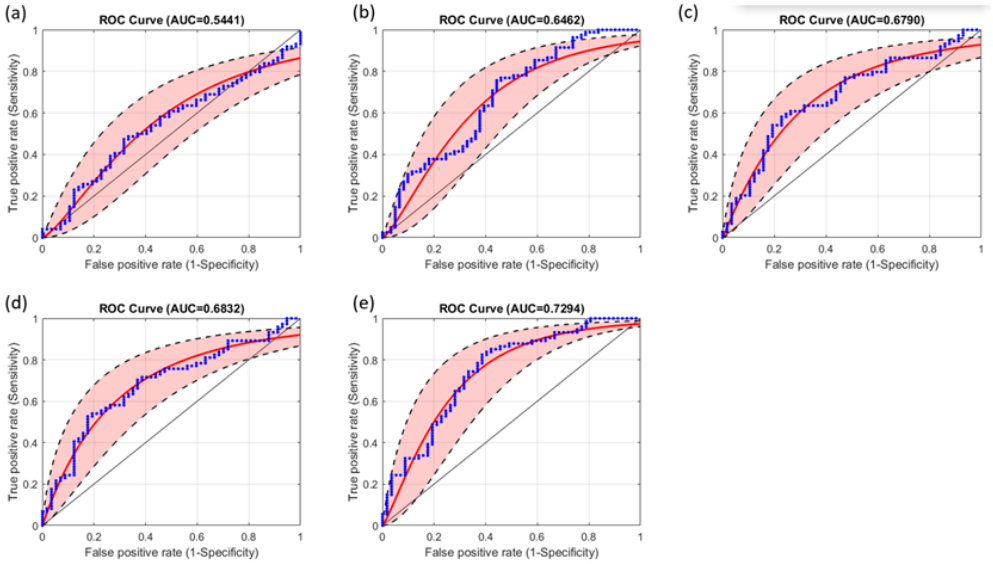


Figure 10 (beta band connectivity, 12m chronic pain classification)

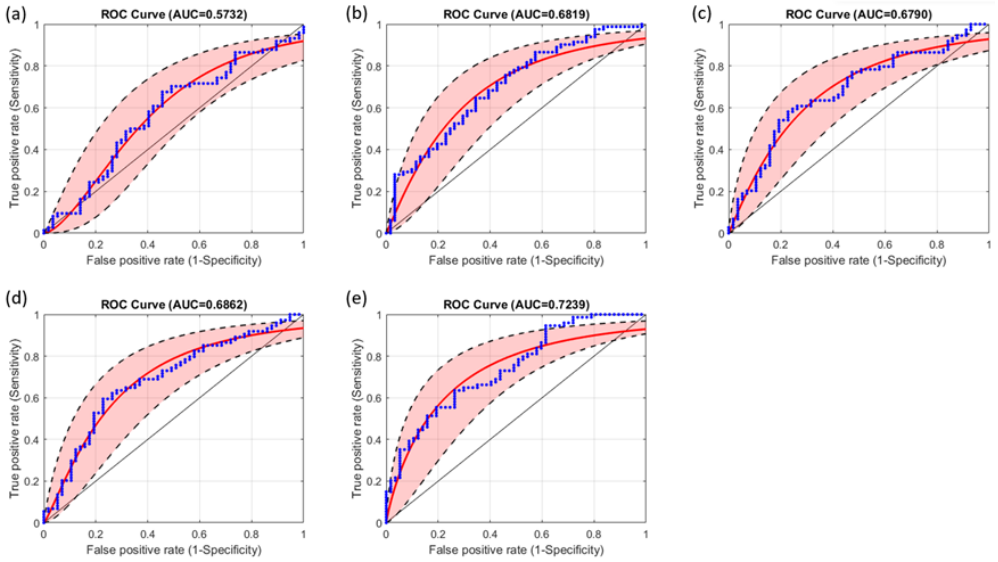
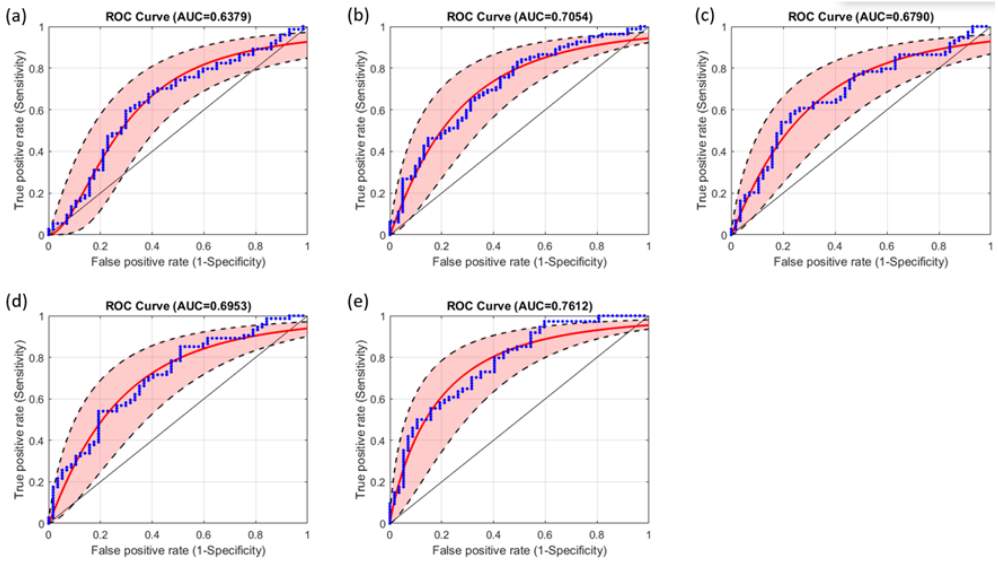


Figure 11 (gamma band connectivity, 12m chronic pain classification)



Frid A, Shor M, Shifrin A, Yarnitsky D, Granovsky Y. A Biomarker for Discriminating Between Migraine With and Without Aura: Machine Learning on Functional Connectivity on Resting-State EEGs. Ann Biomed Eng. 2020 Jan;48(1):403-412. doi: 10.1007/s10439-019-02357-3.

