Design, rationale and feasibility of a multidimensional experimental protocol to study early life stress

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ABSTRACT

There is a rapidly accumulating body of evidence regarding the influential role of early life stress (ELS) upon medical and psychiatric conditions. While self-report instruments, with their intrinsic limitations of recall, remain the primary means of detecting ELS in humans, biological measures are generally limited to a single biological system. This paper describes the design, rationale and feasibility of a study to simultaneously measure neuroendocrine, immune and autonomic nervous system (ANS) responses to psychological and physiological stressors in relation to ELS. Five healthy university students were recruited by advertisement. Exclusion criteria included chronic medical conditions, psychotic disorders, needle phobia, inability to tolerate pain, and those using anti-inflammatory medications. They were clinically interviewed and physiological recordings made over a two-hour period pre, during and post two acute stressors: the cold pressor test and recalling a distressing memory. The Childhood Trauma Questionnaire and the Parental Bonding Index were utilised to measure ELS. Other psychological measures of mood and personality were also administered. Measurements of heart rate, blood pressure, respiratory rate, skin conductance, skin blood flow and temporal plasma samples were successfully obtained before, during and after acute stress. Participants reported the extensive psychological and multisystem physiological data collection and stress provocations were tolerable. Most (4/5) participants indicated a willingness to return to repeat the protocol, indicating acceptability. Our protocol is viable and safe in young physically healthy adults and allows us to assess simultaneously neuroendocrine, immune and autonomic nervous system responses to stressors in persons assessed for ELS.

1. Introduction

1.1. Background

Substantial evidence links early life stress (ELS) to a range of medical and psychological outcomes [1]. Children subjected to stressors, such as physical/sexual abuse, harassment, neglect or war, are at greater risk of developing personality changes, suicidal behaviours, depression and other psychiatric problems [2]. Distress in early life can leave a lasting physiological imprint in the form of a dysregulated hypothalamic-pituitary-adrenal (HPA) axis [3]. This has been found

when there has been the loss of a parent [4,5], divorce of parents [6], childhood abuse (both physical and sexual) [2,7–9] [10], neglect [11], maternal depression [12–14], low socio-economic status [15,16], parenting style (e.g. anger/control) [17–19] and post-traumatic stress disorder (PTSD) [2].

Almost invariably in these studies, ELS is dichotomised into present/absent categories, or there is a comparison between a clinical (high ELS) and control (low ELS) population against a clinical outcome variable such as dermatitis or drug taking behaviour or vulnerability to PTSD. Within these studies ELS has typically been used as the predictor (usually categorical) of specific or systemic pathophysiology and

disease risk. An association between ELS and the presence of altered physiology or pathology appears robust [20]. At the end of these studies the limitation of retrospective recall is invariably and appropriately noted. While behavioural descriptors, rather than subjective judgements, of childhood stress can improve the reliability of questionnaires assessing childhood events, factors such as childhood attachment, emotional neglect and parental warmth remain confounded by the current emotional relationship with the parent [21–23]. Is there a way then to avoid, or at least minimise, the reliance upon retrospective recall?

Given the established links between ELS and altered physiology, we propose that these physiological alterations might be able to be collectively combined into a bio-index that would provide a dose-response curve without relying upon retrospective recall. This would entail the ELS-associated physiological alterations being the predictors, and ELS being the outcome variable. Initially we anticipate the physiological markers being associated with a global measure of ELS, with future refinement being able to predict sub-factors (emotional abuse/neglect) of ELS. To create this bio-index, a wide range of potential physiological predictors needs to be measured simultaneously in the same participants. This paper reports on the feasibility of this approach within a university laboratory setting. The protocol deployed used a dual stress-induction method. The feasibility, including safety, acceptability, and the practical logistics of this complex design, were assessed.

1.2. Aims

The aims of this study were: 1) to develop a protocol to investigate neuro-immuno-endocrine responses to stressors that could form the basis of a physiological (biomarker) index of ELS, and 2) to determine the feasibility, including acceptability and safety, of measuring indicators of ELS, as well as mood and psychological traits linked with ELS, prior to measuring autonomic, immune and endocrine parameters before, during and after inducing acute physical pain, and then psychological stress, in young healthy participants.

2. Methods

2.1. Study design

The study involved a 4-stage process (Fig. 1): i) Online eligibility screening and completion of questionnaires; ii) Clinical interview and recall of distressing memories, paper-based questionnaire, and laboratory familiarisation; iii) Experimental session involving recording of physiological parameters and collection of blood samples during application of stressors; and iv) Completion of a follow-up questionnaire 1–2 weeks after the testing session. The study was approved by the University's Human Research Ethics Committee, approval No. H-2010-1274.

Key feasibility measures were recruitment of participants, acceptability of the screening and experimental protocol, and safety of both the recall of distressing memories and physiological measurement procedures. The Childhood Trauma Questionnaire and the Parental Bonding Index, which are commonly used to measure ELS but rarely concurrently in the same individuals, were both used. Mood and psychological traits linked with ELS were also measured by questionnaires. In the experimental session, physiological parameters that reflect autonomic function, such as electrocardiogram (ECG), blood pressure, ventilation and sudomotor activity were measured simultaneously. Blood samples were obtained for later analysis of stress (neuro-endocrine) and immune markers. These parameters were chosen because of established or probable links to ELS (32).

2.2. Participants

Young adults with no physical or psychotic condition were recruited using posters placed around a University campus. The posters indicated that the study was investigating childhood stress and psychophysiological reactivity, as well as pain. Interested participants were emailed an ID code which enabled them to log on to an intranet website with detailed descriptions of the study, and to access online screening questions to assess their eligibility based on a priori inclusion/exclusion criteria (Table 1). There was no requirement for a history of ELS. If participants provided a response that deemed them ineligible, they were thanked for their interest in the study and no further questions were provided. Participants were informed that they could withdraw from the study at any stage.

2.3. Stage 1: Online screening and questionnaires

Following the eligibility screening and having read the detailed description of the study, participants signified their consent by ticking a box. Participants then completed a series of online questionnaires to provide demographic, health, lifestyle and social data. Questions included their birth weight, the number of addresses they lived at during childhood and the maximum number of children living in their home during their childhood (Table 2). Participants then completed the following standardised questionnaires that measure attachment, mood and personality characteristics. The PBI and CTQ (see 2.3.1 and 2.3.2) were used as predictor variables to measure ELS. On completion of these questionnaires, participants used their unique ID code on the online portal to schedule a meeting time for Stage 2.

