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## 1. CONSORT checklist

Table S1: CONSORT 2010 checklist of information to include when reporting a randomised trial\*

Section/Topic	Item No	Checklist item	Reported on page No
<b>Title and abstract</b>			
	1a	Identification as a randomised trial in the title	N/A
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 4
<b>Introduction</b>			
Background and objectives	2a	Scientific background and explanation of rationale	Pages 5 & 6
	2b	Specific objectives or hypotheses	Pages 7 & 8
<b>Methods</b>			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Page 8
Participants	4a	Eligibility criteria for participants	Page 9
	4b	Settings and locations where the data were collected	Page 8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Pages 9 – 12
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Pages 10 – 12

	6b	Any changes to trial outcomes after the trial commenced, with reasons	Page 8
Sample size	7a	How sample size was determined	Page 15
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	N/A
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	N/A
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	N/A
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N/A
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Page 14
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Pages 15 & 16
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Pages 15 & 16
<b>Results</b>			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Page 16

	13b	For each group, losses and exclusions after randomisation, together with reasons	Page 16
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 8
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Page 17
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Page 17
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Pages 18 – 20
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Page 21
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Supplementary file: Table S5
<b>Discussion</b>			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Page 26
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Pages 25 & 26
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Pages 23 & 24
<b>Other information</b>			

Registration	23	Registration number and name of trial registry	Page 8
Protocol	24	Where the full trial protocol can be accessed, if available	Page 8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Page 2

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Citation: Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Medicine. 2010;8:18.

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\*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist, see [www.consort-statement.org](http://www.consort-statement.org).

## 2. Protocol deviations

*Table S2: Summary of protocol deviations and justifications for the deviations.*

Protocol deviation number	Stated in the protocol	Deviation from the protocol	Justification(s) for deviation
1	We will determine the area under the curve formed by the three estimates of surface area over time since HFS, as the dependent variable for hypothesis 3. The within-participant peak of surface area of secondary hypersensitivity provides insight into the peak severity of secondary hypersensitivity; therefore, we will also compute and report this at the group level.	We included all three measures of surface area across the three timepoints for each participant in our statistical analysis.	We consulted a statistician who informed us that using area under the curve in this study has 3 limitations: (i) it reduces the intra-individual variability in the data, (ii) it reduces the power because of the removing the repeated measures, and (iii) area under the curve is not a stable measurement for just three repeated measures.
2	We calculate the combined arithmetic mean for the three sets of the two pinprick stimuli to obtain a stable estimate of baseline sensory ratings to mechanical punctate stimulation. The magnitude of secondary hypersensitivity will be calculated for each post-HFS time point by subtracting ratings before HFS from each rating after HFS. Again, we will determine the area under the curve formed by the three estimates of magnitude of secondary hypersensitivity over time since HFS, as the dependent variable for hypothesis 3. The within-participant peak magnitude of secondary hypersensitivity provides insight into the peak severity of secondary	We included ratings for each stimuli, at baseline and all three follow-up points for each participant in our statistical analysis.	We consulted a statistician who informed us that using area under the curve in this study has 3 limitations: (i) it reduces the intra-individual variability in the data, (ii) it reduces the power because of the removing the repeated measures, and (iii) area under the curve is not a stable measurement for just three repeated measures.

	hypersensitivity; therefore, we will also compute and report this at the group level.		
3	Broken blinding will be assessed using James' Blinding Index.	Broken blinding will be assessed using chi-squared test.	
4	PRK (data analyst) will remain blinded to natures of the 3 groups to undertake blinded model specification, assessment, and interpretation of the best fitting models. To achieve this, VJM will use a script that is stored outside the analysis repository, out of access to PRK, before formal statistical analyses, and PRK will have no access to preliminary data analysis files or discussion of the preliminary analysis. PRK will choose, assess, and confirm the final statistical models and provide conclusions about each study hypothesis, and this document will be locked online, before PRK is unblinded for further discussion and more detailed interpretation of the study findings.	During data analysis, the data analyst was unblinded participants' CA group allocations.	It was deemed unnecessary for the data analyst to be blinded to group allocation because CA group allocation was merely used to ensure recruitment of a cohort representing a range of CA history. CA group status was not included in any of the statistical analyses.

### 3. Estimates of cytokine levels

To estimate the levels of IL-1b and IL-6, we undertook the following calculations to obtain a standard curve and estimate the observed values for each panel in a reproducible manner. First, the background fluorescence was subtracted from the fluorescence observed to obtain the net fluorescence of each well. Second, we used the standards data to obtain an appropriate standard curve. To obtain an initial standard curve, we fitted a quadratic model to each standard, using expected concentrations (dependent variable) and net observed fluorescence (independent variable). We assessed the accuracy of this model by plotting observed vs expected standards data against a line showing model-predicted values, on both a linear scale and a log-log scale. Poor fit was apparent at the lower end of the range. We assessed this further by calculating the model-predicted values for each standard value, and the

%CV between the model-predicted values and observed values. The %CV increased as the values dropped, showing poor model fit towards the lower end of the range.

To address this systematic bias in model fit, we defined a weight for each analyte and each standard as the inverse of the expected concentration (e.g. weightIL6 = 1/(expected-valueIL6)) and then fitted a quadratic model as before, but now with weighting that increased the influence of each data point in inverse proportion to its expected value. We assessed the accuracy of this weighted quadratic model by plotting observed vs expected data against a line showing model-predicted values, on both a linear scale and a log-log scale. The fit was better at the lower end of the range, although the %CV between the model-predicted values and observed values still increased (but less dramatically) as the values dropped. The weighted quadratic model was carried forward as the standard curve.

Third, we used our weighted quadratic model (standard curve) to predict the true values of the test samples from the observed net fluorescence values. Estimates that fell outside the bounds of the expected range for the panel were flagged as “out of range” and are reported for each analyte (see Results). Given that the fitted model suggested linearity of the data, we interpolated new values for each samples flagged as ‘out of range’ as follows:

- For values above the expected range, we used the net fluorescence value at the maximum of the expected range (i.e. net fluorescence for Standard 1) plus half the difference between the highest (S1) and second-highest (S2) standards.
- For values below the expected range, we used the net fluorescence values at the minimum of the expected range (i.e. predicted value for Standard 6) minus half the difference between the lowest (S6) and second-lowest (S5) standards.
- We then used the weighted quadratic model to predict the values at each of these net fluorescence values. Finally, the mean of the two predicted values was calculated and then multiplied by 30, to account for the sample dilutions.

#### **4. Method for determining individual detection threshold for the high-frequency electrical stimulation**

The individual detection threshold was determined using an established calibration process in which single electrical stimuli (pulse width 2 ms) were delivered with an adaptive staircase method, starting from zero and increasing in 0.1 mA increments (steps) until the participant reported feeling the electrical stimulus (usually felt as a “tiny prick”) at the cathode site. Next, the intensity was decreased by steps of half the previous step size (i.e. 0.05 mA) until the participant reported no longer feeling the electrical stimulus. Next, the intensity was increased by steps of half the previous step size (i.e.

0.025 mA) until the participant reported feeling the electrical stimulus again. This final reporting of feeling the electrical stimulus was used as the individual detection threshold.

## 5. Outcome measures for potential confounders

### 5.1 Positive childhood experiences

Participants reported on positive childhood experiences using the Positive Childhood Experiences Questionnaire [57]. This questionnaire has seven items to which participants endorsed answers using a 5-point Likert scale (0 – never; 1 – rarely; 2 – sometimes; 3 – often; 4 – very often). The total score was computed as the sum of all seven item scores. Positive childhood experiences may buffer the effects of adverse childhood experiences [57]. We tested whether score on the positive childhood experiences questionnaire influenced the relationship childhood adversity and inflammatory reactivity.

### 5.2 Long-term stress

Participants completed the 10-item Perceived Stress Scale [58] by rating each item (e.g. *In the last month, how often have you been able to control the irritations in your life*) on a 5-point Likert scale (1 – never; 2 – almost never; 3 – sometimes; 4 – fairly often; 5 – very often). The total score was computed as the sum of all 10 item scores. Long-term stress alters inflammatory reactivity, as seen by high levels of IL-6 in people exposed to chronic stress [59-65]. Further, individuals exposed to long-term stress of caring for a spouse with dementia showed lower levels of IL-2 after influenza vaccine immune provocation than age- and sex-matched controls [66]. Therefore, we tested whether long-term stress was a potential confounder of inflammatory reactivity, surface area and magnitude of secondary hypersensitivity, CPM, and/or TS.

### 5.3 Depression and anxiety

We screened for a lifetime history of diagnosed major depressive disorder, as well as screened for current symptoms of depression and anxiety using the well-validated and reliable Patient Health Questionnaire-4 [67]. This questionnaire has 4-items to which answers were endorsed using a 4-point Likert scale (0 – not at all; 1 – several days; 2 – more than half the days; 3 – nearly every day). The sum of the first two items screen for anxiety and the sum of the last two items screen for depression (>3 is considered positive for either of these subscales). Depression alters inflammatory reactivity, as seen by a meta-analysis of data from 3212 people reporting higher levels of IL-6 and TNF- $\alpha$ , as well as other cytokines, in peripheral blood of people with major depressive disorder, than in healthy controls [68]. Anxiety is also associated with higher levels of IL-6 and TNF- $\alpha$  [69]. Therefore, we tested whether depression and/or anxiety (total screening score on Patient Health Questionnaire-4, and

previous diagnosis of major depressive disorder) were potential confounders of inflammatory reactivity, surface area and magnitude of secondary hypersensitivity, CPM, and/or TS.

#### **5.4 Asthma**

Participants self-reported history of diagnosed asthma. Asthma alters inflammatory reactivity, as seen by preliminary, unpublished data from our research team. Further, asthma history is associated with reduced levels of the largely anti-inflammatory cytokine IL-10 [70] in LPS-stimulated blood culture, and asthma severity is negatively associated with levels of IL-10 [71]. Therefore, we tested whether diagnosed asthma was a potential confounder of inflammatory reactivity.

#### **5.5 COVID-19 infection**

Participants self-reported history of COVID-19 infection in the six months preceding participation. In participants with a history of COVID-19 infection, we collected data on the timing and severity of known COVID-19 infection and severity of long-COVID symptoms. Recent COVID-19 infection may alter inflammatory reactivity, and could confound interpretation of IL-6 and TNF- $\alpha$  levels in stimulated and unstimulated blood samples. COVID-19 infection stimulates a cytokine-driven inflammatory response that can build into a ‘cytokine storm’ with profound and lasting effects [72, 73]. Further, persistent symptoms after acute COVID-19 infection, termed “long-COVID” are associated with pro-inflammatory cytokine activation [74, 75]. Therefore, history of COVID-19 infection may be associated with inflammatory reactivity. Unfortunately, we were unable to account for undiagnosed COVID-19 infection, which is suspected to be common although the inflammatory consequences are unknown. We tested whether COVID-19 infection was a potential confounder of inflammatory reactivity.

#### **5.6 Chronic and recent acute illnesses**

Participants self-reported chronic illnesses and any recent acute infections in the six months preceding participation, and we tested whether chronic illnesses (e.g. HIV) and recent acute illnesses (e.g. influenza) was a potential confounder of inflammatory reactivity.

