# PEER REVIEW HISTORY

## ARTICLE DETAILS

| TITLE (PROVISIONAL) | The RAISE study protocol; a cross-sectional, multi-level, neurobiological study of studying resilience after individual stress exposure |
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| AUTHORS | Moreno-López, Laura; Sallie, Samantha; Ioannidis, Konstantinos; Kaser, Muzaffer; Schueler, Katja; Askelund, Adrian; Turner, Lorinda; van Harmelen, Anne-Laura |

## VERSION 1 – REVIEW

| REVIEWER | S.J.A. van der Werff<br>Leiden University Medical Center |
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| REVIEW RETURNED | 08-Jul-2020 |

| GENERAL COMMENTS | This is a comprehensive written study protocol, which aims to integrate multiple neurobiological characteristics with respect to resilience to childhood adversity.<br>I have some comments which the authors could address to improve the manuscript:<br><br>1. With respect to the variables addressed in hypothesis 6: How do the authors expect these to distinguish from the variables used to define resilience. To this reviewer they seem to be exactly what the authors refer to as psychosocial functioning, a term they also use to corner resilience.<br><br>2. Are all CA within the context of family? And if yes, how do you control for the presence of childhood trauma outside of the context of family?<br><br>3. By excluding psychiatric disorders the authors are very much limiting the data to the resilience side of the spectrum, and subsequently decreasing variation of the data. If we consider psychiatric disorders as labels referring to psychological and psychosocial dysfunction, shouldn't these individuals also be represented in the study? You would also have the added benefit to check the PCA scores between individuals with psychiatric disorders and those without.<br><br>4. Could the authors be more specific on the type of functional connectivity analysis they have planned? |
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| REVIEWER | Sarah Baracz<br>The University of New South Wales, Sydney, Australia. |
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| REVIEW RETURNED | 27-Oct-2020 |

| GENERAL COMMENTS | Moreno-Lopez and colleagues have produced a very detailed protocol for their ongoing cross-sectional study. Overall, it is well written, and I only have a few comments. |
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| | 1. The introduction is very brief. The inclusion of some more information pertaining to relevant factors that will be addressed in the aims would help improve this section. For example, including information about why increased grey matter is expected in the hippocampus and prefrontal cortex of resilient individuals relative to those who are more vulnerable. |
| | 2. Considering that the composite score of resilient functioning is a crucial component of the study, how this score is calculated should be included in the manuscript, rather than referring the reader to another paper. |
| | 3. How long after phase II will the follow up (phase III) be completed? |
| | 4. In figure 1, the authors refer to early life stress as childhood maltreatment, however in the rest of the manuscript, they refer to this as childhood abuse. Please adjust to be consistent with the rest of the manuscript. |
| | 5. In the supplementary document, the authors have stated that two of the four subtests from the WASI were administered to calculate IQ. Can the authors explain why all four subtests were not administered? |
| | 6. Information pertaining to ethical approval of the study should be included in the body of the manuscript in addition to in the abstract. |

## VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

This is a comprehensive written study protocol, which aims to integrate multiple neurobiological characteristics with respect to resilience to childhood adversity.

I have some comments which the authors could address to improve the manuscript:

1. With respect to the variables addressed in hypothesis 6: How do the authors expect these to distinguish from the variables used to define resilience. To this reviewer they seem to be exactly what the authors refer to as psychosocial functioning, a term they also use to corner resilience.

Thank you for your comment. We have realised that our description of resilient functioning and what it entails was unclear and have edited the introduction accordingly.

Please see bellow and pages 3 and 4 of the manuscript.

'However, although CA is associated with considerably lowered odds of adequate mental and physical health functioning later in life, a significant proportion of individuals with a history of CA function 'better than expected,' or, in other words, are "functioning resiliently" [32]. These individuals may have benefited from protective 'resilience factors' [33,34] which exist across social, cognitive, neuronal, physiological and genetic levels. For example, good mental health after CA has been associated with increased hippocampal volume and greater connectivity between the central executive network and limbic regions as well as a greater ability to regulate emotions [35], higher self-esteem [36,37] and social support [32,38].'