2.3.1. Parental Bonding Instrument (PBI)

The Parental Bonding Instrument consists of 25 items using a 4-point Likert scale and is a retrospective recall of the first 16 years of parental experience that is categorised into the two dimensions of Care and Control. These two dimensions usually yield Cronbach alphas > 0.85 and result in four diagnostic categories: High/Low Care x High/Low Control. Factor analysis of the PBI has repeatedly shown the care dimension to be robust, but three-factor solutions can split the control dimension (also labelled overprotection) suggesting a reduced stability [24]. Instruments used to measure parental attachment or bonding tend to fall into one of two categories: self-report or structured interview. In general, the structured interview instruments require training to conduct, may require inter-rater validity checks, are time-consuming to administer and score. By contrast, the self-report instruments are quick to administer and score, and require no specific training. All parental attachment instruments tend to measure related elements of attachment, which can be categorised into secure/insecure avoidant/insecure pre-occupied/and insecure fearful [25]. The PBI used in our study offers a well validated [24,26], stable [27], easy to use option that is considered comparable to the more labour intensive Adult Attachment Interview in normal populations [28].

2.3.2. The Childhood Trauma Questionnaire (CTQ) short form

This questionnaire was administered offline, during Stage 2, due to the publisher's constraints on its use. The CTQ was originally a 70-item questionnaire that is now widely used in a shorter 28 item format. Each of the 28 items are scored on a 5-point Likert scale (1 = never true to 5 = very often true), yielding scores between 5 and 25 for each question. Of the 28 questions, three are used to assess bias towards minimisation of childhood trauma. The CTQ has been found to contain 5 factors – 3 forms of abuse (emotional, physical, sexual) and 2 forms of neglect (emotional, physical) in clinical and non-clinical populations [29,30]. The five factor structure of the CTQ has been replicated repeatedly across many ethnic groups showing good validity and test-retest reliability, though the physical neglect subscale has been challenged for lack of homogeneity [31] which would need to be
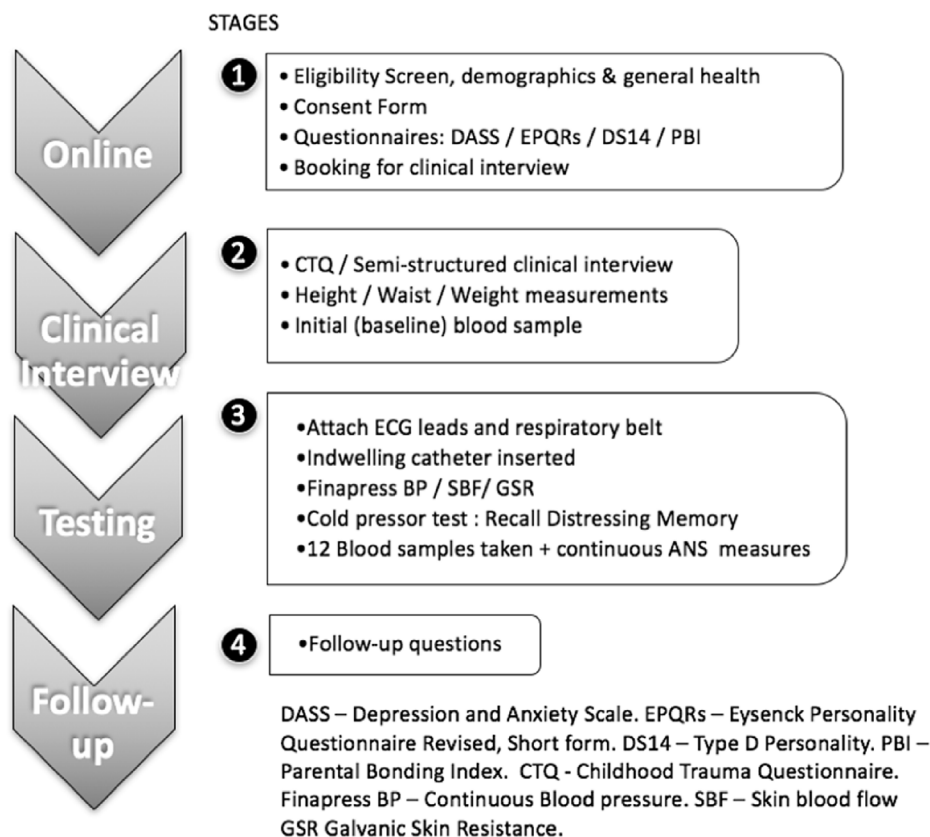
STAGES

Fig. 1. Outline of the 4 stages of the study design, listing the components of each stage.

Table 1
Eligibility criteria.

Inclusion Criteria
- English as a first or main language
- Available to attend 2 appointments at the laboratory
- Able to sit still in a semi-recumbent position for 2 h
- Able to tolerate cold induced pain
Exclusion criteria
- History of psychotic disorders or brain injury or seizures
- Chronic medical condition requiring regular use of anti-inflammatory drugs - diabetes, cancer, autoimmune disease, peripheral vascular disease or recent surgery
- Use of medications affecting the immune system: psychotropics, beta-blockers, ACE inhibitors, ketoconazole (Nizoral), metyrapone (Metopirone)
- Possibly or actually pregnant
- Intellectual disability
- Current drug or alcohol problems (last 12 months)
- Blood or needle phobia

Table listing the inclusion/exclusion criteria for the protocol.

Table 2
Participant characteristics.