#### **5.7 Sleep**

Participants reported sleep quality using the Pittsburgh Sleep Quality Index [76], which consists of 19 self-rated items, the sum of which provides a total score out of 21. Sleep deprivation and/or disturbance can alter inflammatory and neural reactivity, as shown by data from humans under resting and challenged states. A large meta-analysis of data from 3000 participants reported a strong positive association between recent sleep disturbance and circulating IL-6 levels [77]. Additionally, poor sleep quality was associated with higher levels of IL-6 after *in vivo* stimulation using LPS in African

American women [78]. Further, one night of sleep deprivation increased the area of experimentally induced secondary hyperalgesia in healthy male, but not female, subjects [79]. Therefore, we tested whether sleep deprivation (total score out of 21) was a potential confounder of inflammatory reactivity, surface area and magnitude of secondary hypersensitivity, CPM, and/or TS.

## 6. Sensitivity power analysis

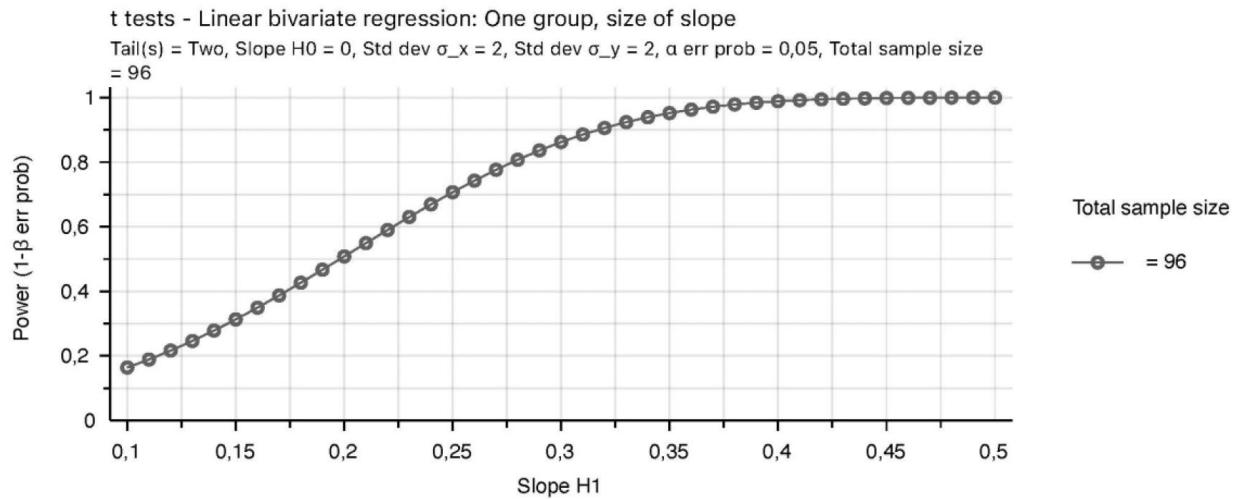


Figure S1: **Sensitivity power analysis** to estimate that our final sample size ( $n=96$ ) provided a priori power to detect an effect size of 0.275 with power 0.8 and alpha 0.05.

## 7. Descriptive statistics

Table S3: Descriptive statistics

Characteristics	Full sample N = 96	History of mild childhood adversity (CTQ-SF score: 25 – 36) N = 32	History of moderate childhood adversity (CTQ-SF score: 37 – 67) N = 32	History of severe childhood adversity (CTQ-SF score: 68 – 125) N = 32	p-value for between- group differences
<b>Positive childhood experiences</b> (Positive Childhood Experiences Questionnaire) <sup>‡</sup>	15.9 ( $\pm$ 6.6)	22.1 ( $\pm$ 5.2)	18.6 ( $\pm$ 3.8)	11.3 ( $\pm$ 4.8)	<0.001
<b>Long-term stress</b> (Perceived Stress Scale) <sup>§</sup>	31.0 (28.0 – 33.0)	30.0 (28.8 – 32.5)	30.0 (28.0 – 33.0)	32 (29.8 – 35.3)	0.32
<b>Depression and anxiety</b>					
Diagnosis of major depressive disorder	7 (7.29%)	2 (6.3%)	1 (3.1%)	4 (12.4%)	-
Patient Health Questionnaire-4 <sup>¶</sup>	4.0 (1.0 – 6.0)	1.5 (0.0 – 5.0)	2.0 (1.0 – 4.0)	5.0 (3.0 – 8.0)	0.01
Depression subscale <sup>#</sup>	2.0 (0.0 – 3.0)	0.0 (0.0 – 2.3)	1.0 (0.0 – 2.0)	2.0 (1.0 – 4.0)	0.02
Anxiety subscale <sup>#</sup>	2.0 (1.0 – 3.3)	1.5 (0.0 – 2.0)	2.0 (1.0 – 2.0)	3.0 (2.0 – 4.0)	0.04
<b>Diagnosis of asthma</b>	4 (4.2%)	1 (3.1%)	2 (6.3%)	1 (3.1%)	-
<b>COVID-19 infection in preceding 6 months</b>	7 (7.29%)	3 (9.4%)	4 (12.4%)	0	-
<b>Chronic and recent acute illness</b>					
Diagnosed chronic illness(es)	11 (11.46%)				

	HIV n = 5  Hypertension n = 2  Polycystic ovarian syndrome n = 2  Chronic allergic rhinitis n = 1  Inverse psoriasis n = 1				
Recent acute illness(es) in preceding 6 months	56 (58.3%)	20 (62.5%)	18 (56.2%)	18 (56.2%)	0.84
Sleep (Pittsburgh Sleep Quality Index)**	7.0 (5.0 – 10.0)	5.0 (3.8 – 9.3)	6.5 (5.0 – 10.0)	8.5 (5.8 – 11.3)	0.095
<b>Self-reported side effects of influenza vaccine</b>					
Participants reporting any side effects	48 (50.0%)	19 (59.4%)	15 (46.9%)	14 (43.8%)	0.42
List of side effects	Pain at the vaccination site n = 39 (40.6%)  Fatigue n = 12 (12.5%)  Headache n = 11 (1.5%)  Flu-like symptoms n = 5 (5.2%)  Body aches n = 6 (6.3%)  Pain in axilla ipsilateral to vaccine site n = 2 (2.1%)  Swollen axillary lymph nodes ipsilateral to vaccine site n = 2 (2.1%)  Chest pain n = 1	Pain at the vaccination site n = 17 (53.1%)  Fatigue n = 2 (6.3%)  Headache n = 4 (12.5%)  Flu-like symptoms n = 2 (6.3%)  Body aches n = 1 (3.1%)  Swollen axillary lymph nodes ipsilateral to vaccine site n = 2 (6.3%)  Chest pain n = 1	Pain at the vaccination site n = 12 (37.5%)  Fatigue n = 6 (18.8%)  Headache n = 4 (12.5%)  Flu-like symptoms n = 1 (3.1%)  Body aches n = 3 (9.4%)  Pain in axilla ipsilateral to vaccine site n = 2 (6.2%)	Pain at the vaccination site n = 10 (31.2%)  Fatigue n = 4 (12.5%)  Headache n = 3 (9.4%)  Flu-like symptoms n = 2 (6.3%)  Body aches n = 2 (6.2%)  Pain in axilla ipsilateral to vaccine site n = 2 (6.2%)	-

	Night sweats n = 1  Dry mouth n = 1  Moody n = 1  Fever n = 1  Stomach ache n = 1  Muscle twitches n = 1		Night sweats n = 1  Dry mouth n = 1  Moody n = 1  Fever n = 1  Stomach ache n = 1  Muscle twitches n = 1	
Overall rated severity of side effects <sup>††</sup>	1.9 ( $\pm 1.1$ )	1.6 ( $\pm 0.9$ )	2.0 ( $\pm 1.1$ )	2.3 ( $\pm 1.1$ )

<sup>‡</sup> 49 (of 96) participants completed the Positive Childhood Experiences Questionnaire; possible total score range: 0 – 28

<sup>§</sup> Possible total score range for the Perceived Stress Scale: 10 – 50

<sup>¶</sup> 48 (of 96) participants completed the Patient Health Questionnaire-4; possible total score range: 0 – 12

<sup>#</sup> Possible total score range for the depression and anxiety subscales of the PHQ-4: 0 – 6

<sup>\*\*</sup> Possible total score range for the Pittsburgh Sleep Quality Index: 0 – 21

<sup>††</sup> Possible score range for rated severity of side effects: 5-point Likert scale, where 1 = “very mild and 5 = “very severe”)

Data are presented as median (IQR), mean ( $\pm SD$ ), or n (%)

## 8. Manipulation checks

*Table S4: Summary of the main effect of condition (before vs during cold water immersion) on pressure pain threshold (PPT) at each test site. PPT = pressure pain threshold.*

<i>Predictors</i>	Main effect of condition on PPT at lumbar site			Main effect of condition on PPT at deltoid site		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	50.02	44.90 – 55.14	<0.001	28.79	25.47 – 32.12	<0.001
During cold water immersion	20.49	17.80 – 23.17	<0.001	13.08	11.37 – 14.80	<0.001
<b>Random Effects</b>						
$\sigma^2$	179.33			73.35		
$\tau_{00}$	560.77	study_id		237.49	study_id	
ICC	0.76			0.76		
N	96	study_id		96	study_id	
Observations	384			384		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.124 / 0.788			0.121 / 0.793		

*Table S5: Summary of main effect of condition (1<sup>st</sup> vs 16<sup>th</sup> stimulation) on SPARS ratings to mechanical stimuli.*

<i>Predictors</i>	Main effect of condition on SPARS rating at lumbar site			Main effect of condition on SPARS rating at deltoid site		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-27.33	-31.19 – -23.46	<0.001	-30.74	-34.34 – -27.14	<0.001
16th mechanical stimulation	9.49	6.99 – 12.00	<0.001	8.57	6.26 – 10.88	<0.001
<b>Random Effects</b>						
$\sigma^2$	155.53			132.50		
$\tau_{00}$	288.07	study_id		251.02	study_id	
ICC	0.65			0.65		
N	96	study_id		96	study_id	
Observations	383			384		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.048 / 0.666			0.046 / 0.670		

