Revisiting Defensive Motivation and the Error-Related Negativity: A Multi-Site Replication Study

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Abstract

A large number of EEG studies have identified a distinct event related potential (ERP) component during error processing known as the Error-Related Negativity (ERN). In an influential study, Hajcak and Foti (2008) explored the idea that errors could trigger defensive motivational reactions and that the ERN might forecast the intensity of defensive reactions following errors. Using a flanker task, thirty-one college-aged participants responded to the direction of a central arrow with acoustic startle probes administered pseudo-randomly throughout. Hajcak and Foti's (2008) findings indicated the ERN is indicative of individual variations in aversive reactions to errors. This has influenced understanding of the ERN being more than a simple error detection mechanism and sheds light on how people differ in their emotional responses to mistakes. As part of the #EEGManyLabs project, an international network of laboratories, we will test the replicability of the results from this influential study. The data will later be combined to compute global effect sizes of the ERN, startle potentiation, and their interaction. Collectively, these replications will help solidify the results from this highly-cited study. Given that the ERN is an integral part of a broader neural system responding to potentially threatening stimuli, this replication will provide a more solid foundation for our understanding of error processing and its relationship to defensive reactivity.

Introduction

One of the most widely studied event-related potentials (ERP) is the Error-Related Negativity (ERN). The ERN is characterized by a large deflection in the ERP waveform that peaks within ~100 milliseconds of an erroneous response (Gehring et al., 2018). Studies utilizing source localization suggest that the ERN is generated in the medial frontal cortex, and more specifically the anterior cingulate cortex (ACC; Dehaene et al., 2003; Holroyd et al., 1998; Miltner et al., 1997) although more recent studies suggest that the ERN may also arise from alternate brain regions such as the supplementary motor area (SMA; Bonini et al., 2014) or the pre-supplementary motor area (preSMA; Fu et al., 2019; Iannaccone et al., 2015). The ERN has been observed across different stimuli and response modalities and is thought to reflect the activity of a generic response monitoring system (Bernstein et al., 1995; Dehaene et al., 2003; Falkenstein et al., 2001), signaling a need to increase cognitive control and make behavioral adjustments (Botvinick et al., 2001). After 25 years of investigation, the ERN's prominence is clear, with new theories about its functional significance continuing to emerge. More recently, it has been listed as a core physiological marker of *Cognitive Control, Sustained Threat,* and *Reward Learning* in the Research Domain Criteria (RDoC) that forms the basis of National Institute of Mental Health's (NIMH) efforts to understand symptoms relevant to psychopathology. The ERN may be positioned within a broader nomological network, integrating with other biomarkers allowing for a more comprehensive understanding of error processing, emotional regulation, and individual differences. This has the potential to refine diagnostic strategies and move toward personalised treatment approaches.

The ERN component is largely regarded as a neural index of response monitoring processes (Bernstein et al., 1995; Holroyd et al., 2003; Luu et al., 2000). However, as affective and motivational variables influence the magnitude of ERN, the ERN may relate to emotional and motivational aspects of error detection. Indeed, individuals and groups characterized by excessive concern over errors – for whom errors may be both more salient and more aversive – show enhanced ERN amplitudes (Riesel et al., 2013). Specifically, the ERN has been implicated as a biomarker of obsessive compulsive disorder (OCD; Gehring et al., 2000), worry (Hajcak et al., 2003) and extreme negative affective experience (Hajcak et al., 2004; Luu et al., 2000). Importantly, the enhanced ERN often observed, is rarely reflected in performance differences, suggesting that the magnitude of the ERN varies as a function of motivational states independent of behavioral measures (Weinberg et al., 2012). Therefore, rather than indexing error or conflict detection per se, the ERN may be sensitive to dynamically established goal states, and reflect the negative affective response to errors (Hajcak et al., 2005).

Following the idea that the ERN may reflect more than simple error detection, Hajcak and Foti (2008) were the first to demonstrate a direct link between errors and defensive motivation. Arguing that error detection should prompt defensive motivation and rapidly activate reflexes and dispositions toward action that protect the organism, they sought to determine whether defensive responding would similarly be increased following errors, compared with correct responses. The authors used startle potentiation, a robust psychophysiological measure of defensive reactivity. This refers to an increase in the intensity of the startle reflex in response to a sudden, intense

stimulus (typically a loud, sudden noise) under aversive or threatening conditions relative to neutral conditions. Hajcak and Foti (2008) hypothesized that startle responses would be larger (i.e., potentiated) after errors than after correct responses, given the aversive nature of error-making. Furthermore, they hypothesized that if the ERN reflects a motivationally relevant correlate of error processing, larger ERNs should predict greater startle potentiation following errors. To test their hypotheses, Hajcak and Foti (2008) recorded ERNs using EEG and startle responses using electromyographic (EMG) activity in thirty-one undergraduate students (26 male, 5 female). Their experimental paradigm included both predictable and unpredictable acoustic startle probes whilst simultaneously measuring error-related brain activity to determine whether individuals with larger ERNs would demonstrate greater startle potentiation following errors. Consistent with their hypotheses, the ERN appeared to be indicative of individual variations in aversive reactions to errors. Subsequent work by these investigators, and others, has led to the proposal that the ERN could be a potential neural marker for defensive motivation, with variance in the ERN reflecting individual differences in the degree to which errors are evaluated as threatening (Weinberg et al., 2016). This perspective aligns with the idea that the ERN could be part of a broader neural system involved in responding to potentially threatening stimuli.