N = 5	Mean (SD) or counts
Gender (F/M)	1/4
Age (y)	21.2 (0.8)
Marital Status (Single/Married/Defacto)	5/0/0
Education (Newstep[a]/Degree)	1/4
Handedness (Left/Right/Mixed)	0/5/0
Low birth Weight (< 2.5 kg) (Yes/No/Don't know)	0/4/1
Premature (< 37 weeks) (Yes/No)	1/4
BMI (kg/m^2)	20.9 (2.8)
Waist (cm)	72.8 (7.5)
Smoking (cigarettes/week)	0
Alcohol consumption (standard drinks/week)	1–5
Tetrahydrocannabinol	0
Number of homes for ≥ 3 months till now	1.8 (0.84)
Max number of children living at home, ≥ 4 days/week, till 15yo	2.2 (0.84)
Annual Gross Household Income (< 60 K/ > 60 K)	3/2

[a] Newstep = University entry course.

considered if performing subscale analysis of this questionnaire. Minimisation/denial scores (M = 0.00 SD = 0.00) for the present sample were optimum. Cronbach's alpha coefficients ranged from 0.71 (emotional abuse) to 0.84 (CTQ total).

2.3.3. CTQ < 13y: the Childhood Trauma Questionnaire (CTQ) short form - (modified and administered at the end of interview)

A second CTQ form was administered, identical to the first except that the printed instructions at the top of the page were altered, from "*as a child and a teenager*" to "*only as a child i.e. less than 13 years of age*". Participants were instructed to attempt answering these questions to the best of their ability while focusing only on events before they were 13 years old. This modification was introduced in an attempt to assess any differential impact that early trauma (< 13 years) may have upon the dependent variables. Minimisation/denial scores (M = 0.00

SD = 0.00) for these responses were optimal. Cronbach's alpha coefficients ranged from 0.70 (emotional abuse) to 0.81 (CTQ < 13 total).

2.3.4. Depression Anxiety and Stress Scale (DASS)

The Depression Anxiety and Stress Scale (DASS) is a 42 item self-report mood (state) measure, using a 4 point Likert scale (0 = did not apply to me at all to 3 = applied to me very much or most of the time). There are well-established norms for university student and clinical populations, and gender, age and education account for marginal variance [32]. The DASS has good psychometric properties (Cronbach

alpha > 0.89), is in the public domain, correlates well with other depression scales (convergent/divergent validity) and also measures anxiety and stress [32–34].

2.3.5. Eysenck Personality Questionnaire Revised short form (EPQRs)

The Eysenck Personality Questionnaire Revised Short-form measures four domains of normal personality, with 12 items per scale using Yes/No answers. These domains are Extraversion (a measure of introversion-extroversion), Neuroticism (a measure of emotional stability), Psychoticism (a measure of tough-mindedness) and a Lie scale (a measure of social desirability responses) [35]. There has been a long debate as to the number of stable factors (domains) that can be reliably extracted from linguistic sources or personality questionnaires [36]. Some have argued for 3, 5 or 16 factors [37] but regardless of the number, there is consistent evidence that these personality related domains are generally stable over time [38] and are linked to childhood stress/trauma [39,40]. Neuroticism and extraversion are two domains that have proved stable and are associated with a number of outcome variables, such as self-reported somatic symptoms [41], morbidity, suicidality, mood, and pain [41,42]. The EPQ was used in our study as it measures both neuroticism and extraversion, is well normed, is associated with a range of ELS factors [42,43], and is in the public domain.

2.3.6. The Type-D scale (DS14)

The Type D scale (DS14) is a 14 item, self-report questionnaire with 5-point Likert type responses, used to assess for the presence of type D personality, a distressed characteristic defined by high negative affect and high social inhibition. Showing strong predictive capability in cardiovascular and other disease studies [44–47], it may be considered as an approximation to dysthymia, as it is stable across time and largely independent of mood fluctuations [48]. Both subscales have good test-retest reliability and internal validity [49]. In addition, it has been found to predict poor health related behaviours such as depression [50] and normative data from an Australian study [51] are now available.

2.4. Stage 2: clinical interview and familiarisation

Participants attended a dedicated human physiology laboratory at an appointed time. Participants first completed the paper-based CTQ, followed by a clinical interview, anthropometry measurements, familiarisation with the experimental set up for Stage 3, and collection of a resting blood sample. Also, a consent form was signed during this session.

2.4.1. Semi-structured clinical interview and recall of distressing memories (RDM)

A series of routine clinical questions were posed covering familial history, developmental relationships, current coping strategies, social network, sleep, energy, libido, appetite, auditory and visual hallucinations, drug and alcohol use, medications and previous psychotherapy history, as part of a brief semi-structured clinical assessment for psychiatric illness conducted by a clinical psychologist. At the end of this interview, participants were asked to provide their three most distressing memories (in brief, summary detail), one of which would be chosen for use in the experimental session (see RDM in 2.5.6). The interview was conducted in the laboratory to assist with habituation to the room where the Stage 3 experimental session would be conducted.

2.4.2. Anthropometry measurements, blood sample and experiment familiarisation

Height, weight and waist measurements (umbilicus level) were then recorded prior to a 10 mL blood sample being drawn (an initial "low-stressed" baseline). This sample was always obtained between 1300 and 1330 h to control for diurnal variation [52]. Further explanations regarding the experimental protocol and familiarisation with the equipment to be used in Stage 3 was facilitated before concluding this laboratory session.

2.5. Stage 3: experimental session

2.5.1. Pre-session preparation

Participants were asked to abstain from alcohol, exercise and anti-inflammatory medication for the 4 hours prior to testing and also from eating or smoking for the 2 h prior to testing. Compliance was verbally confirmed.

2.5.2. Session protocol

Participants reported to the laboratory at 1330 h. The participant was seated, semi-reclined, on an examination chair (Pro-lift Venus, Athlegen, Mascot, Australia). A purpose built foam cradle was used to support the arm that was required to remain motionless for the 2–2.5 h of the session. An indwelling catheter was inserted into a forearm vein by a phlebotomist (nurse). Devices for non-invasively recording heart rate, arterial blood pressure, breathing pattern, skin blood flow, and galvanic skin resistance were attached. A first (baseline) blood sample (time = 0 min) was taken immediately prior to the CPT. Participants then underwent the cold pressor test for up to 4 min; blood samples were collected at 5, 10, 15 and 30 min post baseline. The 30-min sample served as the baseline measure for the RDM test; blood samples were also collected at 40, 45, 50, 55, 70, 85 and 115 min.