We have also clarified (based on the reviewer comments below) the specific analyses used to calculate resilient functioning:

4.1. Preprocessing

'Quantification of resilient functioning

Using the data collected during the first online assessment, we will calculate gender and age-related degree of resilient functioning based on the model described in van Harmelen et al. [32]; see also Ioannidis et al. [39] for a description of the benefits and pitfalls of this method. Specifically, we will conduct two PCAs; one for psychosocial functioning using standard-normally transformed individual total scores on the MFQ, RCMAS, LOI, CBC, K10 and WEMWBS; and another one for CA, including standard-normally transformed sum scores for the MOPS, APQ, FAD and CTQ. From both analyses, we will extract individual scores for the first component to reflect individual current psychosocial functioning and recalled CA experience scores. Next, we will regress the psychosocial functioning component score against the CA score, testing for possible linear, quadratic or cubic relationships. From this model, we will extract the residual scores as a measure of individual degree of resilient functioning: the extent to which an individual has better, or worse, psychosocial functioning than the average score expected given their CA experiences. For parsimony, we will refer to this as degree of 'resilient functioning' with higher scores reflecting better (conditional) psychosocial functioning outcomes. These individual resilient functioning scores will be utilised in the analyses described below.'

We hope the reviewer agrees it is now clearer that whilst resilient functioning is calculated from degree of psychosocial functioning relative to childhood adversity experienced, the variables mentioned in hypothesis 6 refer to resilience factors that aid resilient functioning after CA, but are not included in the calculation of resilient functioning.

After reviewing the variables included in hypothesis 6 and the calculation of psychosocial functioning, and based on the reviewer's comment we realised that we erroneously wrote that we would include the Rosenberg self-esteem scale (RSES) in the calculation of psychosocial functioning. This has never been our intention (and is in line with our previous calculations, see van Harmelen et al., 2017 and 2020). Rather, we believe that higher self-esteem, more friendship and family support, in those who are more resilient lead to lower stress responses in the MIST, including physiological stress responses, as well as lower reported anxiety and perceived stress. We have now edited hypothesis 6. Please see below and page 5 of the manuscript:

'2. Objectives and hypothesis
6) higher self-esteem and more friendship and family support which will aid lower anxiety and perceived stress after the MIST [36, 58].'

2. Are all CA within the context of family? And if yes, how do you control for the presence of childhood trauma outside of the context of family?

In our calculation of Childhood Adversity we do indeed include adverse life experiences outside the family environment too. We now explain this more clearly in the introduction;
'Childhood adversity is the leading preventable risk factor for mental illness and substance abuse [1-8]. This kind of experiences, which can happen within the family environment (e.g., in the form of childhood maltreatment and/or intra-family adversity) or outside the household (e.g., trauma and bullying), can have a detrimental impact on a wide range of functions.'

3.2. Testing protocol and procedure

Phase I: online assessment

The online assessment will include signing an informed consent form (ICF), the completion of a set of self-report questionnaires and two cognitive tasks online. The self-report questionnaires included in this assessment will be the Mood and Feelings Questionnaire (MFQ; [61]), Revised Children's Manifest Anxiety Scale (RCMAS; [62]), Leyton Obsessional Inventory (LOI; [63]), the 10-item version of the Kessler Psychological Distress Scale (K10; [64]), the Child Behaviours Checklist (CBC; [65]), the Warwick-Edinburgh Mental Well-being Scale (WEMWBS; [66]), the Measure of Parenting Style (MOPS; [67]), the Alabama Parenting Questionnaire (APQ; [68]), the Cambridge Friendship Questionnaire (CFQ), Family Assessment Device (FAD; [69,70]), Rosenberg Self-Esteem Scale (RSES; [71]), Childhood Trauma Questionnaire (CTQ; [72]) and the Drugs, Alcohol, and Self-Injury Inventory (DASI; [73]). These questionnaires will be used to calculate a composite measure of resilient functioning as described below. The cognitive tasks will be the Emotional Stroop task [74]

and Emotional Regulation task [75]. More information about these measures is provided in the Supplementary Material.