Since its publication, Hajcak and Foti (2008)'s article has been cited over 450 times according to Google Scholar. Given the influence this result has had on the scientific study of error processing, testing its replicability is especially important to validate further findings that may have clinical implications. Indeed, the reliability and replicability of research findings is a widespread concern in the scientific community. It has raised questions about the robustness of many published studies and the overall credibility of scientific research. To ameliorate the state of study replication, the #EEGManyLabs project is seeking to shed light on the robustness of results in highly cited and influential electroencephalographic (EEG) studies (Pavlov et al., 2021). Replication is essential for ensuring that these EEG findings are robust, credible, and contribute meaningfully to the advancement of our understanding of psychological phenomena, which is particularly prudent for clinically-relevant results. Hajcak and Foti (2008) recruited only 31 participants which was typical in the field at that time. Yet, despite the interest in the ERN and defensive motivation, there have only been two attempts to replicate the original procedure, measures, and analyses. One successful replication was conducted by the original authors who found a significant correlation between ERN magnitude and error-potentiated startle (Riesel et al., 2013). However this was only evident in a subgroup of 16 participants exhibiting higher ERNs. The second replication was an unsuccessful attempt by Lewis and Pitts (https://osf.io/82bwv). Lewis and Pitts failed to find a significant correlation between ERN and startle potentiation ($r=-.25$, $p=.11$, one-tailed). However, it is important to note that the small sample size of 51 participants, though larger than the original study sample size, may not have been sufficiently powered to detect the effect.

In addition to concerns about statistical power, the generalizability of past studies have been questioned. There is also a consistent bias towards studies taking place in the United States which has been met with a recent push towards globalized efforts (Ledgerwood et al., 2022). Therefore it is important to build upon attempted replications of this influential study by utilizing an international network of laboratories. Both the original study and Lewis and Pitts' replication effort was conducted in the USA, therefore diversifying this original study's sample (i.e., applying

a global sample) will increase the generalizability of its results and, ultimately, better test whether the ERN is a marker of defensive motivation in the broader population. While the currently committed participating labs are based in W.E.I.R.D. (Western, Educated, Industrialized, Rich, Democratic) countries, we will seek to recruit more international labs ahead of Stage 2 of this Registered Report.

The focus on replicating highly cited ERP studies is motivated by the field's reliance on these typically smaller sample sizes and the complex data analysis methods that allow for researcher degrees of freedom (Simmons et al., 2011). As part of the #EEGManyLabs project (Pavlov et al., 2021), the current Registered Report proposes a close replication of Hajcak and Foti (2008), involving the collection of data from laboratories across UK, USA, Germany, France, and Belgium to test the robustness of the ERN, in tandem with startle potentiation, as a biomarker of defensive motivation. Having a large international sample will provide clearer evidence into how this study design and results replicate and generalize. The data will later be combined to compute global effect sizes of ERN, startle potentiation, and their interaction. Collectively, these replications will place our understanding of error processing on firmer grounds.

There have been few studies that have since investigated the direct relationship between the startle response and ERN. Riesel et al., (2013) found that an association between ERN and error-potentiated startle was only observed in a subgroup of adults with relatively large ERN amplitude. More recent studies have found mixed evidence in children whereby Meyer et al., (2017) found a relationship between ERN and aversive potentiation of the startle reflex during picture viewing whereas Jackson et al., (2017) found no relationship between the ERN and startle response in 8–14 year old girls. Conversely, Lo et al., (2015) found a significant result but in the opposite direction whereby smaller ERNs were associated with high defensive reactivity (larger startle) in 3-7-year old children. Furthermore, although studies have supported the notion that the ERN reflects motivational reactivity to errors in relation to anxiety (Moser et al., 2013; Moser et al., 2016; Pasion and Barbosa, 2019; Saunders and Inzlicht, 2020), recent work challenges this interpretation. For instance, Clayson (2024), using a multiverse analysis, has highlighted inconsistencies in the relationship between the ERN and anxiety, raising questions about whether heightened ERN amplitudes universally reflect increased defensive emotional reactivity. These findings underscore the need for rigorous, high-powered replications to clarify the functional significance of the ERN. If our replication supports the original findings, it will reinforce the framework linking the ERN to defensive motivation. Conversely, a failure to replicate would necessitate a re-evaluation of the theoretical models underpinning the ERN's functional significance and motivate further investigations into alternative explanations, such as the role of individual differences, task characteristics, or broader contextual factors.