5. GENERAL STUDY CONCLUSIONS:

This study has explored several lines of investigation at the very early stage after mTBI, within 72 hours of the injury. The exploration included clinical, psychophysical, psychological, and neurophysiological lines, as well as imaging by MRI and genetics analysis. All lines found parameters that can predict chronification of pain out of data clollected at baseline. This includes certain psychophysical features mainly of the dynamic testing line, scores of pain catastrophyzing, connectivity of brain areas by EEG, specific areas inter-connection by MRI, and certain genetic state. At this point in time we have examined the combined predicting power of EEG with the psychophysical and psychological parameters, and found an added effect of the the lines, reaching a fairly high predicting ability. We expect to further improve the predicting ability by adding the structural and functional MRI data, as well as the genetic data.

6. CHANGES/ PROBLEMS

The COVID 19 crisis continues to gain momentum and affects all of us. In order to follow the general guidelines of the Israeli Ministry of Health, the Technion labs were closed, and the administration worked very partially. Consequently we couldn't run our protocol, and had to cancel all planned experimental sessions. We asked for two 1-year extensions of the grant period, which were approved by the funder.

7. Publications, Abstracts, and Presentations

Paper 1. "Psychophysical-psychological dichotomy in very early acute mTBI pain: A prospective study ". Kuperman P, Granovsky Y, Granot M, Bahouth H, Fadel S, Hyams G, Ben Lulu H, Aspis O, Salame R, Begal J, Hochstein D, Grunner S, Honigman L, Reshef M, Sprecher E, Bosak N, Sterling M, Yarnitsky D. **Neurology**. 2018 Sep 4;91(10):e931-e938. doi: 10.1212/WNL.0000000000006120. Epub 2018 Aug 1.

Paper 2. "Explaining very early acute mild traumatic brain injury after motor vehicle collision pain variability: additive value of pain sensitivity questionnaire" Pora Kuperman, MPH; Yelena Granovsky, PhD; Hany Bahouth, MD; Shiri Fadel, BSc; Hen Ben Lulu, RN; Noam Bosak, MD; Chen Buxbaum, MD, Elliot Sprecher, PhD; David Yarnitsky, MD, Michal Granot, PhD. **PAIN Reports**. 5(3):e821, May/June 2020.

Paper 3. "Head- and neck-related symptoms post-motor vehicle collision (MVC): Separate entities or two-sides of the same coin? " Pora Kuperman , Yelena Granovsky , Shiri Fadel , Noam Bosak , Chen Buxbaum , Rafi Hadad , Elliot Sprecher , Hany Bahouth , Hen Ben Lulu , David Yarnitsky , Michal Granot, **Injury,**2021 May.

Paper 4. "Dispositional and situational personal features and acute post-collision head and neck pain: Double mediation of pain catastrophizing and pain sensitivity". Granot, M, Srulovic E, Yarnitsky, D, Granovsky, Y, Kuperman, P. **PLOS ONE** (2021).

Abstract 1. Kuperman et al "Acute head pain, low socioeconomic status and less-efficient CPM predict post-whiplash chronic pain occurrence". The poster presentation was accepted to the 10th Congress of the European Pain Federation, EFIC in September 2017.

Abstract 2. Granovsky et al "Whiplash- associated pain chronification; the predictive role of resting stage EEG Alpha power and acute pain". The poster presentation was accepted to the 10th Congress of the European Pain Federation, EFIC in September 2017.

Abstract 3. Kuperman et al "Age as a predictive factor for post-mTBI pain chronification timeline". The poster presentation was accepted to the 2018 IASP in Boston.

Abstract 4. Kuperman et al "Post-mild Traumatic Brain Injury: Pain Chronification Timeline and Distribution". The abstract will be published in the Rambam Maimonides Med J 2018;10 (Suppl 1): 54

Abstract 5. "mTBI and Whiplash Disability Variance 6- Months Post- Motor Vehicle Collision Explained by Different Factors". Submitted by Kuperman et al.

Abstract 6. "Very-Early Acute Clinical Pain, Psychophysical Pain Sensitivity and Psychological Distress Can Predict Pain Behavior in mTBI Post-Collision Patients at One-Year Post-Injury". Submitted by Kuperman et al.

Abstract 7. "Additive Utility of Pain Sensitivity Questionnaire in Explaining Very Early Acute mTBI Post-Motor Vehicle Collision Clinical Head Pain Variability". Submitted by Kuperman et al.

Abstract 8. "Pain Sensitivity Mediates the Link Between Catastrophizing and Mild Traumatic Brain Injury Head Pain Following Motor Vehicle Collision". Submitted by Granot et al.

Abstract 9. "Very-Early Acute Pro-Nociceptive Pain Modulation Predicts Chronic Area-of-Injury Pain in mTBI Patients Six-Month Post Injury". Submitted by Cohen et al.

Abstract 10. "Psychological measures contribute to post-collision mild Traumatic Brain Injury head-related but not to neck-related disability" Rambam HealthCare Campus Research Day 2019.

Papers Submitted to:

1. Submitted to **PLOS Biology**. Structural brain connectivity predicts acute pain after mild traumatic brain injury. Paulo Branco; Noam Bosak; Jannis Bielefeld; Olivia Cong; Yelena Granovsky; Itamar Kahn; David Yarnitsky; A. Vania Apkarian.

Papers in Preparation:

1. Functional connectivity in early-acute mild traumatic brain injury predicts chronic pain. Noam Bosak, Paulo Branco, Pora Kuperman, Chen Buxbaum, Ruth Manor Cohen, Shiri Fadel, Ameer Lawen, Noam Saadon-Grossman, Rabab Zubeidat, Rafi Hadad, Yelena Granovsky, Apkar Vania Apkarian, David Yarnitsky and Itamar Kahn

8. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Prof. David Yarnitsky (Technion)
Project Role:	PI
Researcher Identifier:	ORCID ID: 0000-0002-2293-2090
Nearest person month worked:	3
Contribution to Project:	Prof. Yarnitsky has performed work in the area of supervising and advising all study activities mentioned above in section 1 "Accomplishments", in addition to recruitment of subjects.

Name:	Dr. Yelena Granovsky (Technion)
Project Role:	CI
Researcher Identifier:	Research gate name: Yelena Granovsky
Nearest person month worked:	1
Contribution to Project:	Dr. Granovsky completed the IRB submissions, HRPO submissions, stuff training to PhD students. Dr. Granovsky is responsible for analyzing the psychophysical and neurophysiological data collected in this study.

Name:	Prof. Michal Granot (Haifa University)
Project Role:	CI
Researcher Identifier:	ORCID ID: 0000-0002-5105-1209
Nearest person month worked:	1
Contribution to Project:	Prof. Granot is responsible for the work in the area of psychophysics and neurophysiology data analysis related to our study.