And all CA assessments are included in our analyses:

4.1. Preprocessing

'Quantification of resilient functioning

'Using the data collected during the first online assessment, we will calculate gender and age-related degree of resilient functioning based on the model described in van Harmelen et al. [32]; see also Ioannidis et al. [39] for a description of the benefits and pitfalls of this method. Specifically, we will conduct two PCAs; one for psychosocial functioning using standard-normally transformed individual total scores on the MFQ, RCMAS, LOI, CBC, K10 and WEMWBS; and another one for CA, including standard-normally transformed sum scores for the MOPS, APQ, FAD and CTQ. From both analyses, we will extract individual scores for the first component to reflect individual current psychosocial functioning and recalled CA experience scores. Next, we will regress the psychosocial functioning component score against the CA score, testing for possible linear, quadratic or cubic relationships. From this model, we will extract the residual scores as a measure of individual degree of resilient functioning: the extent to which an individual has better, or worse, psychosocial functioning than the average score expected given their CA experiences. For parsimony, we will refer to this as degree of 'resilient functioning' with higher scores reflecting better (conditional) psychosocial functioning outcomes. These individual resilient functioning scores will be utilised in the analyses described below.'

3. By excluding psychiatric disorders the authors are very much limiting the data to the resilience side of the spectrum, and subsequently decreasing variation of the data. If we consider psychiatric disorders as labels referring to psychological and psychosocial dysfunction, shouldn't these individuals also be represented in the study? You would also have the added benefit to check the PCA scores between individuals with psychiatric disorders and those without.

Thank you for your comment. Although we will include participants with past histories of psychiatric disorders and current subthreshold mental health disorders, we acknowledge that excluding patients with current psychiatric disorders may limit the data to the resilience side of the spectrum. This bias has now been included within the 'strengths and limitations' section and discussion of the study. Please see below. #xa0;

'Strengths and limitations

'The RAISE study will provide a comprehensive evaluation of the neurobiological mechanisms that contribute to adolescent resilience.

We will use standardised and validated instruments of psychological functioning, childhood adversity, cognitive tasks, venepuncture and neuroimaging.

The exclusion of psychiatric patients will restraint the data to the resilience side of the spectrum

Child adversity will be assessed from self-reports that are subjected to reporting biases.

A longer recruitment period may be required due to Covid-19.'

Discussion

Although we will take a dimensional approach, examining individual variation in degree of resilient functioning and including individuals with past histories of psychiatric disorders and subthreshold mental health disorders, the exclusion of patients with current psychiatric disorders will limit the interpretability of the data and our findings. Future studies should include participants with current mental health disorders to ensure the representation of both dimensions of the spectrum.

4. Could the authors be more specific on the type of functional connectivity analysis they have planned?

Please see below and pages 13 and 14 of the manuscript for a description of the functional connectivity analysis planned.

Hypotheses 2-4: Higher resilient functioning is associated with more balanced and integrated neural systems:

'For the event-related fMRI analysis, we will use both whole-brain and regions of interest (ROIs) approaches. To examine the effect of stress, we will examine brain responses to the contrast

'experimental + control condition' vs. 'rest condition'. Then, in a second level analysis, we will conduct a multiple regression analysis with resilience scores as regressor of interest. The ROIs use will be the MPFC, ACC, amygdala, insula, and hippocampus. Finally, we will run a psychophysiological interaction (PPI) analysis to test hypothesis 3 (i.e., increased functional connectivity between the DMPFC and emotion processing regions during the MIST).