2.5.3. Autonomic nervous system measures

Heart rate was recorded by electrocardiograph (ECG) (2.0 kHz) using 3 disposable Ag-AgCl surface electrodes in Lead II configuration. Blood pressure (BP) was recorded non-invasively and continuously using finger pulse plethysmography (Finometer Finapres Medical Systems, The Netherlands) and sampled at 400 Hz. Ventilation (breathing pattern) was recorded using a strain gauge piezoelectric transducer (DC-100hzH; Pneumotrace, UFI Morro Bay CA, USA) wrapped around the chest (sampled at 100 Hz). A photoelectric pulse plethysmograph (ADInstruments, Sydney, Australia) was used to measure skin blood flow from a finger pod. Finally, galvanic skin resistance (GSR Amp, ADInstruments, Sydney, Australia), which is inversely related to sudomotor activity, was recorded. All these physiology devices were applied to the hand ipsilateral to that where the venous catheter was inserted. In addition to the continuous BP measurement, sphygmomanometer measures were taken after each blood sample (see Fig. 2) and recorded.

All physiological recordings were stored on a laptop computer (10 kHz sampling) using a computer based human data acquisition and analysis system (Powerlab 16SP hardware and Labchart Pro 7 software, ADInstruments, Sydney, Australia) for off-line analysis. This allowed assessments such as heart rate variability using power spectral analyses yielding three frequency domain measures of very low frequency (vLF) power $\leq$ (0.04 Hz), Low frequency (LF) power (0.04–0.15 Hz) and High Frequency (HF) power ($\geq$0.15 Hz) [53] to be obtained. Power frequency (Hz) could be converted to ms^2 by fast Fourier transformation using Lab Chart HRV module software (ADInstruments, Sydney, Australia).

2.5.4. Blood sample processing

Blood samples (10 ml) were collected in labelled pre-chilled ethylene-diamine-tetra-acetic acid (EDTA) tubes, from an indwelling venous catheter in the antecubital fossa. After sample collection each tube was inverted 4–5 times, then returned to the fridge till the testing period was completed. Blood samples were spun for 15 min at 1000 g at 0 °C, and then the supernatant from each sample was aliquoted into nine, 1000 µl Eppendorf sample tubes with volumes between 40 and 750 µL. The samples were then frozen at minus 80 °C for later analysis of plasma levels of immune and stress markers.
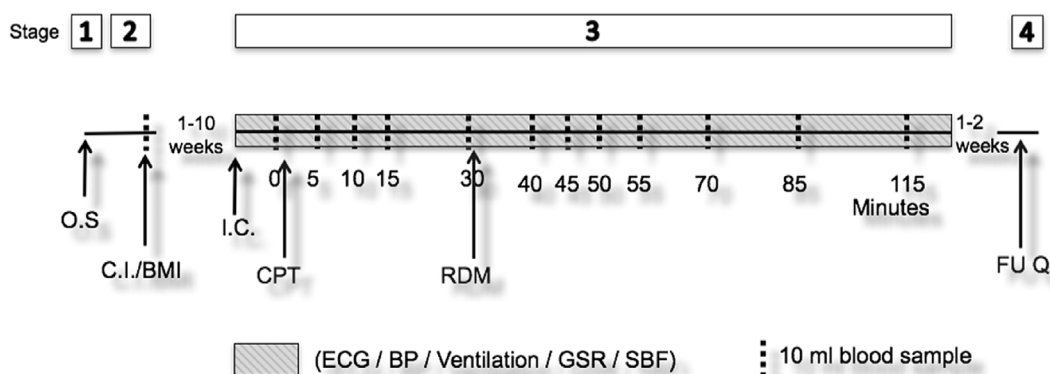
O.S. – Online screen; CI/BMI - Clinical Interview and Body Mass Index;
I.C. - Indwelling catheter; CPT – Cold pressor test (≤ 4 min duration); RDM - Recall of
Distressing Memory (5-8 min duration); FU Q – Follow-up Questions; ECG –
Electrocardiograph; BP – Blood pressure; GSR – Galvanic skin resistance; SBF – Skin blood flow (plethysmography)

Fig. 2. Experimental session overview, showing the chronological relationship of the various stages of the protocol.

2.5.5. Cold pressor test (CPT)

Using an outer container filled with ice to maintain constant water temperature, the inner water bath was maintained between 2-3 °C, to a constant depth (approximately 15 cm), though the water was not circulating. Participants were instructed to keep their hand (up to their wrist, no fist) immersed in the cold water for as long as they could bear up to 4 min (tolerance), notifying the research assistant when the cold sensation was first considered painful (threshold). Participants were instructed to remove their hand from the ice water once 4 min had elapsed (if still immersed), and asked to rate their maximum pain during the CPT on a visual analogue scale (VAS) (no pain to extremely painful) that was quantified on a 0–100 scale on the reverse side of the slide. Once their hand was dried and warmed to body temperature to terminate the effect of the cold stimulus they commenced watching a benign nature DVD till the RDM (~20 min).

2.5.6. Recall distressing memory (RDM)

After the 30-min blood sample was obtained (Fig. 2) the participant was instructed to close their eyes and recall as vividly as they could, a distressing memory. The memory was chosen by the registered clinical psychologist from the three memories related during the clinical interview on the basis of being the most vivid; i.e. able to be recalled and visualised distinctly. In order to enhance the emotional response to the memory, they were instructed to focus on the part of the memory they found to be the most vivid and to describe that in detail. The autonomic measures continued uninterrupted throughout the RDM phase as in Fig. 2. Following the RDM phase (5–8 min), participants were purposely engaged in conversation regarding the DVD content or about their course at university to curtail further active rumination of the memory over the remaining 65 min.

A five step safety procedure was followed with these participants: i) they were debriefed after the experimental procedure; ii) they were invited to phone the clinical psychologist if they felt the need to talk further – not utilised; iii) they were sent a follow-up questionnaire where they could express any concerns and thereby initiate more active follow-up procedures; iv) the university clinical psychology clinic was informed of the experimental paradigm and agreed to accept referrals at short notice – this wasn't needed; and v) being assured that their names would not be recorded or used on any of the experimental records (apart from their consent form) or data forms, assured them of anonymity for all questionnaires and physiological recordings.