## 9. Hypothesis 1: relationship between childhood adversity and provoked cytokine expression

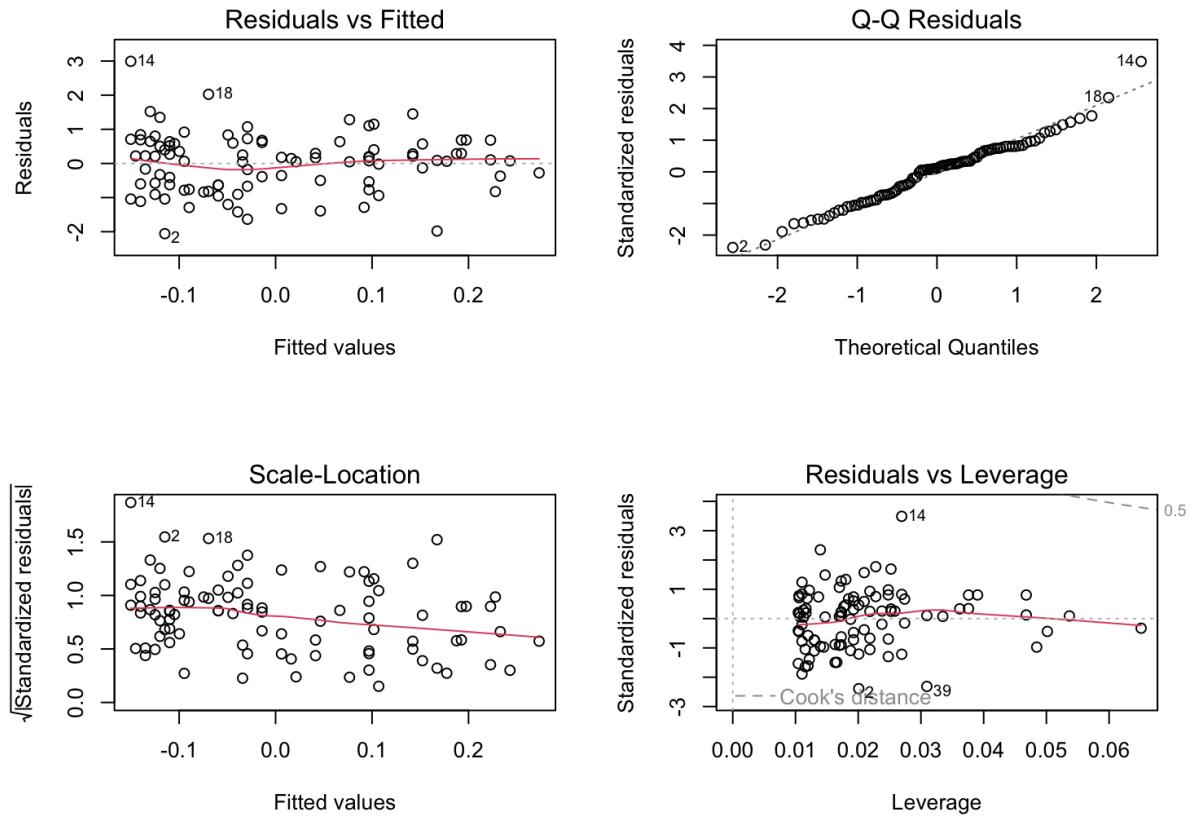


Figure S2: Plots of linear regression model assumptions for hypothesis 1.

*Table S6: Summary of unadjusted and adjusted models for hypothesis 1. CTQ-SF = Childhood Trauma Questionnaire-short form; PSQI = Pittsburgh Sleep Quality Index; PSS = Perceived Stress Scale.*

<i>Predictors</i>	<b>Unadjusted Model</b>			<b>Adjusted Model</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-0.28	-0.72 – 0.17	0.220	-0.42	-1.94 – 1.09	0.580
Total score on CTQ-SF	0.01	-0.00 – 0.01	0.182	0.01	-0.00 – 0.02	0.092
Sex (female)				-0.21	-0.61 – 0.19	0.302
Age				0.01	-0.01 – 0.03	0.476
Positive history of asthma				0.37	-0.62 – 1.35	0.462
Positive diagnosis of chronic illness				0.15	-0.48 – 0.78	0.647
Recent acute illness				0.19	-0.19 – 0.57	0.326
Recent COVID-19				0.48	-0.23 – 1.19	0.184
Total score on PSQI				-0.01	-0.06 – 0.04	0.648
Total score on PSS				-0.00	-0.05 – 0.04	0.899
Diagnosis of major depressive disorder				-0.54	-1.31 – 0.22	0.162
Observations	96			96		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.019 / 0.008			0.105 / 0.000		

## 10. Hypothesis 2: relationship between provoked cytokine expression and induced secondary hyperalgesia

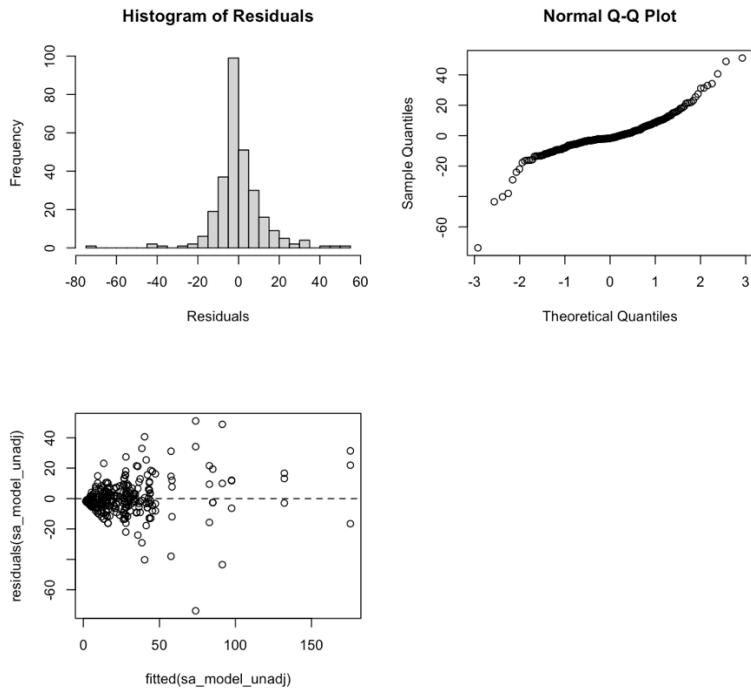


Figure S3: Model assumptions for conventional linear regression model for the surface area of secondary hyperalgesia.

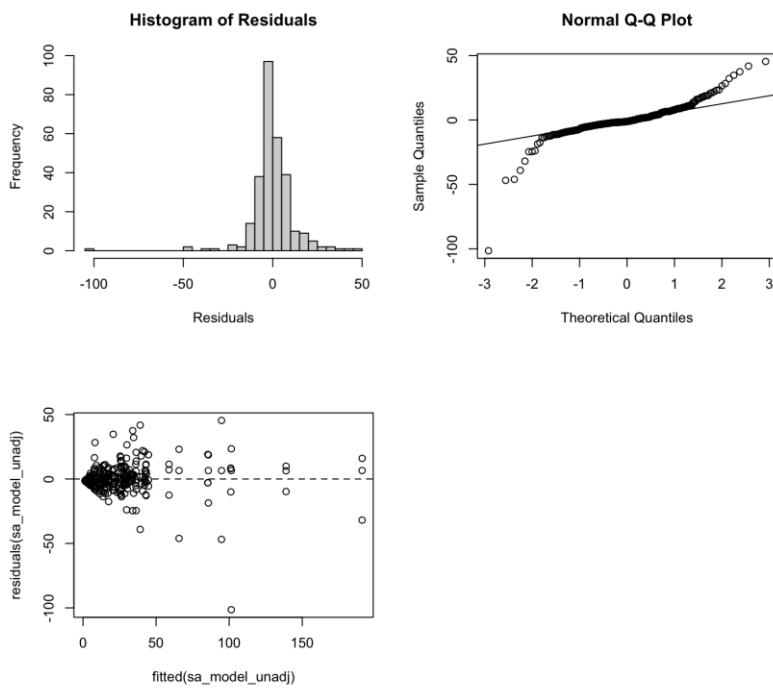


Figure S4: Model assumptions for robust linear regression model for the surface area of secondary hyperalgesia.

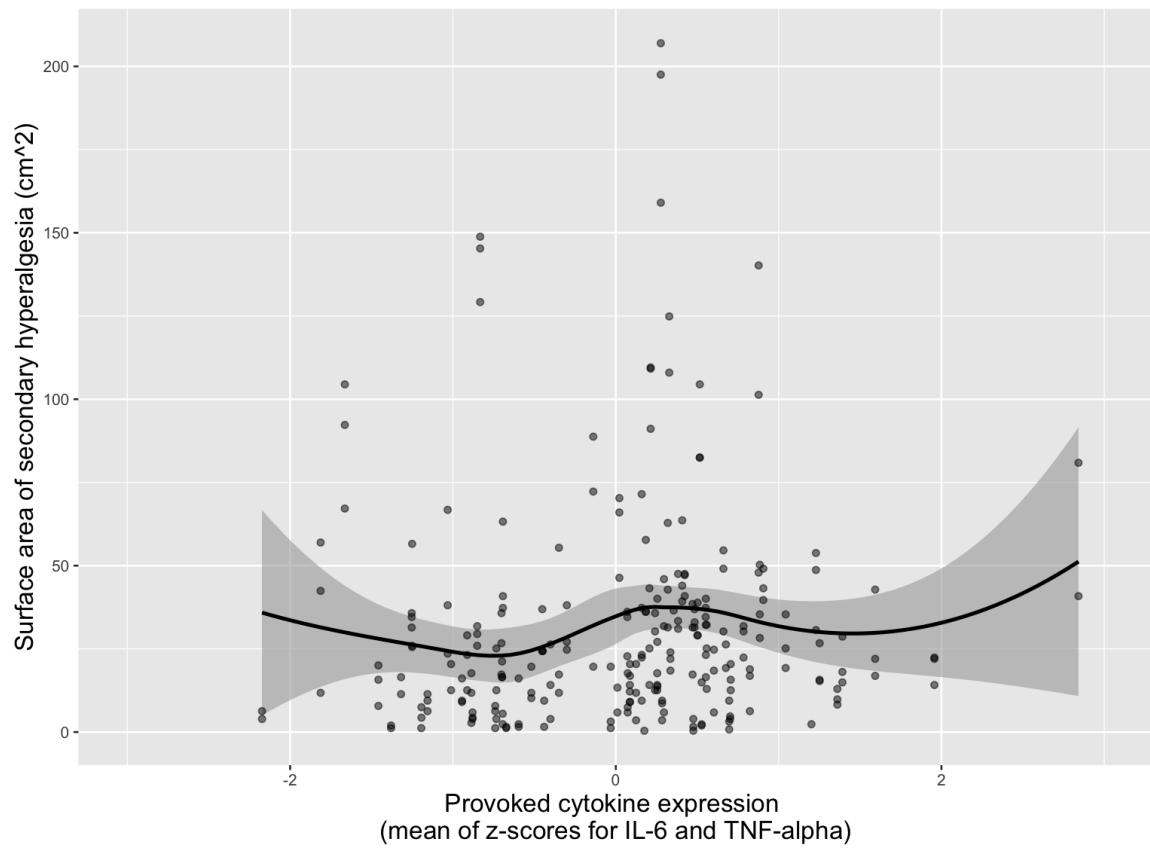


Figure S5: Relationship between provoked cytokine expression and surface area, where the area is > 0 cm<sup>2</sup>, of secondary hyperalgesia after neural provocation (i.e. HFS induction).