For the analysis of resting state fMRI, we will use whole-brain and ROIs approaches. Our first approach will be a data-driven analysis. We will use intrinsic connectivity contrast (ICC; [93]). The use of ICC does not require a priori selection of a seed region but instead objectively defines how well each voxel is connected to the rest of the brain. Following the calculation of a resting state ICC map for each participant, we will test associations between ICC maps and resilient functioning. Specifically, we will correlate resilient functioning with the voxel level ICC using a multiple regression analyses in SPM. Finally, we will calculate functional connectivity maps and network involvement of the regions found to be associated with resilient functioning.'

Reviewer: 2
Moreno-Lopez and colleagues have produced a very detailed protocol for their ongoing cross-sectional study. Overall, it is well written, and I only have a few comments.
1. The introduction is very brief. The inclusion of some more information pertaining to relevant factors that will be addressed in the aims would help improve this section. For example, including information about why increased grey matter is expected in the hippocampus and prefrontal cortex of resilient individuals relative to those who are more vulnerable.
Thank you for your comment. We have now extended the introduction to include some of the factors that will be addressed in the study. Please see bellow and pages 3 and 4 of the manuscript.
'However, although CA is associated with considerably lowered odds of adequate mental and physical health functioning later in life, a significant proportion of individuals with a history of CA function 'better than expected,' or, in other words, are "functioning resiliently" [32]. These individuals may have benefited from protective 'resilience factors' [33,34] which exist across social, cognitive, neuronal, physiological and genetic levels. For example, good mental health after CA has been associated with increased hippocampal volume and greater connectivity between the central executive network and limbic regions as well as a greater ability to regulate emotions [35], higher self-esteem [36,37] and social support [32,38].'
2. Considering that the composite score of resilient functioning is a crucial component of the study, how this score is calculated should be included in the manuscript, rather than referring the reader to another paper.
We have expanded the description of how resilient functioning is calculated in the new manuscript to be more clear on page 11.
4.1. Preprocessing
'Quantification of resilient functioning
Using the data collected during the first online assessment, we will calculate gender and age-related degree of resilient functioning based on the model described in van Harmelen et al. [32]; see also Ioannidis et al. [39] for a description of the benefits and pitfalls of this method. Specifically, we will conduct two PCAs; one for psychosocial functioning using standard-normally transformed individual total scores on the MFQ, RCMAS, LOI, CBC, K10 and WEMWBS; and another one for CA, including standard-normally transformed sum scores for the MOPS, APQ, FAD and CTQ. From both analyses, we will extract individual scores for the first component to reflect individual current psychosocial functioning and recalled CA experience scores. Next, we will regress the psychosocial functioning component score against the CA score, testing for possible linear, quadratic or cubic relationships. From this model, we will extract the residual scores as a measure of individual degree of resilient functioning: the extent to which an individual has better, or worse, psychosocial functioning than the average score expected given their CA experiences. For parsimony, we will refer to this as degree of 'resilient functioning' with higher scores reflecting better (conditional) psychosocial functioning

outcomes. These individual resilient functioning scores will be utilised in the analyses described below.'

3. How long after phase II will the follow up (phase III) be completed?

The follow-up and final assessment will be completed online within a month from the in-unit assessment and will include the third and last online ICF, the MFQ, RCMAS, and LEQ, as well as the following measures described in the Supplementary Material: Perceived Stress Scale (PSS), Interpersonal Sensitivity Measure (IPSM) and the 10-item Ruminative Response Scale (RRS-10). We mention this on page 10 of the new manuscript.

4. In figure 1, the authors refer to early life stress as childhood maltreatment, however in the rest of the manuscript, they refer to this as childhood abuse. Please adjust to be consistent with the rest of the manuscript.

We apologise that this was unclear in the manuscript. We use Figure 1 to exemplify that resilient functioning has been associated with various components, ranging from genes and cellular mechanisms to higher-order biological systems and the social environment. However, the aim of the RAISE study is to examine the factors and mechanisms that facilitate resilient functioning after childhood adversity (CA) including adverse life experiences and/or childhood adversity within the family environment before the age of 16.