2.5.7. End of experimental session

At the end of the recording period, participants were debriefed, provided with remuneration ($100 AUD) for their time, and asked to

contact the investigators if there were any after-effects or concerns arising from their participation in the study. Participants were asked to complete and return the follow-up questionnaire (see 2.7) regarding the acceptability of the study components.

2.6. Blood sampling

Stress response profiles for the following endocrine (ACTH, cortisol, adrenaline, noradrenaline, DHEA) and immune (IL-6, IL-1β, TNFα, IL-10) variables are planned. ACTH and cortisol plasma levels are more regularly assessed in response to different stressors. There is evidence from samples of controls and depressed/traumatised participants that ACTH/cortisol levels increase rapidly (< 15 min) in response to stressors [54–57]. There is also evidence that IL-6 and IL-1β can remain significantly elevated 90–120 min following social stress [58,59]. The blood sampling protocol was therefore constructed to maximise the possibility of detecting such changes in these stress profiles.

2.7. Stage 4. Follow-up questions

After a 1–2 week period, participants were emailed a list of questions regarding their experience during the study. The questions covered their recovery from the CPT and the RDM stressor. Space was provided for further open-ended comments regarding the experimental protocol.

2.8. Data analysis and sample size

Analyses are planned to be performed on JMP 11 statistical software and IBM SPSS statistics version 24. Mean ± SD, counts, and graphical exploration will be carried out using the JMP software, that is more versatile than SPSS in visual representation of data. The results from the CTQ and PBI subscales will be utilised as predictor variables to measure ELS. The dependent (outcome) variables will include the psychological measures (DS14, EPQ, DASS, life-stressors), physiological measures (blood samples, ANS measures) and pain measures (threshold, tolerance and max pain). We propose to carry out a series of univariate regressions to assess the association between our predictor variables (CTQ/PBI) and the various outcome variables from the different systems (psychological, ANS, immune, HPA, endocrine). If significant relationships are found from the univariate analyses, then multivariate analyses will be carried out to identify the relationship (including interactive effects) between those significant dependent variables (potentially from different systems) and the predictor variables.

Linear mixed models will be considered for those outcome measures

with repeated measures. The CTQ and PBI have not been utilised previously as joint predictor variables, and only once measured together to assess main effects for odds ratio calculations [60]. To our knowledge, no interaction has ever been reported between them.

The overall plan for the study is to compare non-clinical and clinical groups. The first stage was to assess feasibility in five participants; the next phase is to extend this to 25 non-clinical participants to further evaluate the protocol; the final phase is to extend the study to 25 participants from a clinical population (chronic pain). The primary research question is concerned with the effect of ELS on adult physiology. Cortisol was chosen as the index analyte for conducting a power analysis. Data from Yehuda 2004 [61] was used as the basis for the calculations. A sample size of 22 in each group (non-clinical/ clinical) would allow detection of a 60% difference between the groups in the Pre/Post acute-stressor analysis with power = 0.80 and alpha = 0.05, mean(SD) baseline cortisol of 11(8) (ug/dl), and correlation between pre and post = 0.37.

3. Results

3.1. Participant recruitment and characteristics

Nineteen participants inquired about the study (Fig. 3) and five completed the experimental session and returned the follow-up questionnaire. Table 2 summarises the psychosocial and biometric characteristics of the participants. The results indicate we were able to recruit a group of young adults, with primarily normal BMI (Range: 17.2–24.6 kg.m-2), normal mood and with characteristics consistent with a stable psychosocial history. The DASS mean(SE) scores for depression/anxiety/stress were 7.4(3.1)/7(4.2)/15.2(7.5), which were in the normal (or just mild) range.

3.1.1. General health

Few participants had a presence (or history) of any of the 11 common health complaints (Table 3). One 22yo female who reported a PTSD diagnosis was raped when 14yo. She was already linked to mental health services for treatment. Based on DSM V criteria, it is questionable whether she currently met criteria for PTSD as she mainly reported avoidance behaviours i.e. 9 + hours sleep each night, and "keeping herself busy". Her mood was described as "good" and "happy" and she didn't rate herself depressed or of having a depressive history. This trauma was the only event deliberately not chosen in the RDM section of the laboratory testing. This same participant accounted for 4 of the 8

Table 3
General health of participants.

N = 5	Past or Present
High BP	
Asthma	2
GI Disorder	1
CFS	
OA/RhA	
Cancer	
Stroke	
Diabetes	
PTSD	1
Depression	2
Anxiety	2

BP: Blood Pressure.
GI Disorder: Gastro-Intestinal disorder.
CFS: Chronic Fatigue Syndrome.
OA/RhA: Osteo and Rheumatic Arthritis.
PTSD: Post-traumatic Stress Disorder.
List of common health complaints (past and present) often associated with various form of stress.

"past or present" responses (PTSD/Depression/GI disorder/asthma) reported in Table 3.

3.2. Feasibility of protocol

3.2.1. Physiological recordings

Fig. 4a shows the raw data obtained from one participant. While a clear signal to noise ratio was obtained in all physiological traces at rest (ECG, BP, ventilation, skin blood flow and resistance), we found that the use of conversation with the participants to distract them from ruminating over the RDM stressor compromised the ventilation record (Fig. 4b). The introduction of a flashing light was used in subsequent participant sessions to signal a 3-min period of silence for the participant, commencing one minute prior to each blood collection point; this facilitated collecting 2 min of quality ventilation recording in subsequent recordings. While 2–3 participants evinced a transient stiffness in the immobilised arm after the 2–2.5 h of recording, none complained of prolonged discomfort.