*Table S7: Summaries of unadjusted and adjusted hurdle models for hypothesis 2. Outcome: surface area of secondary hyperalgesia. PSS = Perceived Stress Scale; HFS = high-frequency electrical stimulation; PSQI = Pittsburgh Sleep Quality Index; MDD = major depressive disorder.*

<b>Predictors</b>	<b>Unadjusted Model</b>		<b>Adjusted Model</b>	
	<i>Estimates</i>	<i>CI (95%)</i>	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	2.86	2.62 – 3.09	1.03	-1.21 – 3.27
hu_Intercept	-3.36	-4.83 – -2.39	-1.32	-12.58 – 9.29
Provoked cytokine expression	0.25	0.01 – 0.51	0.18	-0.09 – 0.44
Total score on CTQ-SF	-0.42	-1.35 – 0.46	-0.72	-2.22 – 0.55
hu_Provoked cytokine expression			0.01	-0.00 – 0.02
Total score on PSS			0.04	-0.02 – 0.10
Mean SPARS rating for HFS			0.00	-0.02 – 0.02
HFS current intensity			-0.17	-0.44 – 0.11
Total score on PSQI			-0.02	-0.09 – 0.04
Diagnosis of MDD			-0.26	-1.09 – 0.66
Recent COVID-19			0.33	-0.58 – 1.31
Recent acute illness			-0.19	-0.72 – 0.32
Diagnosis of chronic illness			-0.53	-1.33 – 0.29
Sex (female)			-0.20	-0.71 – 0.31
Age			0.04	0.01 – 0.06
hu_Total score on PSS			-0.09	-0.37 – 0.17
hu_Mean SPARS rating for HFS			0.04	-0.06 – 0.17
hu_HFS current intensity			0.21	-1.09 – 1.61
hu_Total score on PSQI			-0.07	-0.41 – 0.25
hu_Diagnosis of MDD			-3.48	-10.59 – 1.19
hu_Recent COVID-19			-0.22	-5.23 – 4.35
hu_Recent acute illness			1.98	-0.65 – 5.27
hu_Diagnosis of chronic illness			4.37	0.78 – 9.22
hu_Sex (female)			0.63	-1.96 – 3.64
hu_Age			-0.13	-0.32 – 0.03
<b>Random Effects</b>				
$\sigma^2$	1501.32		1059.49	
$\tau_{00}$	218.50		730.93	
ICC	0.88		0.61	
N	96_study_id		96_study_id	
Observations	288		288	
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.023 / 0.740		0.254 / 0.755	

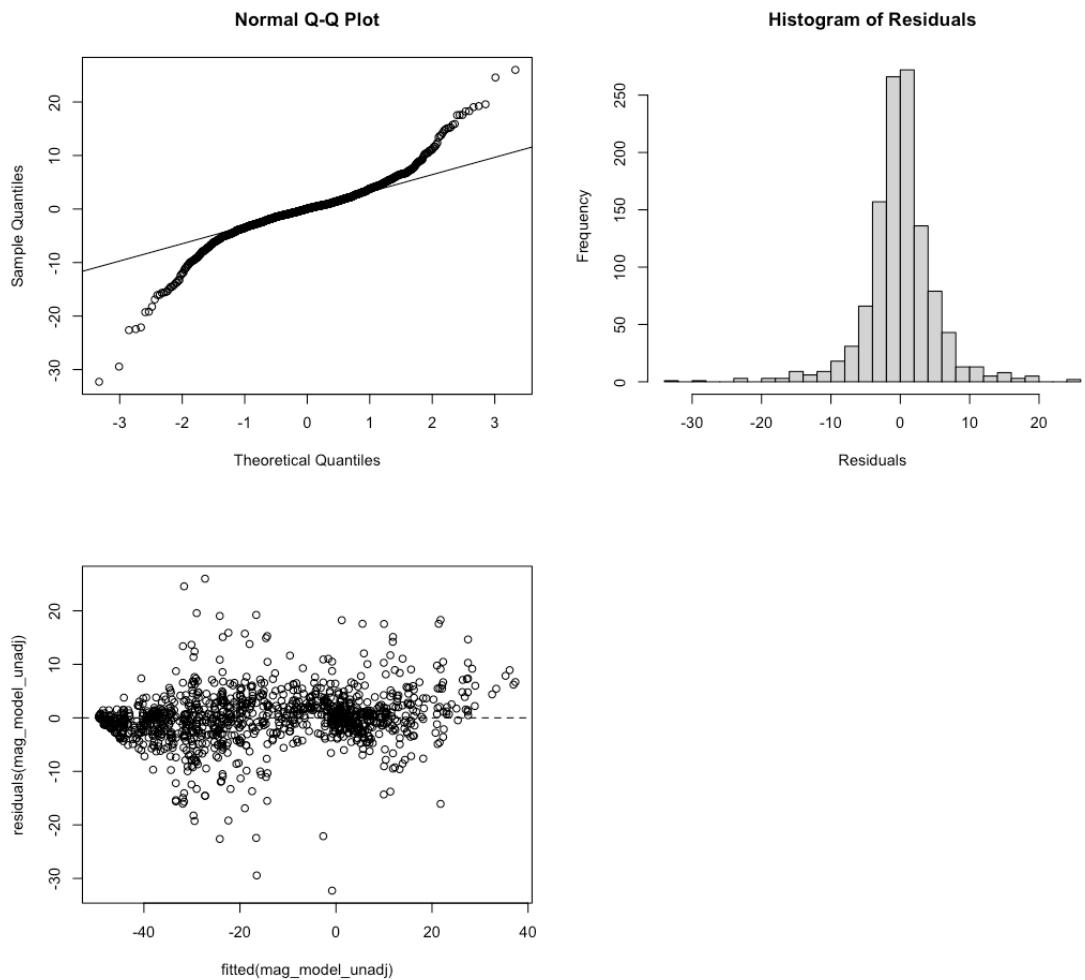


Figure S6: Model assumptions for conventional linear regression model for magnitude of secondary hyperalgesia

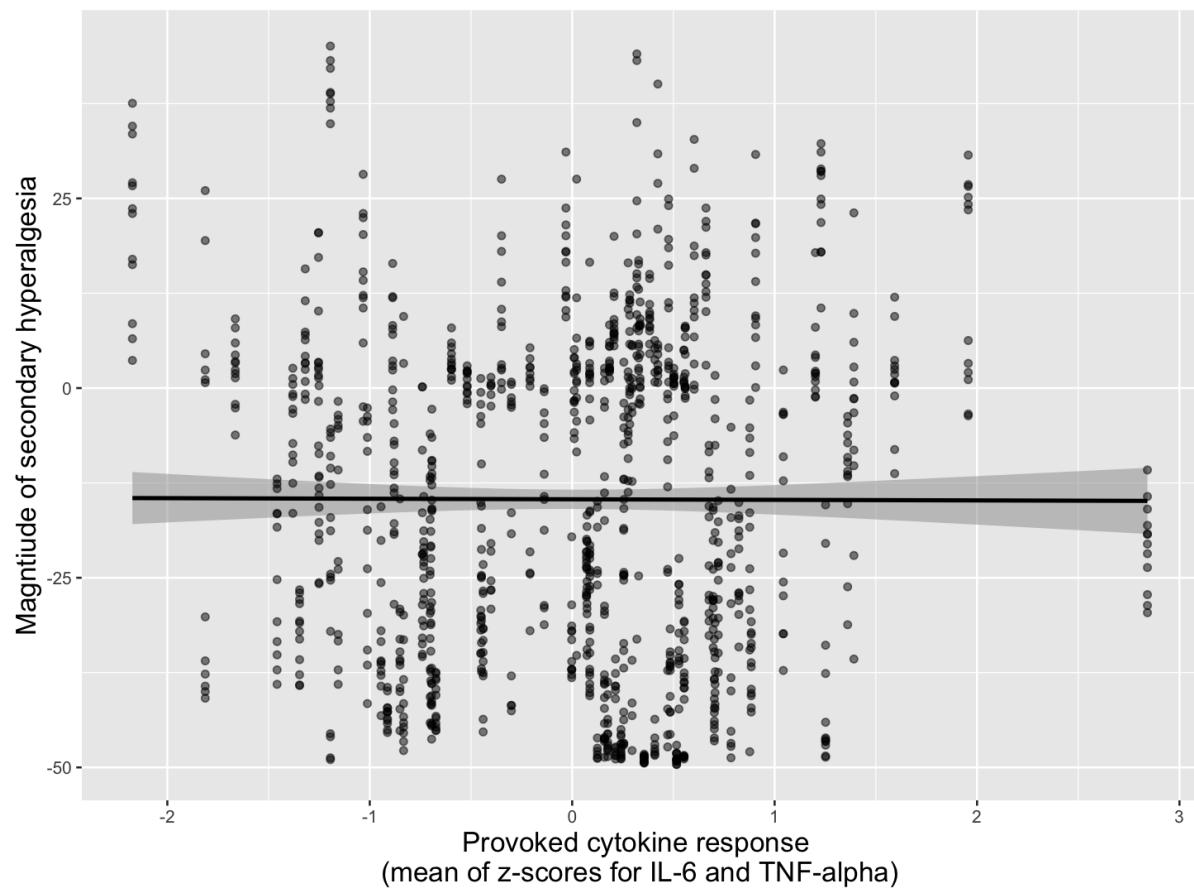


Figure S7: Relationship between provoked cytokine expression and magnitude of secondary hyperalgesia after neural provocation (i.e. HFS induction) for all participants ( $n=96$ ).

*Table S8: Summary of unadjusted and adjusted models for hypothesis 2. Outcome: magnitude of secondary hyperalgesia.*  
*HFS = high-frequency electrical stimulation; CTQ-SF = Childhood Trauma Questionnaire-Short Form; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index.*

Predictors	Unadjusted Model			Adjusted Model		
	Estimates	CI	p	Estimates	CI	p
Intercept	-11.51	-15.47 – -7.55	<0.001	2.88	-31.92 – 37.68	0.870
Timepoint (before HFS)	-6.29	-8.26 – -4.33	<0.001	-6.29	-8.25 – -4.33	<0.001
Provoked cytokine expression	-0.19	-4.75 – 4.37	0.935	-0.65	-5.09 – 3.78	0.770
Total score on CTQ-SF	0.23	-2.03 – 2.49	0.842	0.23	-2.03 – 2.49	0.842
Timepoint (before HFS)*provoked cytokine expression				-0.08	-0.25 – 0.09	0.326
Sex (female)				-5.85	-14.25 – 2.55	0.170
Age				-0.28	-0.72 – 0.15	0.201
Total score on PSS				-0.60	-1.49 – 0.28	0.177
Mean SPARS rating for HFS				0.69	0.35 – 1.02	<0.001
HFS current intensity				-1.43	-5.92 – 3.06	0.529
Total score on PSQI				0.17	-0.87 – 1.21	0.747
Diagnosis of major depressive disorder				9.32	-5.36 – 23.99	0.210
Recent COVID-19				5.70	-10.21 – 21.60	0.478
Recent acute illness				-3.23	-11.72 – 5.26	0.451
Diagnosis of chronic illness				7.78	-5.05 – 20.61	0.231
<b>Random Effects</b>						
$\sigma^2$	47.05			47.10		
$\tau_{00}$	37.92	pinprick:time:study_id		37.90	pinprick:time:study_id	
	40.69	time:study_id		40.54	time:study_id	
	354.99	study_id		294.63	study_id	
ICC	0.90			0.89		
N	2	pinprick		2	pinprick	
	4	time		4	time	
	96	study_id		96	study_id	
Observations	1152			1152		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.020 / 0.904			0.200 / 0.910		

## 11. Hypothesis 3: relationship between provoked cytokine expression and change in CPM and TS

Table S9: Summary of the main effect of session (before vs after influenza vaccination) on conditioned pain modulation (CPM) at each test site.