Name:	Prof. A Vania Apkarian (Northwestern University)
Project Role:	CI
Researcher Identifier:	ORCID ID: 0000-0002-9788-7458
Nearest person month worked:	1
Contribution to Project:	Prof. Apkarian approved the MRI protocol and scans. He is responsible for the work in the area of Imaging.

Name:	Dr. Luda Diatchenko (McGill University)
Project Role:	CI
Researcher Identifier:	ORCID ID: 0000-0002-1350-6727
Nearest person month worked:	1
Contribution to Project:	Dr. Diatchenko is responsible for all the work in the area of Genetic data related to our study.

Name:	Michele Sterling (Prof. Sterling changed her institution, and now works in the University of Queensland. She will prepare papers to be submitted to the DoD grant officer regarding the institution change)
Project Role:	CI
Researcher Identifier:	ORCID ID: 0000-0001-8242-2685
Nearest person month worked:	1
Contribution to Project:	Prof. Sterling is responsible for all the work in the area of psychological data related to our study.

Name:	Shiri Fadel (Technion)
Project Role:	Project administrator
Researcher Identifier:	
Nearest person month worked:	4
Contribution to Project:	Shiri is responsible for all the administrative work related to our study, HRPO submissions and communications, pain application development, purchases, FITBIR accounts, preparing all study documentations relates to the study, preparing study checklists for MRI team, ER team, pain team, working together with ER coordinators to identify new subjects.

Name:	Rabab Zubiedat
Project Role:	Project administrator
Researcher Identifier:	
Nearest person month worked:	10
Contribution to Project:	Rabab is responsible for all the administrative work related to our study, preparing all study documentations relates to the study, preparing study checklists for MRI team, ER team, pain team, working together with ER coordinators to identify new subjects.

Name:	Tzipora Miriam Kuperman (Technion)
Project Role:	PhD student
Researcher Identifier:	
Nearest person month worked:	4
Contribution to Project:	Tzipora is responsible for preparing all study documentations relates to the study, work together with the ER team, pain team, recruitment of subjects and performing study procedures.
Name:	Ruth Cohen (Technion)
Project Role:	PhD student
Researcher Identifier:	
Nearest person month worked:	12
Contribution to Project:	Ruth is responsible for preparing all study documentations relates to the study, work together with the ER team, pain team, recruitment of subjects and performing study procedures.

Name:	Shoshana Cristal (Technion)
Project Role:	PhD student
Researcher Identifier:	
Nearest person month worked:	3
Contribution to Project:	Shoshana assists Shiri with all study procedures and administrative tasks.

Name:	Dr. Noam Bosak (Rambam Health Care Campus affiliated to the Technion)
Project Role:	Study Physician
Researcher Identifier:	
Nearest person month worked:	8
Contribution to Project:	Dr. Bosak identifies potential patients in the ER, complete the recruitment procedure in the ER, as well as conduct the neurological assessments during 6 and 12 months visits.

Name:	Dr. Chen Buxbaum (Rambam Health Care Campus affiliated to the Technion)
Project Role:	Study Physician
Researcher Identifier:	
Nearest person month worked:	8
Contribution to Project:	Dr. Buxbaum identifies potential patients in the ER, complete the recruitment procedure in the ER, as well as conduct the neurological assessments during 6 and 12 months visits.

Name:	Elliot Sprecher (Rambam Health Care Campus affiliated to the Technion)
Project Role:	Statistician
Researcher Identifier:	ORCID ID: 0000-0001-8564-1090
Nearest person month worked:	1
Contribution to Project:	Elliot performs the statistics and advises the study team regarding the statistical analysis of the data collected in the study.

Name:	Dr. Alex Frid (Technion)
Project Role:	Post Doc
Researcher Identifier:	ORCID ID: 0000-0003-3487-9060
Nearest person month worked:	7
Contribution to Project:	Alex assists Dr. Granovsky with analysis of EEG data

Name:	Taha Abdullah (Northwestern University)
Project Role:	Technician
Researcher Identifier:	ORCID ID: 0000-0003-3373-7597
Nearest person month worked:	12
Contribution to Project:	Taha assists Prof. Apkarian with analysis of brain images. He downloads data provided from the Technion, performs data quality checks on a subset of the images using independent component analysis to identify general sources of noise.

Name:	Diane Rackziegel (Northwestern University)
Project Role:	Technician
Researcher Identifier:	
Nearest person month worked:	12
Contribution to Project:	Diana assists Prof. Apkarian with analysis of brain images

Name:	Rami Jabakhanji (Northwestern University)
Project Role:	Technician
Researcher Identifier:	ORCID ID: 0000-0002-9100-5071
Nearest person month worked:	12
Contribution to Project:	Rami assists Prof. Apkarian with analysis of brain images

Name:	Ryan Lichtenwalter, (McGill University)
Project Role:	Research Analyst

Researcher Identifier:	
Nearest person month worked:	4
Contribution to Project:	Under Dr. Diatchenko's supervision, Ryan was responsible for assisting with all aspects of the data cleaning and management, as well as data management related to the study.

Name:	Nancy Levesque, (McGill University)
Project Role:	Research Coordinator
Researcher Identifier:	
Nearest person month worked:	2
Contribution to Project:	Under Dr. Diatchenko's supervision, Nancy was responsible for assisting with all aspects related to the assays conducted on the samples of the study.