3.2.2. Venous catheter insertion

In order to obtain 12 blood samples over a 2.5 h period an indwelling venous catheter was used. As indicated in Table 4 the
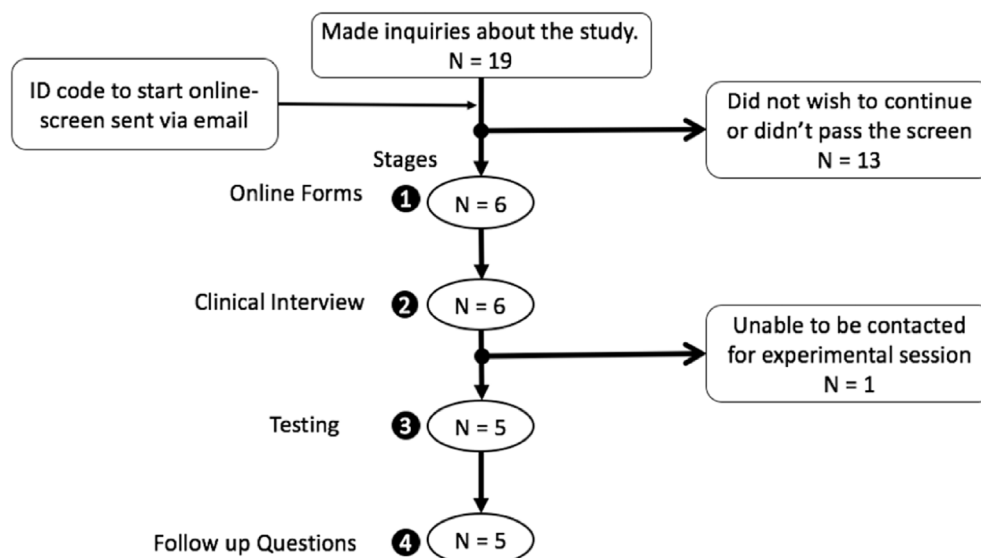

Fig. 3. Flowchart of trial participant numbers through the 4 stages of the protocol.
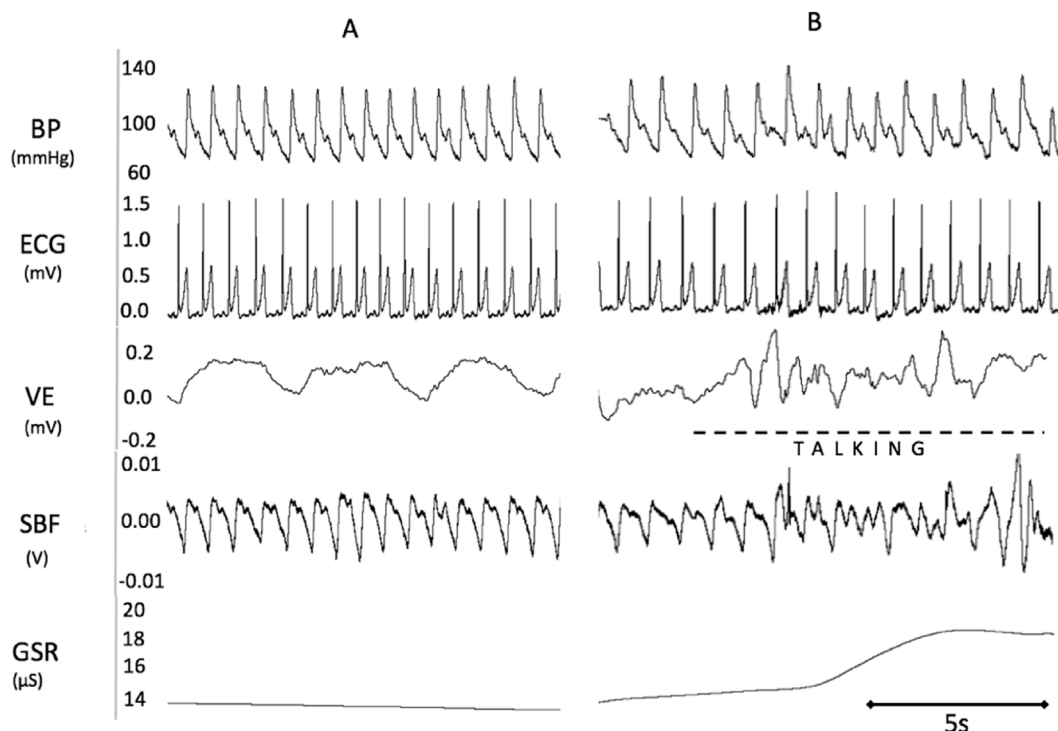
Fig. 4. Example of signals recorded during a segment of the experimental protocol in one participant while silent (panel A) and talking (panel B – dashed line). The recordings in Panels A and B were separated by less than 30s. BP – Blood pressure; ECG – Electrocardiograph; VE – Ventilation; SBF – Skin blood flow; GSR – Galvanic skin resistance.

Table 4
Questions and responses from the follow-up questionnaire pertaining to the cold pressure test (CPT).

	"No"
1. Did you experience any after-effects following the cold-water test?	5
2. Did your hand feel abnormal by the time you left the lab?	5
3. Were there any other concerns regarding your hand?	5

follow-up questionnaire revealed that most participants (4/5) tolerated the catheter insertion and blood sampling. One participant described pain during the catheter insertion and bruising at the site of catheterisation, which took longer than one week to resolve, however this participant did not seek medical care for the bruising. Another participant fainted and developed a mild tremor for several minutes upon seeing 1–2 ml of blood escape around the insertion point. No after-effects were verbally reported at the end of the testing session or in response to the follow-up questions. These events highlight the need for a skilled phlebotomist as part of the research team to reduce the number of participants whose data may be compromised, as pain induced by the blood collection may confound the study design.

3.2.3. Cold pressor test (CPT)

A stable ice water temperature (2–3 °C) was achieved for each participant. Ambient room temperature ranged between 22.2 and 23.4 °C, and humidity between 45 and 62%. Tolerance for the CPT had a mean($\pm$ SD) of 133 $\pm$ 85 s and a wide range from 17 to 240 s. Threshold, the time to pain being first reported, had a mean of 21 $\pm$ 19s, and a wide range of 4–93 s. The participants mean max pain experienced during the CPT using the VAS rating was 55 $\pm$ 13 s and a wide range of 16–73 s on the 0–100 scale. These values are comparable to other non-clinical populations undertaking the CPT [62]. The use of the towel and rapid hand warming (3–5 min) terminated the pain and cold sensations following removal of the hand from the ice water. Hand warming for all participants continued till they reported they were comfortable.