We have now edited the text to reflect this and be consistent through the text. Please see below and pages 3, 4 and 6 of the protocol.

1. Introduction

'Childhood adversity is the leading preventable risk factor for mental illness and substance abuse [1–8]. This kind of experiences, which can happen within the family environment (e.g., in the form of childhood maltreatment and/or intra-family adversity) or outside the household (e.g., trauma and bullying), can have a detrimental impact on a wide range of functions. For example, CA has been associated with physical (e.g., failure to thrive, poor adult health, and high mortality), cognitive (e.g., impaired inhibitory control and emotion regulation), and personal and interpersonal problems (e.g., negative self-cognitions, suicidal behaviours, increased peer rejection, social withdrawal, sexual maladjustment, aggression, and criminality) [8–16].'

2. Objectives and hypothesis

'Resilient functioning will be quantified as the degree to which an individual functions better or worse than expected given their self-reported childhood adversity experiences ([32]; Figure 2).'

3.1. Recruitment and eligibility

'The inclusion criteria will be: aged 16-26 years old; able and willing to give informed consent; able to speak, write, and understand English; BMI between 18.5 and 29.9 kg/m2; have experienced adverse life experiences and/or CA within the family environment including childhood maltreatment (e.g., emotional, sexual and/or physical abuse, emotional and/or physical neglect) and intra-family adversity (e.g., marital distress/conflict, parental mental health problems and/or parental alcohol dependence, violence and/or aggressive behaviour) before the age of 16; and willing to abstain from strenuous exercise for 72 hours prior to the in-unit assessment.'

5. In the supplementary document, the authors have stated that two of the four subtests from the WASI were administered to calculate IQ. Can the authors explain why all four subtests were not administered?

We used the Matrix Reasoning and Vocabulary subtests from the WASI in line with previous studies using the Neuroscience in Psychiatry Network (NSPN) cohort (e.g., Kievit et al., 2017). These subtests constitute measures of two core cognitive domains, fluid reasoning and vocabulary and have shown excellent interrater reliability (rs = 0.98 and 0.95), split half reliability (rs = 0.90 and 0.92), and concurrent validity (rs = 0.71 and 0.92) with comparable tests, such as the fourth editions of the Wechsler Intelligence Scale for Children (WISC-IV) and the Wechsler Adult Intelligence Scale (WAIS-IV) (McCrimmon & Smith, 2013).

McCrimmon AW, Smith AD. Review of the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II). J. Psychoeduc. Assess. 2013; 31: 337-341.

Kievit RA, Brandmaier AM, Ziegler G, van Harmelen A-L, de Mooij SMM, Moutoussis M, Ian M Goodyer IM, Bullmore E, Jones PB, Fonagy P, NSPN Consortium; Lindenberger U, Dolan RJ (2017). Developmental cognitive neuroscience using latent change score models: A tutorial and applications. Review Dev Cogn Neurosci. 2017; 33: 99-117. doi: 10.1016/j.dcn.2017.11.007. Corrigendum: Mutualistic Coupling Between Vocabulary and Reasoning Supports Cognitive Development During Late Adolescence and Early Adulthood. Psychol Sci. 2019; 30: 955-959. doi: 10.1177/0956797619843895

6. Information pertaining to ethical approval of the study should be included in the body of the manuscript in addition to in the abstract.

Thank you for highlighting that the information relating to the ethical approval was missing in the main text of the manuscript. We have now added this information to the current status of the manuscript. Please see below and page 14 of the protocol.

'This study has been reviewed and given favourable opinion by the National Research Ethics Service, NRES Committee East of England – Cambridge Central and external reviewers from the Royal Society (RGF\R1\180064 and RGF\EA\180029).'

## VERSION 2 – REVIEW

| REVIEWER | Sarah Baracz<br>UNSW, Sydney Australia |
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| REVIEW RETURNED | 14-Dec-2020 |

| GENERAL COMMENTS | The authors have addressed all of my concerns. |
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