9. REPORTABLE OUTCOMES: see abstracts and papers below in appendix B.

10. OTHER ACHIEVEMENTS: see posters and papers below in appendix B.

11. REFERENCES: Mentioned in paper #1 appendix B.

12. APPENDICES:

Appendix A: Quad Chart
Appendix B: Papers and abstracts

Appendix A: Quad Chart

Why does acute post whiplash injury pain transform into chronic pain?
Multi-modal assessment of risk factors and predictors of pain chronification

MR130308; To construct a specific and sensitive tool for prediction and for understanding of the mechanisms relevant for transition from acute to chronic pain in mild traumatic brain injury / whiplash head and neck pain patients

Award Number: W81XWH-15-1-0603

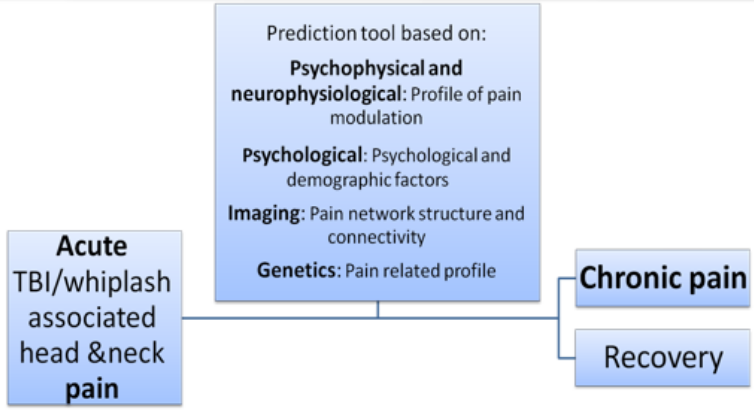
PI: David Yarnitsky **Org:** Technion – Israel Institute of Technology **Award Amount:** $1,499,904

Study Aim(s)

- Construction of a tool that predicts, based on parameters collected at time of entry into the study, the prognosis of mild traumatic brain injury (TBI)/whiplash related acute pain into either chronic pain or recovery
- Understanding of the processes that lead to chronification, based on data collected at entry, 6 months and 12 months after injury.

Approach

A prospective, non-intervening longitudinal study, assessing (i) relevant brain structure and connectivity (ii) neurophysiology and psychophysics, (iii) pain-related genetics, (iv) psychological and demographic parameters, for predicting the transition of acute head and neck pain due to mild TBI/whiplash into chronic pain.



Each of the parameters of pain modulation, brain structure and connectivity, pain genetics and psychological factors contributes to transition to chronic pain. We will combine them in one cohort of mild TBI to construct a specific and sensitive prediction tool for pain chronification

Timeline and Cost

Activities	CY	Y1- 16	Y2-17	Y3-18	Y4-19	Y5-20	Y6-21
Building experimental setup							
Patients recruitment							
Patients follow-up							
Interim and final data analysis							
Reports and papers preparation							
Estimated Budget ($K)		253	245	269	339	394	

Updated: (Oct 20,2020)

Goals/Milestones (Example)

CY16 Goal – Building experimental setup and start of recruitment

☑ Functionality tests of the equipment; study's personal training, starting of the patients recruitment, initiation of the data collection.

CY17-20 Goal – Data collection phase

☑ Experimental and clinical data collection including the follow-up, initial data analysis.

CY20-21 Goal – Completion of data collection and final data analysis

☑ Continuation and finalization of the data collection; data analysis

☑ Final statistical analysis, study report and papers preparation

Comments/Challenges/Issues/Concerns

Cohort will include civil populations.

Budget Expenditure to Date

Projected Expenditure: $1,499,904

Actual Expenditure: Around $1,317,325

Appendix B:

Paper #1:

Accepted for publication in Neurology attached to our email.
(NEUROLOGY/2017/871228)

“Psychophysical-psychological dichotomy in very early acute mTBI pain: A prospective study”.
Pora Kuperman, Yelena Granovsky, Michal Granot, Hany Bahouth, Shiri Fadel, Gila Hyams, Hen Ben Lulu, Osnat Aspis, Rabia Salame, Julia Begal, David Hochstein, Shahar Grunner, Liat Honigman, Maya Reshef, Elliot Sprecher, Noam Bosak, Michele Sterling, David Yarnitsky
Neurology. 2018 Sep 4.

The paper is attached in a PDF format, as well as available in the following link:

https://pubmed.ncbi.nlm.nih.gov/30068635/

Kuperman 2018.pdf

Paper #2:

"Explaining very early acute mild traumatic brain injury after motor vehicle collision pain variability: additive value of pain sensitivity questionnaire" Pora Kuperman, MPH; Yelena Granovsky, PhD; Hany Bahouth, MD; Shiri Fadel, BSc; Hen Ben Lulu, RN; Noam Bosak, MD; Chen Buxbaum, MD, Elliot Sprecher, PhD; David Yarnitsky, MD, Michal Granot, PhD. PAIN Reports. 5(3):e821, May/June 2020.

The paper is attached in a PDF format, as well as available in the following link:

https://pubmed.ncbi.nlm.nih.gov/32903910/

,.Kuperman et al pdf.2020

Paper #3:

Head- and neck-related symptoms post-motor vehicle collision (MVC): Separate entities or two-sides of the same coin?

Pora Kuperman, Yelena Granovsky, Shiri Fadel, Noam Bosak, Chen Buxbaum, Rafi Hadad, Elliot Sprecher, Hany Bahouth, Hen Ben Lulu, David Yarnitsky, Michal Granot, Injury,2021 May.

,.Kuperman et al INJURY.pdf 2021

Paper #4:

Dispositional and situational personal features and acute post-collision head and neck pain: Double mediation of pain catastrophizing and pain sensitivity.

Granot, M, Srulovic E, Yarnitsky, D, Granovsky, Y, Kuperman, P. PLOS ONE (2021).

PSQ and PCS as Dispositional and situa

Abstract #1:

ACUTE HEAD PAIN, LOW SOCIOECONOMIC STATUS AND LESS-EFFICIENT CPM PREDICT POST-WHIPLASH CHRONIC PAIN OCCURRENCE

Pora Kuperman, Yelena Granovsky, Michal Granot, Hany Bahouth, Shiri Fadel, Gila Hyams, Hen Ben Lulu, Osnat Aspis, Rabia Salama, Yulia Begal, David Hochstein, Shahar Grunner, David Yarnitsky

Background

Research has shown that 50% of individuals involved in mild car accidents (GCS 13-15) will suffer chronic pain.

Aim

To assess the relationship between acute head/neck pain, Quantitative Sensory Testing (QST) measures, and demographic data on chronic pain development 3 months post-accident.

Methods

Head/neck pain, static and dynamic QST measures, and demographic data were compiled within 72h post-accident, and taken into a logistical regression model to predict chronic post-traumatic pain occurrence. At 3-months 38 patients had follow-up data, 27 of which expressed clinically significant pain (VAS□30), and 11 not (VAS≤30).

Results

An overall logistical regression model was significant (p=0.020). Of the parameters included, acute head pain was significant (p=0.0345), with pressure pain threshold- conditioned pain modulation (PPT-CPM) and monthly salary evidencing trends (p=0.0524 and 0.0714, respectively).