3.2.4. RDM details

The examples of distressing memories provided by the participants included: bereavement (death of a valued grandparent/cousin/father), physical stressor (severe acne), violence (raped at 14yo), relational stress (tense parental relationship, breaking up with boyfriend, bullied at dance classes, being locked in the kitchen by mother), social stress (father's mistress tried to take family home). While only one participant felt the need to discuss their feelings with someone (22yo female referred to previously), this was with her partner rather than a mental health professional. Another participant who during the clinical interview was advised to seek professional help regarding assertiveness training, reported they acted on this advice (see Table 5). During the RDM phase, participants' non-verbal cues (e.g. facial muscle changes, altered skin colour) along with tonal changes during their vocalised recall of remembered events, indicated that they focused on the subject matter. In addition, an elevated systolic BP recording (Mean Systolic/

Table 5
Questions and responses from the follow-up questionnaire pertaining to the recall of a distressing memory (RDM) and acceptability of the study protocol.

	No	Yes
1. Did you experience any after-effects following the recall of a distressing memory?	4	1
2. Did you experience a sense of relief or freedom afterwards?	3	2
3. Did you experience a sense of distress afterwards? If you answered "yes" how long did it last? __hours __days	3	2 / 1 day, x2
4. Did you feel the need to speak to anyone about your feelings afterwards?	4	1[a]
5. Did you feel the need to seek professional help afterwards?	4	1[b]
6. Did you feel the need to contact the CI?	5	
7. Would you be willing to participate in a similar experiment again?	Maybe	4

Additional comments: Hand bruised for one week, and each blood draw was painful.
[a] Discussed with partner, not health professional.
[b] Participant sought professional advice regarding assertiveness, not about the RDM.

Diastolic BP, 0 min: 110/65 & 40 min: 124/76 mmHg) during the RDM phase confirmed physiological reactivity occurring.

3.3. Follow-up data

Questions about the recovery from the study experience and the two stressors (CPT/RDM) were sent 1–2 weeks after the experimental session. Four participants returned their questions within 1 week, one participant however took 5 weeks. All five participants reported no after-effects, no altered sensation in their hand and no concerns in general from the CPT after they left the laboratory (see Table 4).

The follow-up questions about the RDM indicate that 5/5 participants managed the experience tolerably well (see Table 5). The aspect of the protocol that was most negatively perceived was in regard to bruising following difficulty with cannula insertion. In addition, two of the participants reported some emotional distress, which lasted one day for both. Of note was that four of the five participants were willing to repeat the testing procedure.

4. Discussion

This is the first stage of a study to develop a biological index of ELS. This stage investigated the logistical feasibility, acceptability and safety of the protocol in five participants. We have demonstrated that cardiovascular, endocrine and stress responses can be safely and simultaneously recorded in young adults being assessed for relational ELS and that this does not require a clinical setting. This protocol has demonstrated that in addition to various psychological measures, it is possible to concurrently examine an individual's physiological reactivity to both an acute psychological (RDM) and a physiological (CPT) stressor. This has been achieved by combining online screening, clinical interviews, physiological recordings and the collection of sequential blood samples. Although the protocol is demanding, requiring participants to remain stationary in a semi reclined position for a moderate period of time while being challenged by both psychological and physiological stressors, participant feedback indicated that they not only tolerated the protocol well but would also be prepared to return and repeat the protocol. These findings provide support for proceeding to the second stage of this study using this protocol in a larger number of healthy participants (n = 25), which should be sufficient to indicate any further changes that may be required before proceeding to a clinical population, stage 3.

4.1. Safety and logistic issues

Given the university setting, feasibility and safety issues needed to be addressed in formulating this protocol. Screening for food/exercise/alcohol compliance, connecting and calibrating five physiological transducers (ECG/respiratory/BP/GSR/SBF) and inserting an indwelling catheter within 30min does require coordinated teamwork. Developing a standardised routine for attaching the transducers facilitated this process. All transducers used were recording devices, not requiring any electrical stimulation of the participants. AD instruments meet IEC6060-1 safety standards for medical electrical equipment. The three components in the experimental protocol where safety was a potential concern were: i) blood collection, ii) CPT, and iii) RDM which are discussed below.

i) Blood collection: A luer lock injection port worked well over the 2–3 h. Routine hygiene and safety standards (gloves, sterile needles etc.) were handled under the direction of the nurse. The indwelling catheter was taped in place to minimise unwanted movement over the 2–3 h. A medical officer was also based less than 2 min away. Additional screening questions covering haemophilia, blood clotting disorders, or known infections (MRSA, HIV) or allergies, will be added to the routine induction protocol prior to the catheter being inserted.

ii) CPT: Reported CPT protocols vary in maximum exposure time limit (2–4 min) and in water-temperature used (0–10 °C). The 4-min limit was used because hand withdrawal from the iced water was voluntary and once 4 min had passed, numbness could defeat the pain/stress induction purpose. The water-temperature (2–3 °C) was selected by default given the use of an outer ice-container and inner ice-water container filled to a constant height.

iii) RDM: We found seven previous studies that had utilised direct trauma recall (excluding trauma narrative/scripts) as part of the acute stressor protocol, though none in relation specifically to early life events. These included recent physical/sexual assault [63–67] motor vehicle or war [68,69]. Of note was that none of these report precautionary steps to monitor the psychological reaction of the participant after the testing session. One study evaluated the potential risks of this form of psychological stressor on recently traumatised participants and reported an absence of detrimental effects [70], and as here, an overwhelming majority would be happy to repeat the assessment process. Caution is indicated though in that no long-term follow-up was undertaken as noted by the authors. We therefore would consider it advisable that an experienced clinician be involved with the RDM process to select stressful memories and make on-site decisions regarding the wellbeing of participants. This would be all the more important when extending this protocol to a clinical population, which would be necessary to evaluate any putative bio-index of ELS. One could anticipate more serious childhood ELS within a clinical population, and the emotional responses post-RDM stress would need careful management.