Predictors	Main effect of session on CPM at lumbar site			Main effect of session on CPM at deltoid site		
	Estimates	CI	p	Estimates	CI	p
Intercept	21.38	18.24 – 24.51	<0.001	13.26	11.01 – 15.50	<0.001
Session 2 (i.e after the immune provocation)	-1.78	-5.30 – 1.75	0.321	-0.34	-2.55 – 1.87	0.761
<b>Random Effects</b>						
$\sigma^2$	153.31			60.30		
$\tau_{00}$	88.93	study_id		64.51	study_id	
ICC	0.37			0.52		
N	96	study_id		96	study_id	
Observations	192			192		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.003 / 0.369			0.000 / 0.517		

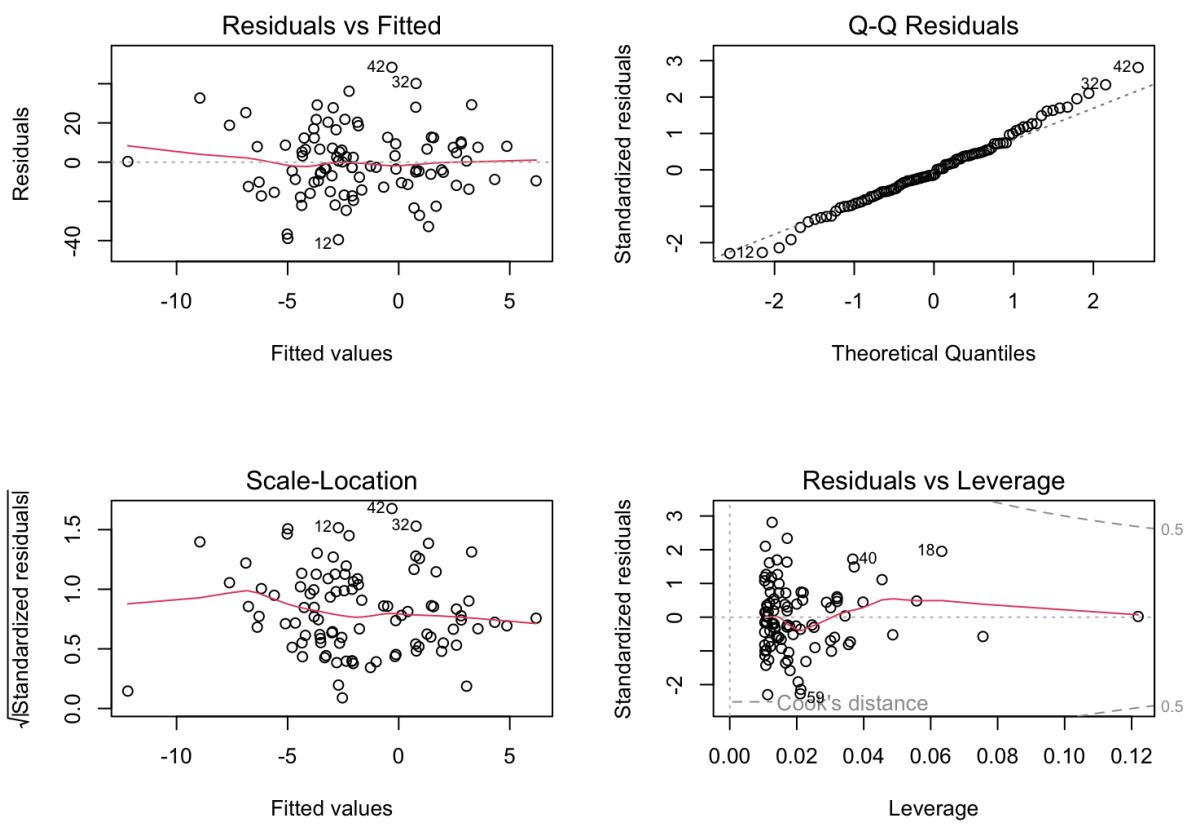


Figure S8: Plots of linear regression model assumptions for hypothesis 2. Outcome: change in CPM; test site: lumbar.

*Table S10: Summary of unadjusted and adjusted models for hypothesis 3. Outcome: change in conditioned pain modulation; test site: lumbar. CTQ-SF = Childhood Trauma Questionnaire-Short Form; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index.*

<i>Predictors</i>	<b>Unadjusted Model</b>			<b>Adjusted Model</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-1.78	-5.29 – 1.73	0.317	10.37	-19.68 – 40.42	0.494
Provoked cytokine expression	-3.67	-7.70 – 0.37	0.075	-3.97	-8.30 – 0.36	0.072
Total score on CTQ-SF				-0.09	-0.26 – 0.08	0.286
Sex (female)				-1.51	-9.37 – 6.35	0.704
Age				-0.40	-0.82 – 0.02	0.062
Total score on PSQI				-0.34	-1.32 – 0.64	0.495
Total score on PSS				0.11	-0.76 – 0.98	0.800
Diagnosis of major depressive disorder				-9.23	-23.44 – 4.98	0.200
Recent COVID-19				-2.53	-16.72 – 11.67	0.724
Recent acute illness				-1.48	-9.37 – 6.41	0.710
Diagnosis of chronic illness				14.45	2.11 – 26.79	<b>0.022</b>
Annual influenza vaccine (2023)				2.51	-5.85 – 10.86	0.552
Observations	96			96		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.033 / 0.023			0.152 / 0.041		

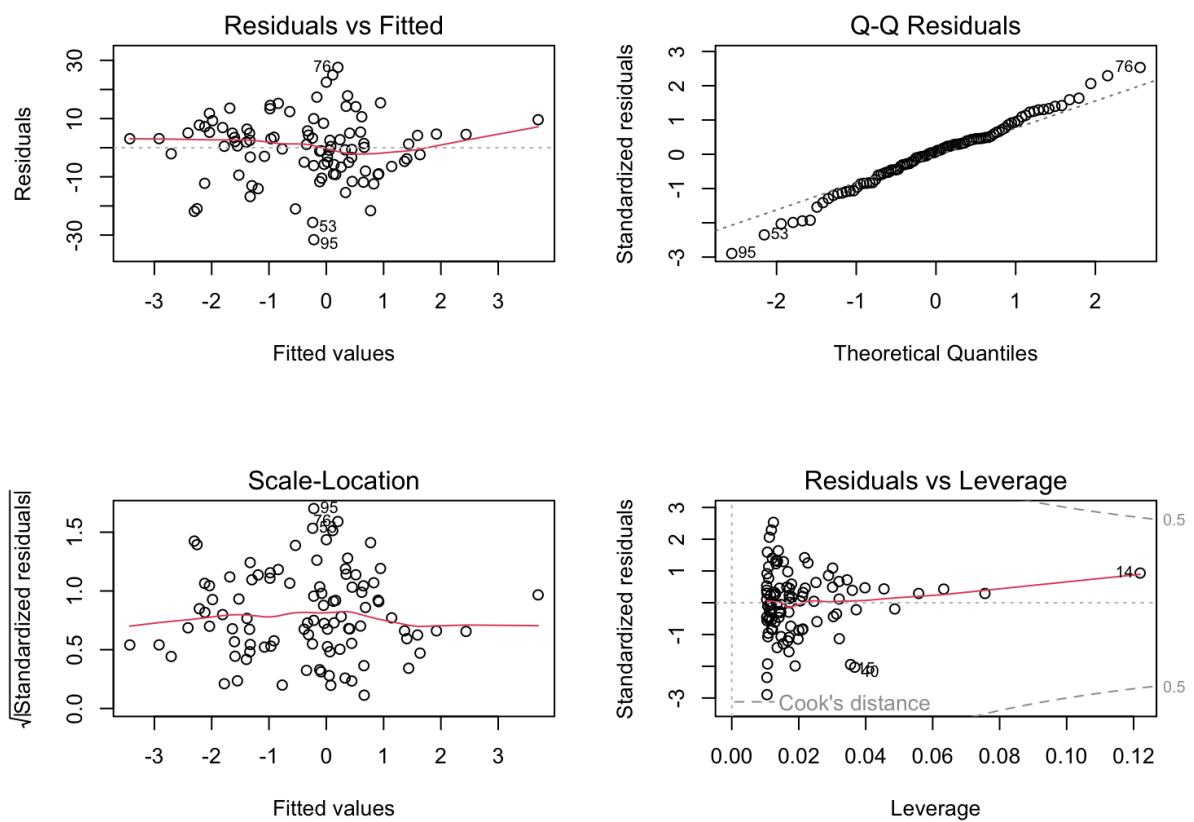


Figure S9: Plots of linear regression model assumptions for hypothesis 3. Outcome: change in CPM; test site: detloid.

*Table S11: Summary of unadjusted and adjusted models for hypothesis 3. Outcome: change in conditioned pain modulation; test site: deltoid. CTQ-SF = Childhood Trauma Questionnaire-Short Form; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index.*

<i>Predictors</i>	<b>Unadjusted Model</b>			<b>Adjusted Model</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-0.34	-2.56 – 1.88	0.761	4.39	-14.46 – 23.25	0.644
Provoked cytokine expression	1.42	-1.14 – 3.98	0.273	1.35	-1.37 – 4.06	0.328
Total score on CTQ-SF				-0.01	-0.12 – 0.09	0.795
Sex (female)				-4.90	-9.84 – -0.03	0.051
Age				-0.10	-0.36 – 0.17	0.472
Total score on PSQI				0.49	-0.13 – 1.10	0.120
Total score on PSS				-0.02	-0.56 – 0.53	0.954
Diagnosis of major depressive disorder				-5.49	-14.40 – 3.42	0.224
Recent COVID-19				-6.35	-15.26 – 2.56	0.160
Recent acute illness				0.74	-4.21 – 5.69	0.767
Diagnosis of chronic illness				6.33	-1.41 – 14.08	0.108
Annual influenza vaccine (2023)				-1.10	-6.34 – 4.14	0.678
Observations	96			96		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.013 / 0.002			0.151 / 0.040		