A model based on these three measures was found to be significant (p<0.001). Acute head pain (p=0.002) and monthly salary (p=0.033) were significant, with higher pain values and low salary associated with greater likelihood of developing chronic pain. In this model, PPT-CPM did not maintain significance. However, when PPT-CPM is divided based on chronicity and compared to controls significance is found (p=0.004) with less-efficient CPM-PPT in chronic pain vs. controls (post-hoc p=0.003).

Conclusions

The occurrence of post-traumatic head/neck pain can be predicted by a combination of acute head pain and low monthly salary.

Independently, a pro-nociceptive pain modulation profile (PMP) as expressed by less-efficient PPT-CPM also influences chronic pain development.

Acknowledgment: Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #2:

WHIPLASH-ASSOCIATED PAIN CHRONIFICATION; THE PREDICTIVE ROLE OF RESTING-STATE EEG ALPHA POWER AND ACUTE PAIN

Yelena Granovsky, Pora Kuperman, Michal Granot, Hany Bahouth, Shiri Fadel, Gila Hyams, Hen Berkovich, Osnat Aspis, Rabia Salama, Yulia Begal, David Hochstein, Shahar Grunner, David Yarnitsky

Background and Aims. Acute pain intensity is an important factor for pain chronicity. Resting-state EEG alpha activity characterizes various pain states. Chronic post-traumatic pain is common after whiplash. We assessed the predictive value of acute headache/neck pain, and EEG alpha power on chronic whiplash pain intensity.

Methods. Head/neck pain and midline resting-state EEG were assessed within 72h after mild road accident. Thirty-eight patients (ages 19-67 yrs; 21 F) had follow-up data, and were determined as having clinically meaningful pain (>30 VAS; N=27) or no (N=11).

Results. Chronic head/neck pain group was characterized by higher acute head ($p<0.001$) or neck pain ($p=0.034$) scores, and by higher peak alpha power ($p=0.009$, Pz). In line, acute headache correlated with chronic headache ($r=0.479$; $p=0.003$); acute neck pain correlated with chronic neck pain intensity ($r=0.492$, $p=0.002$). Similarly, high peak alpha power was associated with higher chronic pain scores (Pz, $r=0.598$, $p=0.002$, head; $r=0.525$, $p=0.007$, neck). Regression model ($p=0.012$) including age and gender, confirmed the predictive effect of alpha power ($p=0.006$) but not acute headache ($p=0.102$) on chronic headache intensity. For the neck pain ($p=0.001$), both alpha power ($p=0.012$) and acute neck pain ($p=0.008$) predicted chronic pain intensity.

Conclusions. High EEG resting-state alpha power, possibly due to acute pain or stressful situation, predicts chronification of post-whiplash pain. Stronger contribution of acute neck pain and not headache to chronic pain intensity may suggest the primarily role of neck trauma in chronicity of whiplash.

Acknowledgment. Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #3:

Age as a predictive factor for post-mild Traumatic Brain Injury pain chronification timeline

Pora Kuperman, Noam Bosak, Yelena Granovsky, Michal Granot, Hany Bahouth, Shiri Fadel, Hen Ben Lulu, Avihu Marco, Chen Buxbaum, Elliot Sprecher, David Yarnitsky

Aim of Investigation: To assess post-whiplash mTBI head and neck pain evolution from the very early acute stage (<72h) to 1-year post-injury. This is of interest as status quo literature holds that those who will recover post-whiplash do so within 3-6 months post-injury, with up to 50% of individuals experiencing long term persistent pain, but offers no further delineation of pain change and/or progression within this time period. This time window may in fact be crucial for appropriate intervention to avert the time-course to chronicity, and as such should be investigated.

Methods: 116 mTBI patients (46F, age range 19-67, median age 35) underwent baseline QST testing, filled out pain-related psychological and demographic questionnaires and provided mean pain ratings for head and neck (0-100 NPS). Pain ratings were provided again at 3, 6, and 12 months post-injury. For analysis the patient group was split, by median age, in to young (19-35yr) and old (36-67yr) as it has been suggested that age might affect post-mTBI symptom development. A mixed model ANOVA for repeated measures tested the effect of month after the injury, gender, pain site (head/neck), age group (young/old), and the significant psychological parameters (Pain Catastrophizing Total and TIPI Agreeableness) on pain levels at months 1,3,6 and 12. Separate models were built to investigate the head-neck pain correlation at each of the time points, as well as the ability of head and neck pain at baseline to predict subsequent head and neck pain along the 1-year time axis.

Results: The ANOVA model was significant ($p <0.001$), where month from injury ($p <0.001$), gender ($p <0. 001$), pain site ($p =0.038$) and month*age group interaction ($p =0.006$) were significant components. Overall pain reduction is observed, where baseline pain is significantly higher than that of all subsequent timepoints; months 3,6, and 12, are statistically similar. Month by age group interaction shows that older

patients' pain stabilizes in the acute stage (month 1), whereas pain remains unchanged only at month 3 (chronic stage) for the younger group. Females express significantly more pain than males, and the neck is a significantly more painful site than head.

Head/Neck pain is always significantly correlated, with neck pain the significantly more dominant pain at months 1 (r=.68, p=0.004) and 3 (r=.63, p=0.037).

Separately, both head and neck pain at baseline are predictive of subsequent pain in those areas (head: month 1,3 p <0.001, month 6 p =0.012, month 12 p=0.016; neck month 1,3 p<0.001, month 6 p =0.006, month 12 p =0.048).

Conclusions: Overall post-whiplash mTBI patients express a reduction in self-reported head and neck pain from the time of their accident to 1-year following, where pain levels remain statistically unchanged from the 3-month mark, reinforcing this time window as crucial for pain intervention. Throughout the year patients continue to express more pain in the neck, and females remain with higher levels of pain. Interestingly, age does seem to affect symptom development, where those aged 36 and above enter their chronic level of pain already at 1-month post-accident, and those younger than that only at 3-months post-injury. Seeing as baseline pain values remain predictive of subsequent pain it reinforces the need to be attuned to patients' self-reported pain at the time of injury. That is to say, taken together, pain intervention for those above 35 years of age should take place within the first month post-accident with the hope of averting pain chronicity, while those younger than that seem to have a slightly longer preventative window.