4.2. Justification for design

4.2.1. Use of RDM

While use of the Trier Social Stress Test may seem a preferable psychological stressor because of the convenience of standardization, RDM was chosen as the psychological stressor for four reasons. *First*, the logistics of the TSST require the availability of 2–3 other personnel (in addition to the 3 researchers busy with experimental measures) and additional equipment (camera/seats) making the data collection impractical in our compact laboratory setting; in addition, we wanted to limit movement artefacts from our physiological recordings. *Second*, we considered that recall of a distressing memory would be very salient to most chronic clinical populations and would have better face validity, e.g., chronic pain patients would more frequently be alone with intrusive distressing memories than facing a panel for an impromptu speech. *Third*, recent investigations into the association between altered memory for early life trauma and depression/affect regulation made this approach of relevance when exploring the impact of ELS [71–74]. We are aware of only one other group who have employed a similar stress induction paradigm other than PTSD [75,76] and this was done in relation to recent anger/sadness, not ELS. *Fourth* we are seeking to monitor how the participants' ELS history impacts upon their physiology. We consider that ELS factors are more likely linked with past memories, than a generalised social stress. For instance, in PTSD subjects, rumination (not subjective distress levels upon recall) was reportedly related to physiological reactivity [67]. Further, there is evidence that autobiographical traumatic material is recalled differentially (and counter-intuitively) depending upon the intensity and age of trauma [77]. Together, these observations point to the inadequacy of using a general psychosocial stressor and the potential advantage of using more specific memories.

4.2.2. Two stressors

Using two distinct stressors (physical and psychological) is consistent with the understanding that the psychophysiological reaction is idiosyncratic to the nature of the stressor and the individual participant [78,79] and conceivably modified by the nature/severity of their ELS.

This would presumably permit an evaluation of the physiological reactivity, so that identification of what elements are consistently triggered and which are stressor-specific can be made.

4.3. Strengths

4.3.1. Range of variables

To our knowledge, there has not been a previous attempt to measure this breadth of variables in the same individuals in relation to ELS although there has been an increased awareness of the need for measurement diversity in the psychosomatic domain [80]. In spite of the complexity and length of the protocol it is clear that when performed as reported here it is acceptable, at least by young healthy individuals, with 4 of the 5 participants indicating they would be happy to return to repeat the protocol.

4.3.2. Measuring trauma and attachment

Remarkably, previous studies measuring ELS have not examined the role of attachment as a possible moderator of the impact of childhood trauma. That is to say, for any given childhood trauma, the impact upon the child may vary depending upon their attachment status, i.e., secure or insecure-avoidant, or insecure-anxious, etc. While we found 15 articles reporting the use of the CTQ (the most commonly used childhood trauma scale) and the PBI in the same study, none reported assessing if they interact when measuring a behavioural, psychological or physiological dependent variable. We consider this absence most surprising.

4.3.3. Linear vs dichotomous design

In addition, we plan to seek factors that show a continuum (linear or non-linear) with ELS, rather than simply seek factors with statistically different means in relation to high/low ELS. By contrast, previous work has linked the presence/absence of ELS categories (e.g. emotional abuse or physical neglect) or composite subscale totals, to the specific pathology/risk-factor being investigated.

4.4. Limitations

4.4.1. Population characteristics

There were various conceptual (outcome measures and stressors) and methodological (laboratory or clinical setting, blood sample collection, ventilation record, ANS measures) considerations that have been identified that may need refining when testing a clinical cohort. With the exception of one participant (rape victim), our pilot cohort was relatively free of comorbidities and possible confounders present in the community and known to be more frequently present in clinical samples. For example, medication usage (only one participant used medication - an SSRI antidepressant) was consistent with that expected among the general population but less than persons with clinically significant ELS levels [81,82]. These factors need to be considered when determining the sample size, design and statistical analysis of a study involving a clinical population. In this regard it is also important, as exemplified by the single person with an ELS memory of a sexual assault, that future studies using the protocol reported here need to carefully take note of the nature and clinical circumstances of the ELS under investigation.

In the next stage, N = 25, we expect a relatively low-moderate range of ELS based on CTQ/PBI results. We therefore anticipate that any positive skew of these predictor variables will either be handled using a transformation, or the use of statistical methods that don't require the assumption of normal distribution.

4.4.2. Unilateral recordings

A potential methodological weakness of this study was that the physiological measurements, such as real-time BP (Finometer) and skin sweating (GSR), were obtained from the same arm as that in which the indwelling catheter was inserted. This was necessary to allow the alternate hand to be free of instrumentation so that it could be inserted into the ice water to perform the CPT. The events associated with the cannulation of two of the study participants also highlight the need for a skilled phlebotomist as part of the research team to reduce the number of participants whose data may be influenced by pain induced by the blood collection, which may confound the study design. We did not observe gross modulation of the physiological parameters measured in the same arm while taking blood samples during the study. Nevertheless, to minimise any potential impact, as the recordings are continuous, inspection of the physiological data sets can be performed at time points corresponding to the blood sampling, to assess for this possibility.

4.4.3. Fixed order of stressors

We chose a fixed order of stressor presentation, as we reasoned that while the ANS system would recover in under 30 min, the immune and endocrine systems would have a delayed response of at least 30–90 min [83]. As there is no previous study focusing on ELS to consider, we anticipated that the endocrine-immune response would be of greater duration for the RDM than the CPT. It should also be remembered that even for the TSST, there is a fixed order of stressors that presents confounds for delayed responses [84]. Whether the assumptions justify this order will be examined in the next phases of the study.

Given the continuing growth of evidence linking ELS with adult depression and chronic physical conditions [85], the capacity to measure endocrine, immune, and a range of autonomic functions will allow us to explore interactions between these systems and ELS. This in turn may reveal a more reliable estimate of ELS's effect upon adult physiology and a better understanding of the clinical presentation of persons with ELS.

5. Conclusion

This initial phase of the study has established the viability, safety and acceptability of the protocol as outlined here. While acknowledging the ambitious nature of the study, we need to complete our target of assessing 25 participants (phase 2) from this university population with a view to investigating whether there are ELS related physiological effects that can be detected even in a physically healthy student population. It is anticipated that modifications to our protocol will be required before we move to a clinical population (phase 3). In addition, the introduction of RDM as the psychological stressor, while utilised in PTSD studies has not been previously employed in ELS research and brings novel challenges with its deployment. The effectiveness of RDM within this population remains to be established. However, and more importantly, we remain of the opinion that viewing ELS as a continuum, rather than a dichotomous factor, will reveal new understanding as to how ELS factors impact upon adult physiology. Finally, ELS may prove to be a very elusive variable, but if it can be objectively quantified, could hold substantial explanatory power for a wide range of psycho-physiological factors.

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