## 11.1 Relationship between provoked cytokine expression and change in CPM stratified by sex

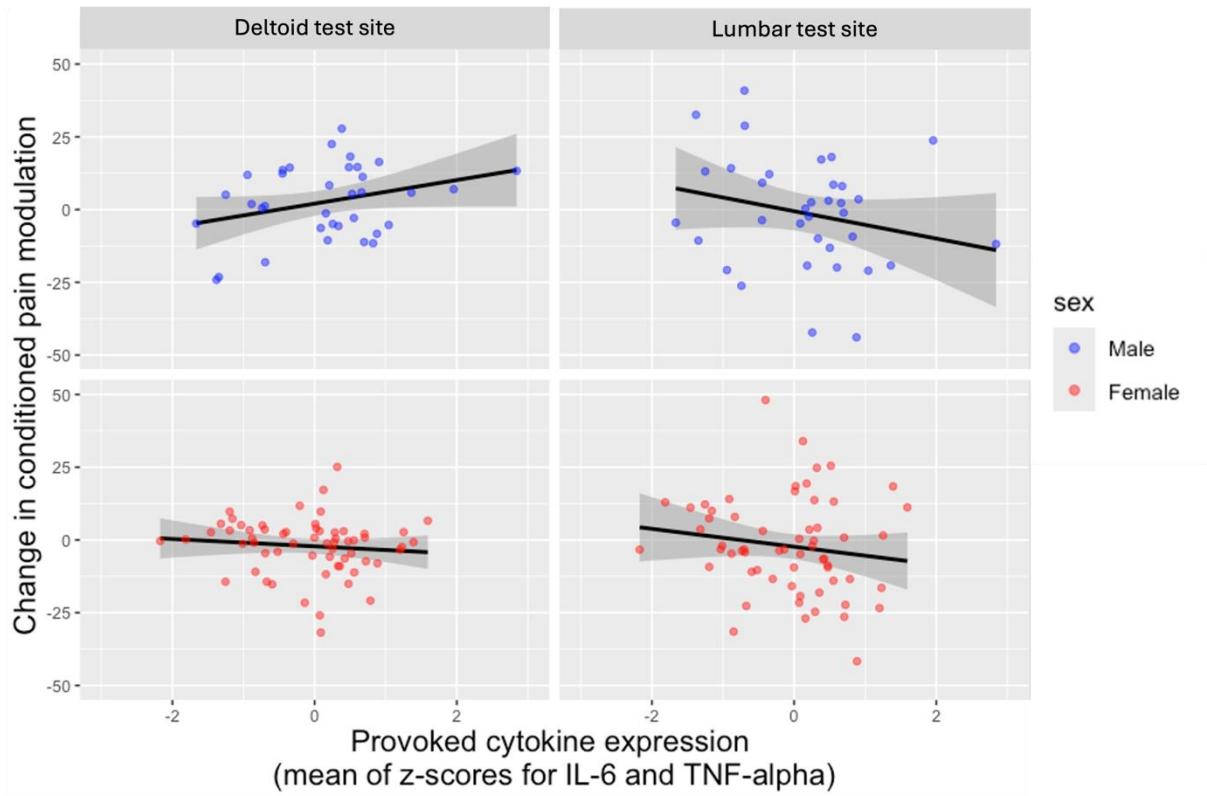


Figure S10: The relationship between provoked cytokine expression and change in conditioned pain modulation stratified by sex at the deltoid and lumbar test sites.

Table S12: Summary of the main effect of session (before vs after influenza vaccination) on temporal summation (TS) at each test site.

Predictors	Main effect of session on TS at lumbar site			Main effect of session on TS at deltoid site		
	Estimates	CI	p	Estimates	CI	p
Intercept	11.27	8.47 – 14.07	<0.001	10.17	7.79 – 12.55	<0.001
Session 2 (i.e after the immune provocation)	-3.05	-6.58 – 0.47	0.088	-3.19	-5.96 – -0.42	0.024
<b>Random Effects</b>						
$\sigma^2$	150.09			93.44		
$\tau_{00}$	41.78	study_id		46.24	study_id	
ICC	0.22			0.33		
N	96	study_id		96	study_id	
Observations	191			192		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.012 / 0.227			0.018 / 0.343		

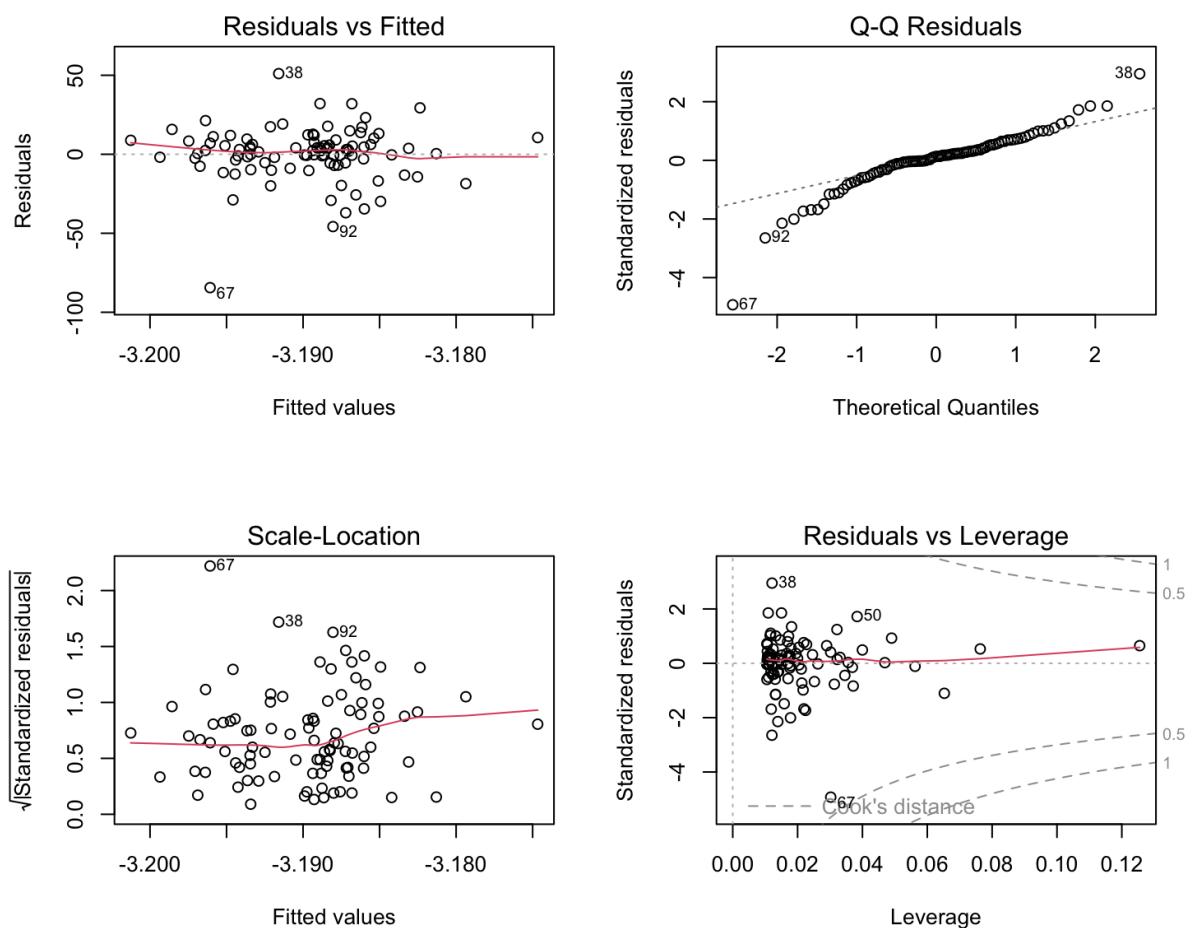


Figure SII: Plots of linear regression model assumptions for hypothesis 3. Outcome: change in TS; test site: lumbar

*Table S13: Summary of unadjusted and adjusted models for hypothesis 3. Outcome: change in temporal summation; test site: lumbar. CTQ-SF = Childhood Trauma Questionnaire-Short Form; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index.*

<i>Predictors</i>	<b>Unadjusted Model</b>			<b>Adjusted Model</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-3.19	-6.74 – -0.36	0.077	-40.85	-70.41 – -11.29	<b>0.007</b>
Provoked cytokine expression	0.01	-4.10 – 4.11	0.998	-0.21	-4.41 – 3.99	0.920
Total score on CTQ-SF				-0.19	-0.36 – -0.02	<b>0.027</b>
Sex (female)				-4.74	-12.31 – 2.83	0.216
Age				0.42	0.01 – 0.83	<b>0.044</b>
Total score on PSQI				0.00	-0.94 – 0.95	0.994
Total score on PSS				1.15	0.29 – 2.02	<b>0.010</b>
Diagnosis of major depressive disorder				-2.98	-16.67 – 10.71	0.666
Recent COVID-19				0.44	-13.33 – 14.20	0.950
Recent acute illness				5.63	-2.02 – 13.27	0.147
Diagnosis of chronic illness				-7.38	-19.53 – 4.78	0.231
Annual influenza vaccine (2023)				0.82	-7.33 – 8.98	0.841
Observations	95			95		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.000 / -0.011			0.197 / 0.090		

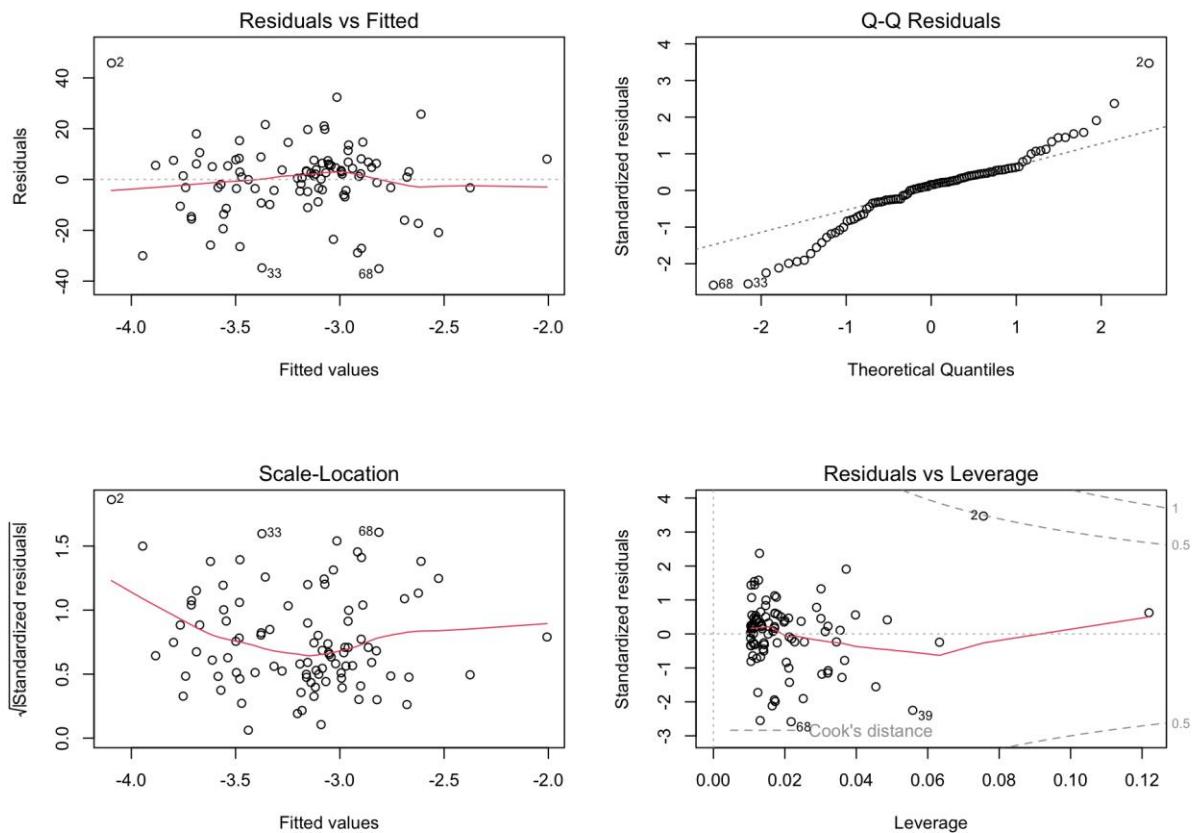


Figure S12: Plots of linear regression model assumptions for hypothesis 3. Outcome: change in TS; test site: detloid.