Acknowledgment: Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #4:

Post-mild Traumatic Brain Injury: Pain Chronification Timeline and Distribution

Pora Kuperman, M.P.H.[1], Noam Bosak, M.D.[2], Yelena Granovsky, Ph.D.[1], Michal Granot, Ph.D.[3], Hany Bahouth, M.D.[4], Shiri Fadel, B.Sc.[2], Hen Ben-Lulu, R.N., M.A.[4], Avihu Marco, B.Sc.[1], Chen Buxbaum, M.D.[2], Elliot Sprecher, Ph.D.[2], and David Yarnitsky, M.D.[2]

[1]The Ruth & Bruce Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel; [2]Department of Neurology, Rambam Health Care Campus, Haifa, Israel; [3]Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel; and [4]Trauma Center, Rambam Health Care Campus, Haifa, Israel

Background: The 2004 WHO Collaborating Centre Task Force on mild traumatic brain injury (mTBI) reported that overall most patients recovered 3 months to 1-year post-injury. Additionally, the 2014 International Collaboration on mTBI Prognosis found that more than 50% of mTBI patients post-car accident still reported symptoms such as headache, neck pain, or sleep disturbances after 1-year. Current work, however, offers no further delineation of pain change and/or progression within this time period.

Study Aims: The aim of this study was to investigate area-of-injury pain distribution in two phases: very-early acute (<72 hours) and chronic phases (<12 months>, as well as the chronicity timeline of mTBI post-car accident participants.

Materials & Methods: The study cohort consisted of 103 consecutive patients (age range 19-67 years, 43F), recruited between March 2016 and September 2017, who provided pain scores at baseline and for at least 6 months post-accident.

A reciprocal time regression model was used. Distribution plots were calculated for head and neck pain at baseline and 12-months post-accident; 12-month distributions were found to be skewed with a long tail at higher pain levels.

Results: Visual examination of the model suggested pain stabilization occuring between months 2 and 4.

Head and Neck Pain distribution at baseline showed similar mean (head: 50.9±28.8; neck: 55.2±27.7) and median (head: 50; neck: 60) values, suggesting a more normally-distributed plot.

Both sites (n=84) had a median value of 0 at 12 months (NPS 0: head n=47, neck n=49). However, mean pain scores were clinically significant (>20, 0-100 NPS) (head: 25.6±33.8, neck: 23.9±32.5), whereas 27 individuals scored pain values of 50 to 100 (NPS) in their head and 25 in their neck, suggesting non-normally distributed plots (Shapiro-Wilk $p<0.001$).

Conclusions: Area-of-injury pain chronification in mTBI post-accident patients lends support to the latest guidelines for WHO's International Classification of Diseases, which holds that chronic pain is persistent or recurring pain lasting longer than 3 months. When examined in greater depth, although at 1-year 57% of the cohort was entirely pain-free, 72% of those who did have pain reported moderate-severe levels (≥ 50), creating what seems to be an "all or nothing" pain dichotomy. This distribution is unlike persistent post-surgical pain, wherein nearly 60% of patients are pain-free and only 40% of painful participants reported moderate-severe pain (≥30). One potential explanation for this difference rests on the traumatic nature of the mTBI injury, which can result in concurrent "polytrauma." This dichotomy warrants further investigation, particularly to determine factors that influence high area-of-injury pain at the 1-year juncture.

Acknowledgement: This research was supported by the US Department of Defense, Health Affairs Office, Grant No. 281XWH-15-1-0603.

Citation: Rambam Maimonides Med J 2018;10 (Suppl 1): 67

Abstract #5:

Title: MTBI AND WHIPLASH DISABILITY VARIANCE 6- MONTHS POST- MOTOR VEHICLE COLLISION EXPLAINED BY DIFFERENT FACTORS

Background and Aims:

Collision-related physical injuries are associated with negative physical and psychological sequelae which may result in long-term functional impairment and disability.

Study aim- to determine which psychological, psychophysical and clinical pain factors can explain head and neck disability variance at 6 months post-injury.

Methods:

53 mTBI post-MVC participants, with neck pain at baseline, participated in follow-up visit at 6m (age range 19-64, mean±SD 37 ±12, 24F).

Head pain, neck pain, painful body areas; static and dynamic QST measures, psychological and disability-related questionnaires (NDI, Rivermead Post Concussion, PTSD) amassed.

Initial correlation analysis performed between mean pain scores and relevant disability questionnaires. Correlation analysis also performed between disability measures and clinical, psychophysical and pain-related psychological factors.

Initial regression analysis performed separately for each group of factors (clinical, psychophysical, psychological) and disability measure. Significant factors then added to final regression model per disability measure.

Results:

Numerous initial significant correlations found with disability measures, e.g. number of painful body areas ($p<.001$) and stress ($p<.001$).

Final Regression Analysis found NDI variance (r=.87, p<.001) explained by neck pain (ß=.26, p=.035) and painful body areas (ß=.47, p<.001); Rivermead Head (r=.76, p<.001) by head pain (ß=.26, p=.033) and PTSD (ß=.34, p=.005); Rivermead General (r=.80, p<.001) by painful body areas (ß=.27, p=.018) and PTSD (ß=.37, p=.006).

Conclusion:
Post-whiplash (somatic) component of collision disability explained only by clinical factors, while post-mTBI component explained by both clinical and post-traumatic factors. Additionally, whole body pain strongly contributes to both forms of disability.

Acknowledgment:
Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #6:

Title: VERY-EARLY ACUTE CLINICAL PAIN, PSYCHOPHYSICAL PAIN SENSITIVITY AND PSYCHOLOGICAL DISTRESS CAN PREDICT PAIN BEHAVIOR IN MTBI POST-COLLISION PATIENTS AT ONE-YEAR POST-INJURY

Background and Aims:
12-month post-accident pain distribution demonstrates that although 60% of individuals are entirely pain-free, above 70% of painful individuals report moderate-severe levels (≥50), creating “all or nothing” pain dichotomy, one not seen at baseline.
Study aim - to determine if very-early acute pain-related personality features, pain modulation profile and clinical factors can explain this dichotomy.

Methods:
117 post-MVC patients with an mTBI were recruited and followed for 1-year. Patients split into 4 groups based on 12m pain levels in head and/or neck: (1) 0,0 (2) 1-49 in one or both (3) ≥50 in one (4) ≥50 in both sites
Head pain, neck pain, number of painful body areas; static and dynamic QST measures, and questionnaires compiled within 72h post-accident. Pain scores collected again at 12m.
Linear correlation performed for all groups, Mann-Whitney U Test performed between groups 1 and 4 to find explanatory factors for edge-group behavior.