*Table S14: Summary of unadjusted and adjusted models for hypothesis 2. Outcome: change in temporal summation; test site: deltoid. CTQ-SF = Childhood Trauma Questionnaire-Short Form; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index.*

<i>Predictors</i>	Unadjusted Model			Adjusted Model		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	-3.19	-5.97 – -0.41	<b>0.025</b>	-12.57	-37.11 – 11.96	0.311
Provoked cytokine expression	0.42	-2.79 – 3.62	0.797	0.79	-2.74 – 4.33	0.657
Total score on CTQ-SF				-0.02	-0.16 – 0.12	0.787
Sex (female)				6.40	-0.02 – 12.81	0.051
Age				0.01	-0.33 – 0.35	0.955
Total score on PSQI				-0.17	-0.96 – 0.63	0.683
Total score on PSS				0.26	-0.45 – 0.97	0.469
Diagnosis of major depressive disorder				-5.21	-16.81 – 6.39	0.374
Recent COVID-19				2.49	-9.10 – 14.09	0.670
Recent acute illness				-2.15	-8.59 – 4.29	0.508
Diagnosis of chronic illness				-4.21	-14.28 – 5.87	0.409
Annual influenza vaccine (2023)				0.78	-6.04 – 7.60	0.821
Observations	96			96		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.001 / -0.010			0.072 / -0.049		

## 12. Blinding assessment

*Table S15: Summary of unadjusted and covariate-adjusted models for hypothesis 1 for the full sample and sensitivity analyses excluding 2 (of 96) participants who were unblinded to hypothesis 1. PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.*

Predictors	Unadjusted Model			Adjusted Model			Unadjusted Sensitivity Analysis			Adjusted Sensitivity Analysis		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-0.28	-0.72 – 0.17	0.220	-0.42	-1.94 – 1.09	0.580	-0.29	-0.74 – 0.17	0.215	-0.43	-1.97 – 1.11	0.583
Total score on CTQ	0.01	-0.00 – 0.01	0.182	0.01	-0.00 – 0.02	0.092	0.01	-0.00 – 0.01	0.181	0.01	-0.00 – 0.02	0.093
Sex (female)				-0.21	-0.61 – 0.19	0.302				-0.22	-0.63 – 0.20	0.299
Age				0.01	-0.01 – 0.03	0.476				0.01	-0.01 – 0.03	0.481
Positive history of asthma				0.37	-0.62 – 1.35	0.462				0.36	-0.63 – 1.36	0.470
Positive diagnosis of chronic illness				0.15	-0.48 – 0.78	0.647				0.15	-0.49 – 0.79	0.646
Recent acute illness				0.19	-0.19 – 0.57	0.326				0.19	-0.20 – 0.57	0.343
Recent COVID-19 diagnosis				0.48	-0.23 – 1.19	0.184				0.48	-0.24 – 1.21	0.186
Total score on PSQI				-0.01	-0.06 – 0.04	0.648				-0.01	-0.06 – 0.04	0.687
Total score on PSS				-0.00	-0.05 – 0.04	0.899				-0.00	-0.05 – 0.04	0.892
Diagnosis of major depressive disorder				-0.54	-1.31 – 0.22	0.162				-0.54	-1.32 – 0.24	0.170
Observations	96			96			94			94		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.019 / 0.008			0.105 / 0.000			0.019 / 0.009			0.106 / -0.002		

*Table S16: Summary of unadjusted and covariate-adjusted models for hypothesis 3 (change in CPM at the lumbar site) for the full sample and sensitivity analyses excluding 4 (of 96) participants who were unblinded to hypothesis 3. PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.*

Predictors	Unadjusted Model			Adjusted Model			Unadjusted Sensitivity Analysis			Adjusted Sensitivity Analysis		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-1.78	-5.29 – 1.73	0.317	12.88	-17.16 – 42.92	0.396	-2.00	-5.55 – 1.55	0.267	10.41	-19.95 – 40.78	0.497
Provoked cytokine expression	-3.67	-7.70 – 0.37	0.075	-3.97	-8.30 – 0.36	0.072	-3.37	-7.45 – 0.70	0.104	-3.86	-8.30 – 0.58	0.087
Total score on CTQ-SF				-0.09	-0.26 – 0.08	0.286				-0.08	-0.25 – 0.09	0.365
Sex (female)				-1.51	-9.37 – 6.35	0.704				-0.83	-8.88 – 7.21	0.837
Age				-0.40	-0.82 – 0.02	0.062				-0.38	-0.80 – 0.04	0.078
Total score on PSQI				-0.34	-1.32 – 0.64	0.495				-0.29	-1.28 – 0.69	0.558
Total score on PSS				0.11	-0.76 – 0.98	0.800				0.10	-0.79 – 0.98	0.830
Diagnosis of major depressive disorder				-9.23	-23.44 – 4.98	0.200				-9.45	-23.65 – 4.76	0.189
Recent COVID-19				-2.53	-16.72 – 11.67	0.724				-1.71	-15.93 – 12.51	0.812
Recent acute illness				-1.48	-9.37 – 6.41	0.710				-1.28	-9.32 – 6.76	0.753
Diagnosis of chronic illness				14.45	2.11 – 26.79	<b>0.022</b>				13.78	1.45 – 26.12	<b>0.029</b>
Annual influenza vaccine (2023)				2.51	-5.85 – 10.86	0.552				3.56	-4.91 – 12.02	0.406
Observations	96			96			92			92		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.033 / 0.023			0.152 / 0.041			0.029 / 0.018			0.145 / 0.028		

*Table S17: Summary of unadjusted and covariate-adjusted models for hypothesis 3 (change in CPM at the deltoid site) for the full sample and sensitivity analyses excluding 4 (of 96) participants who were unblinded to hypothesis 3. PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.*

Predictors	Unadjusted Model			Adjusted Model			Unadjusted Sensitivity Analysis			Adjusted Sensitivity Analysis		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-0.34	-2.56 – 1.88	0.761	3.29	-15.55 – 22.14	0.729	0.09	-2.16 – 2.33	0.939	7.94	-10.54 – 26.42	0.395
Provoked cytokine expression	1.42	-1.14 – 3.98	0.273	1.35	-1.37 – 4.06	0.328	0.85	-1.72 – 3.43	0.513	0.52	-2.18 – 3.22	0.703
Total score on the CTQ-SF				-0.01	-0.12 – 0.09	0.795				-0.01	-0.12 – 0.09	0.826
Sex (female)				-4.90	-9.84 – 0.03	0.051				-6.18	-11.07 – -1.28	<b>0.014</b>
Age				-0.10	-0.36 – 0.17	0.472				-0.09	-0.35 – 0.16	0.471
Total score on PSQI				0.49	-0.13 – 1.10	0.120				0.50	-0.10 – 1.09	0.104
Total score on PSS				-0.02	-0.56 – 0.53	0.954				-0.15	-0.69 – 0.39	0.579
Diagnosis of major depressive disorder				-5.49	-14.40 – 3.42	0.224				-6.47	-15.11 – 2.18	0.141
Recent COVID-19				-6.35	-15.26 – 2.56	0.160				-7.03	-15.68 – 1.63	0.110
Recent acute illness				0.74	-4.21 – 5.69	0.767				2.18	-2.71 – 7.07	0.378
Diagnosis of chronic illness				6.33	-1.41 – 14.08	0.108				6.78	-0.72 – 14.29	0.076
Annual influenza vaccine (2023)				-1.10	-6.34 – 4.14	0.678				-1.47	-6.63 – 3.68	0.571
Observations	96			96			92			92		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.013 / 0.002			0.151 / 0.040			0.005 / -0.006			0.188 / 0.077		

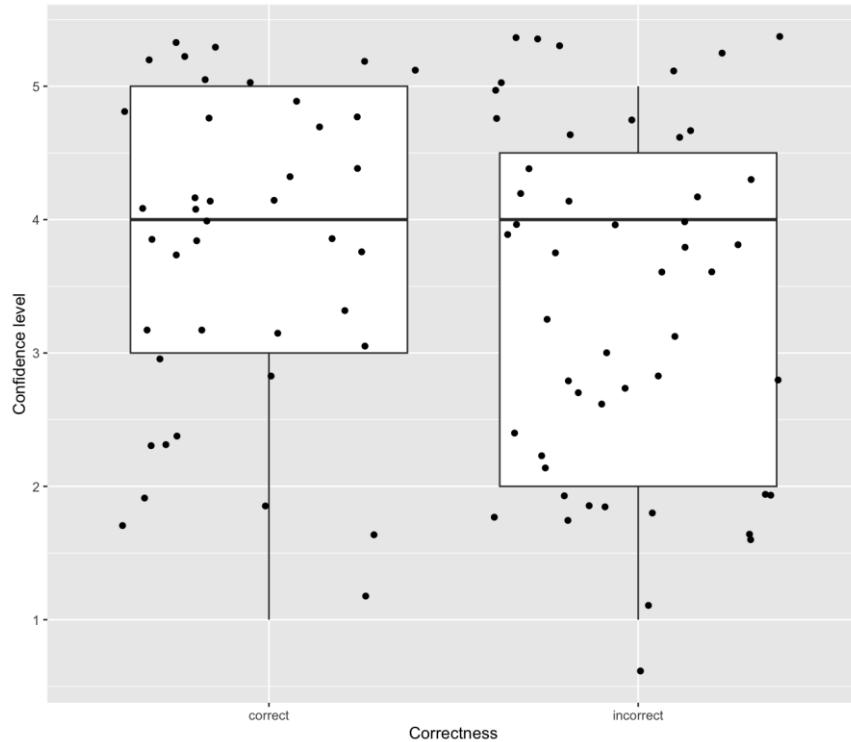
Table S18: Summary of unadjusted and covariate-adjusted models for hypothesis 3 (change in TS at the lumbar site) for the full sample and sensitivity analyses excluding 4 (of 95) participants who were unblinded to hypothesis 3. PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.

Predictors	Unadjusted Model			Adjusted Model			Unadjusted Sensitivity Analysis			Adjusted Sensitivity Analysis		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-3.19	-6.74 – -0.36	0.077	-40.85	-70.41 – -11.29	<b>0.007</b>	-3.17	-6.87 – -0.53	0.093	-39.49	-70.31 – -8.68	<b>0.013</b>
Provoked cytokine expression	0.01	-4.10 – 4.11	0.998	-0.21	-4.41 – 3.99	0.920	0.04	-4.23 – 4.31	0.985	-0.01	-4.43 – 4.40	0.995
Total score on the CTQ-SF				-0.19	-0.36 – -0.02	<b>0.027</b>				-0.20	-0.37 – -0.03	<b>0.025</b>
Sex (female)				-4.74	-12.31 – 2.83	0.216				-4.92	-12.87 – 3.02	0.221
Age				0.42	0.01 – 0.83	<b>0.044</b>				0.41	-0.01 – 0.83	0.056
Total score on PSQI				0.00	-0.94 – 0.95	0.994				-0.02	-1.00 – 0.95	0.964
Total score on PSS				1.15	0.29 – 2.02	<b>0.010</b>				1.18	0.28 – 2.08	<b>0.011</b>
Diagnosis of major depressive disorder				-2.98	-16.67 – 10.71	0.666				-2.73	-16.77 – 11.31	0.700
Recent COVID-19				0.44	-13.33 – 14.20	0.950				-0.06	-14.20 – 14.09	0.994
Recent acute illness				5.63	-2.02 – 13.27	0.147				5.42	-2.57 – 13.41	0.181
Diagnosis of chronic illness				-7.38	-19.53 – 4.78	0.231				-7.09	-19.55 – 5.37	0.261
Annual influenza vaccine (2023)				0.82	-7.33 – 8.98	0.841				0.26	-8.21 – 8.72	0.952
Observations	95			95			91			91		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.000 / -0.011			0.197 / 0.090			0.000 / -0.011			0.202 / 0.090		

*Table S19: Summary of unadjusted and covariate-adjusted models for hypothesis 3 (change in TS at the deltoid site) for the full sample and sensitivity analyses excluding 4 (of 96) participants who were unblinded to hypothesis 3. PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.*

Predictors	Unadjusted Model			Adjusted Model			Unadjusted Sensitivity Analysis			Adjusted Sensitivity Analysis		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-3.19	-5.97 – -0.41	<b>0.025</b>	-12.57	-37.11 – 11.96	0.311	-3.18	-6.03 – -0.32	<b>0.030</b>	-11.51	-36.98 – 13.97	0.371
Provoked cytokine expression	0.42	-2.79 – 3.62	0.797	0.79	-2.74 – 4.33	0.657	0.15	-3.12 – 3.43	0.927	0.48	-3.22 – 4.18	0.797
Total score on the CTQ-SF				-0.02	-0.16 – 0.12	0.787				-0.02	-0.16 – 0.12	0.813
Sex (female)				6.40	-0.02 – 12.81	0.051				5.61	-1.10 – 12.31	0.100
Age				0.01	-0.33 – 0.35	0.955				0.02	-0.33 – 0.37	0.898
Total score on PSQI				-0.17	-0.96 – 0.63	0.683				-0.11	-0.93 – 0.71	0.789
Total score on PSS				0.26	-0.45 – 0.97	0.469				0.19	-0.55 – 0.92	0.614
Diagnosis of major depressive disorder				-5.21	-16.81 – 6.39	0.374				-5.37	-17.20 – 6.47	0.370
Recent COVID-19				2.49	-9.10 – 14.09	0.670				2.54	-9.31 – 14.39	0.671
Recent acute illness				-2.15	-8.59 – 4.29	0.508				-1.42	-8.12 – 5.28	0.674
Diagnosis of chronic illness				-4.21	-14.28 – 5.87	0.409				-3.91	-14.19 – 6.38	0.452
Annual influenza vaccine (2023)				0.78	-6.04 – 7.60	0.821				1.00	-6.06 – 8.05	0.779
Observations	96			96			92			92		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.001 / -0.010			0.072 / -0.049			0.000 / -0.011			0.055 / -0.075		

The relationship between guess accuracy and guess confidence (N = 92)



*Figure S13: Blinding assessment: The relationship between the assessor's guess accuracy and guess confidence*

## 13. Exploratory analyses

### 13.1 Relationship between provoked cytokine expression and static and dynamic light touch and single electrical stimulation

Table S20: Summary of unadjusted and adjusted models for exploratory outcomes. HFS = high-frequency electrical stimulation; PSS = Perceived Stress scale; PSQI = Pittsburgh Sleep Quality Index.

Predictors	Static light touch unadjusted model			Static light touch adjusted model			Dynamic light touch unadjusted model			Dynamic light touch adjusted model			Single electrical stimulation unadjusted model			Single electrical stimulation adjusted model		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
Intercept	-38.25	-40.83 – -35.67	<0.001	-19.27	-42.22 – -3.69	0.099	-46.22	-47.22 – -45.22	<0.001	-43.29	-52.49 – -34.10	<0.001	-3.12	-7.26 – 1.02	0.138	18.26	-15.22 – 51.73	0.281
Timepoint (before HFS)	-2.43	-3.51 – -1.35	<0.001	-2.43	-3.51 – -1.35	<0.001	-0.68	-1.17 – -0.18	0.007	-0.68	-1.17 – -0.18	0.007	0.40	-0.98 – 1.78	0.572	0.40	-0.98 – 1.78	0.572
Provoked cytokine expression	1.13	-1.84 – 4.10	0.452	1.06	-1.90 – 4.02	0.479	-0.55	-1.70 – 0.60	0.347	-0.74	-1.93 – 0.45	0.222	-1.79	-6.55 – 2.98	0.459	-2.32	-6.61 – 1.98	0.286
Timepoint (before HFS)*Cytokine expression	0.47	-0.77 – 1.71	0.457	0.47	-0.77 – 1.71	0.458	0.49	-0.08 – 1.06	0.095	0.49	-0.08 – 1.06	0.095	-0.06	-1.65 – 1.53	0.943	-0.06	-1.65 – 1.53	0.943
Total score on the CTQ-SF		-0.07	-0.18 – 0.05	0.245					0.01	-0.04 – 0.05	0.747				-0.14	-0.30 – 0.02	0.094	
Sex (female)		-6.07	-11.61 – -0.53	0.032					-2.40	-4.62 – -0.18	0.034				-10.13	-18.21 – -2.05	0.015	
Age		-0.25	-0.54 – -0.03	0.084					-0.10	-0.22 – -0.01	0.073				-0.30	-0.72 – -0.11	0.152	
Total score on PSS		-0.25	-0.83 – -0.33	0.400					0.05	-0.18 – 0.28	0.680				-0.88	-1.73 – -0.03	0.042	
Mean SPARS rating for HFS		0.21	-0.01 – 0.43	0.061					0.00	-0.08 – 0.09	0.933				0.90	0.58 – 1.22	<0.001	
HFS current intensity		-2.38	-5.34 – -0.59	0.114					-0.27	-1.46 – 0.91	0.647				-0.20	-4.52 – 4.12	0.927	
Total score on PSQI		0.37	-0.32 – 1.05	0.289					0.10	-0.18 – 0.37	0.489				0.02	-0.98 – 1.02	0.968	
Diagnosis of major depressive disorder		6.85	-2.83 – 16.53	0.163					-0.77	-4.65 – 3.11	0.694				6.91	-7.20 – 21.03	0.333	
Recent COVID-19		7.23	-3.26 – 17.73	0.174					2.39	-1.81 – 6.60	0.261				0.31	-14.99 – 15.61	0.968	
Recent acute illness		-5.17	-10.77 – -0.43	0.070					-1.76	-4.00 – 0.48	0.123				-1.40	-9.56 – 6.77	0.735	
Diagnosis of chronic illness		1.16	-7.31 – 9.62	0.786					1.17	-2.22 – 4.56	0.495				4.21	-8.13 – 16.55	0.500	
<b>Random Effects</b>																		
$\sigma^2$	43.35			43.35			9.18			9.18			71.21			71.21		
$\tau_{00}$	147.84	study_id		129.51	study_id		21.27	study_id		20.40	study_id		394.20	study_id		278.90	study_id	
ICC	0.77			0.75			0.70			0.69			0.85			0.80		
N	96	study_id		96	study_id		96	study_id		96	study_id		96	study_id		96	study_id	
Observations	575			575			576			576			576			576		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.015 / 0.777			0.174 / 0.793			0.008 / 0.701			0.109 / 0.723			0.005 / 0.848			0.299 / 0.857		

## 13.2 Relationship between each subscale of the CTQ-SF and provoked cytokine expression

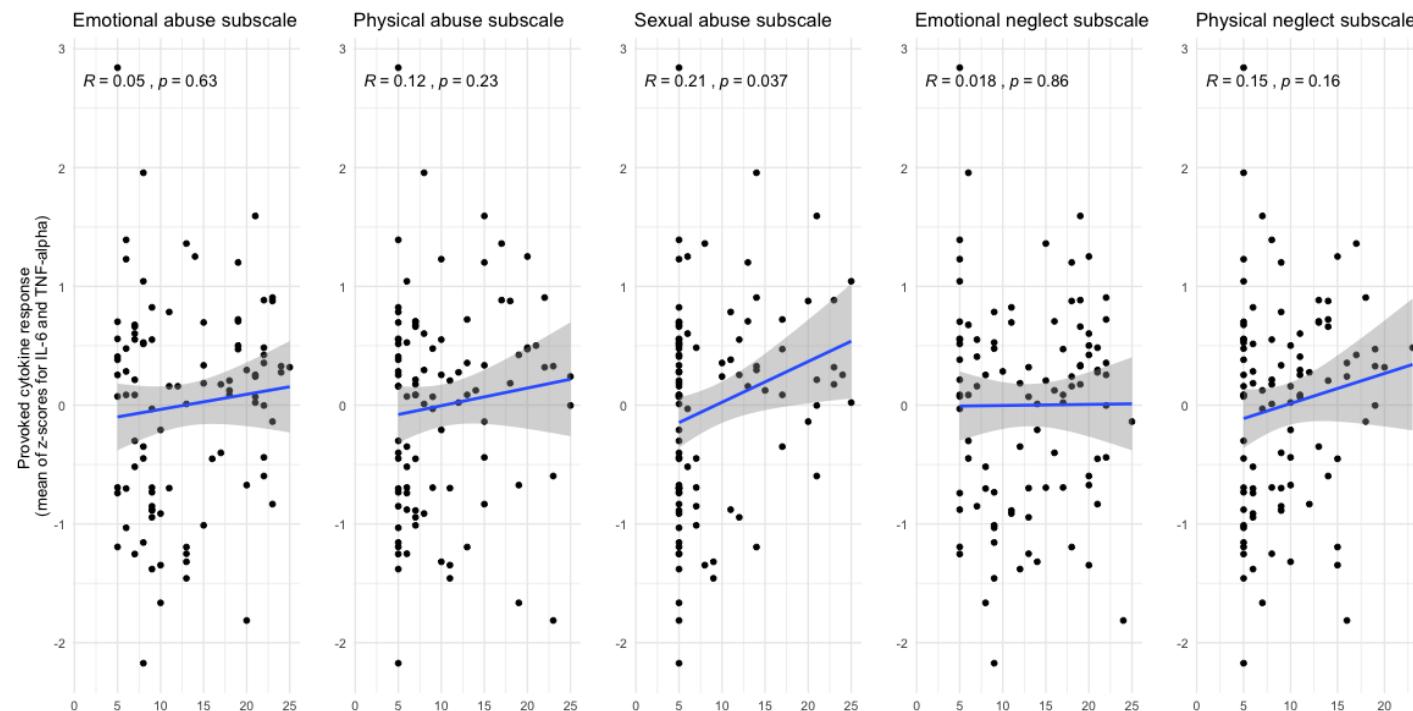


Figure S14: Correlations between each subscale of the CTQ-SF and provoked cytokine expression.

### 13.3 Interaction between positive childhood experiences and adverse childhood experiences on provoked cytokine expression

*Table S21: Summary of models for hypothesis 1 when included positive childhood experiences as a moderator in the subsample of 49 participant for whom we had data on positive childhood experiences. CTQ = Childhood Trauma Questionnaire.*

<i>Predictors</i>	Model with positive childhood experiences as a moderator			Model without positive childhood experiences as a moderator		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	0.21	-0.82 – 1.24	0.683	0.32	-0.48 – 1.12	0.420
Total score on CTQ	-0.01	-0.02 – 0.01	0.359	-0.01	-0.02 – 0.01	0.387
Total score on CTQ*total score on Positive Childhood Experiences Questionnaire	0.00	-0.00 – 0.00	0.728			
Observations	49			49		
R <sup>2</sup> / R <sup>2</sup> adjusted		0.019 / -0.024			0.016 / -0.005	