Results:
Linear correlation found for painful body areas (2.92±1.3, 2.85±1.2, 3.47±1.9, 3.82±1.2; p=.004), head (43.75±30.6, 39.9±20.8, 51.0±32.1, 67.6±25.0; p= .001) and neck (49.0±30.0, 48.1±25.5, 57.1±30.2, 67.9±24.1; p=.006) pain, and pain50 temperature(45.6±3.2, 45.0±3.6, 44.4±3.2, 43.7±3.0; p=.018) and a trend for pressure-pain-threshold (3.3 ±2.0, 3.1±2.1, 2.9±1.4, 2.4±1.2; p=.054).
Mann-Whitney found significant differences in: painful body areas (p=.004), head (p=.002) and neck (p=.014) pain, pain50 (p=.005), PPT (p=.032), depression (p=.020), and stress (p=.038).

Conclusions:
Higher clinical pain, pro-nociceptive pain behavior, and psychological distress at baseline can predict pain non-recovery at 12m post-injury.

Acknowledgment:
Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #7:

ADDITIVE UTILITY OF PAIN SENSITIVITY QUESTIONNAIRE IN EXPLAINING VERY EARLY ACUTE MTBI POST-MOTOR VEHICLE COLLISION CLINICAL HEAD PAIN VARIABLITY

Background and Aims:
Quantitative Sensory Testing (QST) provides a current measure of nociceptive response. Pain Sensitivity Questionnaire (PSQ) reflects a trait-like cognitive representations of previous or expected pain experiences. The role of these measures in determining pain variability in acute situations is not well-explored.
Study aim - to determine the additive role of PSQ in expressing headache at very-early acute post-injury stage above using QST alone.

Methods:
Patients post-MVC with an mTBI (n=133) were recruited.
Head pain, neck pain, number of painful body areas; static and dynamic QST measures, and pain-related psychological questionnaires compiled within 72h post-accident.
Correlation analysis performed to determine relationship between PSQ and: 1) state personality measures 2) clinical and experimental pain.
Linear Regression Models built to examine factors contributing to the headache variance. Partial correlation analysis provided influence of each predictor on headache intensity.

Results:
PSQ and psychological measures (catastrophizing, depression and stress); clinical pain and psychophysical measures were correlated.
Regression model (R-squared=.160, $p<0.001$) showed high PSQ, enhanced mechanical TS and less efficient PPT-CPM explain elevated reports of headache. State features were not significantly correlated. Partial analysis showed strongest contribution provided by PSQ (partial R=.235), PPT-CPM (-.223), mTS (.199).
Neck pain model (R-squared .176, $p=0.072$) not significant.

Conclusions:
Appraisal of cognitive pain representations and imagined daily-life pain situations provides an additional trait-like facet to explain the variability in the clinical pain experience above and beyond assessing nociceptive responsiveness to experimentally-induced pain. Head and neck pain seem to have different cognitive representations.

Acknowledgment:
Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #8:

PAIN SENSITIVITY MEDIATES THE LINK BETWEEN CATTASTROPHYZING AND MILD TRAUMATIC BRAIN INJURY HEAD PAIN FOLOWWING MOTOR VEHICLE COLLISION

Background: Understanding the variability of mTBI head-pain consequent to motor vehicle collision (MVC) is integral for determining proper acute and long-term intervention. Recent publication point to catastrophizing as a key cognitive factor in pain perception. However, it is also important to consider other cognitive features that may not be reflected by the pain catastrophizing scale (PCS) such as the pain sensitivity questionnaire (PSQ) which addresses daily life potentially painful situations.

Aim: To investigate the manner in which PSQ alongside PCS affects head pain intensity at the very early stage of mTBI.

Methods: 117 mTBI post-MVC patients (n=133, 55F) were assessed for head and neck pain intensity, PCS and PSQ within the 72-h after accident. The association between these two cognitive features and pain intensity were explored using correlation analyses and Hayes mediation model.

Results: No correlation was observed between level of PCS and head or neck pain intensity. However, the mediation model showed that the association between PCS and headache is fully mediated by PSQ (R^2=.129, p=0.006), demonstrating that without the PSQ there is no direct association between catastrophizing and head pain. Age or gender were not significant factors.
Conclusion: The PSQ, which represents trait-like memory and imagined facets of pain, offers insight into the cognitive representation dimension of pain experience. Using both PCS and PSQ together may add significant contribution to the construction of the individual post-accident pain evaluation and may be relevant to clinical setting.

Acknowledgment:
Supported by US Department of Defense, Health Affairs Office, No. W81XWH-15-1-0603

Abstract #9:

VERY-EARLY ACUTE PRO-NOCICEPTIVE PAIN MODULATION PREDICTS CHRONIC AREA-OF-INJURY PAIN IN MTBI PATIENTS SIX-MONTH POST INJURY

Background and aims: 20-30 million people worldwide are involved in traffic collisions. Up to 50% will suffer from chronic pain, which has enormous personal, social and financial cost. Previous research has found that static QST testing in whiplash patients can predict occurrence of chronic pain.
Study aim - To identify improved, more accurate, very early-acute post-collision psychophysical predictors for chronic pain among mTBI patients with neck pain at baseline.

Methods: 66 post-MVC (age range 19-67, 25F) patients with an mTBI were recruited and followed for6 months. Scores of head pain, neck pain, static and dynamic QST measures were compiled within 72h post-accident. Pain scores collected again at 6m. Linear correlation performed between patients mean area-of-injury pain ratings at 6 month and CPM-related psychophysical correlates: a.) Pain50 temperature (the temperature which participants defined as pain of 50 on scale of 0-100) b.) Average of 30 phasic heat stimuli pain scores standalone c.) Averaged pain score of 30 phasic heat stimuli while under conditioning (cold water immersion) d.) CPM value (the difference between c&d)

Results: Higher 6m pain was associated with lower Pain 50 temperature ($r=0.27$, $p=0.026$), higher heat pain magnitude tested under conditioning ($r=0.32$, $p=0.009$) and less-efficient CPM ($r=0.28$, $p=0.021$).

Conclusions: Pro- nociceptive pattern of pain modulation at the very-early acute post-accident stage can predict chronic mTBI-associated pain. In that, functional evaluation of pain inhibitory control holds the potential for a useful predictive tool for pain chronification.

Acknowledgments: Supported by US Department of Defense, Health Affair s Office, No. W81XWH-15-1-0603

Abstract #10:

Psychological measures contribute to post-collision mild Traumatic Brain Injury (mTBI) head-related but not to neck-related disability

Pora Kuperman [1], Noam Bosak[1,2], Chen Buxbaum[2,3], Yelena Granovsky [1,2], Michal Granot[3], Hany Bahouth[2], Hen Ben Lulu[2], Shiri Fadel[2], Elliot Sprecher[2,3], David Yarnitsky [1,2]
[1]The Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel
[2]Neurology Department, Rambam Healthcare Campus, Haifa, Israel [3]Trauma and Emergency Center, Rambam Healthcare Campus, Haifa, Israel
[4]Faculty of Welfare and Health Sciences, University of Haifa, Haifa, Israel

Background and Aims

Chronic motor vehicle collision (MVC) resultant injury comprises physical and psychological sequelae, which may result in long-term functional impairment and disability.

Post-collision disability is often defined by the type of physical injury incurred, either whiplash associated disorder (WAD), or mild traumatic brain injury (mTBI), with WAD classically receiving the most research attention.

Although symptoms are overlapping, WAD and mTBI are assigned their own tools. Neck Disability Index (NDI) addressing post-whiplash and Rivermead Post Concussion (RPQ) mTBI-related disability.

This study aimed to examine the interplay between head and neck pain, and head- and neck-related disabilities, in a cohort of patients with an mTBI 6 months post-MVC, in order to explore whether, and to what extent, clinical, psychophysical, and psychological factors, can explain these disabilities.

Methods

53 mTBI post-MVC participants returned for a follow-up visit (age range 19-64, mean±SD 37 ±12.1, 24♀).

Study cohort primarily met mTBI criteria, but also defined as whiplash as they reported neck pain on the day of baseline testing.

Collected Measures

Patient and Clinician Administered Clinical Pain Assessment:
Mean Level of Pain in the Head and Neck (NPS), Post-Accident Areas of Residual Pain, Hebrew Validated NDI, Neurological Examination and RPQ (two sub-scores RPQ-3 (RPQ Head) and RPQ-13 (RPQ General)). Sub-scores investigated separately to provide more comprehensive evaluation.
Pain-Related Psychological Assessment:
Hebrew Validated versions of Pain Catastrophizing Scale (PCS), Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale (PSS) and Post Traumatic Stress Disorder (PTSD, DSM-IV Version).
Partial QST Protocol:
Pain50 temperature, electrical temporal summation (eTS), mechanical TS, pressure pain threshold- conditioned pain modulation (PPT-CPM) Heat-CPM

Statistical Analysis

Simple correlations between mean pain scores and disability questionnaires.

Correlation analysis between disability measures and clinical, psychophysical and pain-related psychological factors.

Initial regression analysis performed within each pain sub-group.

Significant predictors combined across subgroups in secondary regressions.

Results

Follow-up Values for Clinical Pain and Disability

Patients reported median head pain 10 (IQR: 0 to 90 NPS) and neck pain 20 (IQR: 0 to 80 NPS).

NDI: 18/ 53 (34%) recovered (0-8%), 20 (37.7%) mild levels of disability (10-28%) and 15 (28.3%) moderate/severe levels of disability (>30%) (as per Sterling's NDI classification).

RPQ: 18/ 53 (34%) completely recovered (total score of 0) vs. 18 (34%) diagnosis of Post-Concussion Syndrome (PCS) .

Strong correlations between NDI and RPQ Scores (r=.65, p<0.001 for RPQ- 3 and r=.64, p<0.001 for RPQ-13), with only partial overlap:
15 (28.3%) participants showed complete recovery (head and neck)
15 (28.3%) disability in both
11 (20.8%) diagnosed with PCS and self-reported no-mild levels of neck disability.
12 (22.6%) no diagnosis of PCS but self-reported neck disability.

Correlation between Disability and Area-of-Injury Pain

Moderate-strong correlations: neck pain and NDI (Fig. 1), head pain and RPQ-3 (Fig. 2a), head pain and RPQ-13 (Fig. 2b).

Head and neck pain also moderately cross-correlated: head pain and NDI (r=.53, p<0.001), neck pain and RPQ-3 (r=.51, p<0.001), neck pain and RPQ-13 (r=.36, p=0.008).

Fig 1. Neck Pain Mean by NDI

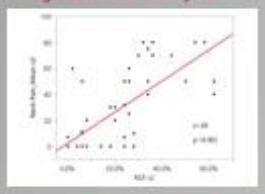


Fig 2a. Head Pain Mean by RPQ_3

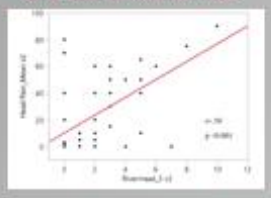
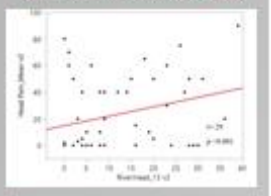


Fig 2b. Head Pain Mean by PRQ_13



Correlation between Disability and Clinical, Psychological, and Psychophysical Variables

Numerous correlations between disability measures and clinical, psychophysical and pain-related psychological factors. Several correlated to all three, e.g. number of painful body areas (p<0.001) and stress (p<0.001).

Age and sex not correlated with disability level.

Regression Analysis for Disability Separately by Sub-Groups

Regression analysis to determine which factors were most strongly correlated found that only number of painful body areas remained strongly correlated with all three measures.

Secondary Regression Models to Explain Disability

NDI variance (r=.87, p<0.001) explained by: neck pain (ß=.26, p=0.035) and painful body areas (ß=.47, p<0.001)

Rivermead Head (r=.76, p<0.001) explained by: head pain (ß=.26, p=0.033) and PTSD (ß=.34, p=0.005)
Rivermead General (r=.80, p<0.001) explained by: painful body areas (ß=.27, p=0.018) and PTSD (ß=.37, p=0.006)

Regression showed that variance of neck-related disability sufficiently explained by clinical pain measures. In contrast, variance of post-mTBI disability requires both clinical pain reports and level of self-reported PTSD symptoms.

Conclusions

It would appear that different mechanisms control head- and neck-related disability in a cohort of patients with an mTBI 6-months post-MVC. Where the post-whiplash (somatic) component of collision disability is explained only by clinical factors, while the post-mTBI component is explained by both clinical and post-traumatic (affective) factors.

Additionally, whole body pain strongly contributes to both forms of disability and should be more heavily studied among post-collision individuals.

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