By clarifying the ERN's reliability and theoretical underpinnings, this replication effort will inform both basic research and clinical applications, contributing to a more comprehensive understanding of neural mechanisms underlying error processing and emotional reactivity. Our primary hypothesis (H.1) is that the magnitude of the ERN correlates with the degree of startle potentiation following errors: participants with larger ERNs demonstrate greater startle potentiation following errors. Additionally (H.2), we will test whether startle responses will be larger after errors than after predictable and unpredictable correct responses.

Methods

This manuscript adheres to the recommended open science practices for psychophysiological research outlined by Garrett-Ruffin et al. (2021). All study materials, including comprehensive details about each site, including equipment and electrode specifications, site-specific language, and participant recruitment procedures, and code for stimulus presentation and data processing will be stored in a project repository on the Open Science Framework (https://osf.io/fbqtm/). All raw EEG data will be shared publicly in a suitable repository (https://gin.g-node.org/). The OSF repository functions as a central hub, connecting to raw EEG data as well as data processing and analysis code.

This replication is a global initiative involving replicating labs that encompass multiple independent study sites geographically dispersed. At the time of the initial Stage 1 report submission, there are 8 replicating labs. Each lab will secure approval from their local institutional review board/ethics committee to conduct the study and share de-identified data.

Participants

Participants will be recruited from the local community which is likely to be predominantly, but not limited to, undergraduate and postgraduate students. Participants will be required to give written informed consent before participating. The inclusion criterion is that participants must be 18 years of age or older with no known hearing problems and typical-to-corrected vision needed in order to properly complete the behavioral task. Individuals recruited from undergraduate courses will receive compensation in the form of course credit for their time, while those from the community will be remunerated in accordance with local policies governing participant compensation.

Power Analysis

A power analysis was conducted using G*Power (Kang et al., 2021) based on an effect size estimate from the original study for the central finding (H.1). The statistics from the original study showed a correlation between the amplitude of the ERN and the degree to which errors potentiated the startle response as $r = -.38$, $p<.05$. To counteract potential overestimation of the true effect size, we took into account that the effect size in pre-registered studies is about half the size of that in large-scale replications (Open Science Collaboration, 2015) and studies without pre-registration (Schäfer & Schwarz, 2019). Assuming that the effect size is half of what was observed in the original study (i.e., -.19), a total of 303 participants will be needed to achieve 90% statistical power, in a one-sided test. For H.2, regarding the effect stating that startle magnitudes are larger following errors than following correct trials (original study results: $t(30) = 2.51$; estimated dz = 0.451), the power analysis indicated that a sample of 57 participants is required. The anticipated data collection involves each replicating lab gathering EEG data from as many as necessary to collectively gather data from at least 303 participants overall.

Procedure

The procedure will follow, as closely as possible, the process described in Hajcak and Foti (2008) with any deviations stated explicitly. Participants will be recruited via Institutional Review Board or Ethics Committee approved channels, using approved language. Upon their arrival to the lab, they will receive a verbal description of the experiment, have an opportunity to ask questions, and provide informed consent.

Participants will then be prepared for the EEG and EMG recordings in the participant room. First, the EEG cap will be positioned on the participant's head, followed by four EOG facial electrodes which will be placed on the orbicularis oculi and two EMG electrodes which will be placed under the left eye to record eyeblink startle response. Once all sensors are correctly placed, participants will have a chance to familiarize themselves with the task and response buttons with a practice block of 30 trials, which will not be included in analysis. The training session will last approximately 3 minutes. Participants will then complete the flanker task. comprising 8 blocks of 30 trials (240 total trials) with feedback text written in between each block, using the mouse button to respond. If the participant's accuracy is below 75% or above 90%, the text will encourage the participant to make more accurate or faster responses, respectively.

As part of the broader initiative on EEG replicability (#EEGManyLabs), in addition to replicating the study, labs will also collect resting state EEG data and certain personality measures (see https://osf.io/sp3ck/). The analysis of both EEG and personality data is not within the scope of the current study; however, they will be consolidated across sites for a future replication project, the findings of which will be reported separately. However, the inclusion of these measures will also allow us to address exploratory subsidiary research questions such as the influence of trait anxiety on the variability of ERN and the startle response. It will also allow us to test for potentially confounding variables such as whether participants have experienced neurological, psychiatric or sleep issues as well as current medications (for a full list of measures please see https://osf.io/sp3ck/).

Following the Flanker task, participating labs will gather 8 minutes of resting state EEG, and participants will complete three brief questionnaires (translated into the local language where applicable), including the Karolinska Sleepiness Scale (KSS; Åkerstedt & Gillberg, 1990), the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), and the State Anxiety Inventory Version (STAI; Spielberger et al., 1970). Participating labs will also collect the Edinburgh Handedness Inventory (EHI; Oldfield, 1971), the Behavioral Inhibition and Approach System Scales (BIS-BAS; (Carver & White, 1994), the Center for Epidemiologic Studies Depression Scale (Radloff, 1977), the Short Version of the Big Five Inventory (Gerlitz & Schupp, 2005) and the Trait Anxiety Inventory Version (STAI; Spielberger et al., 1970) in advance of the participant's lab visit.

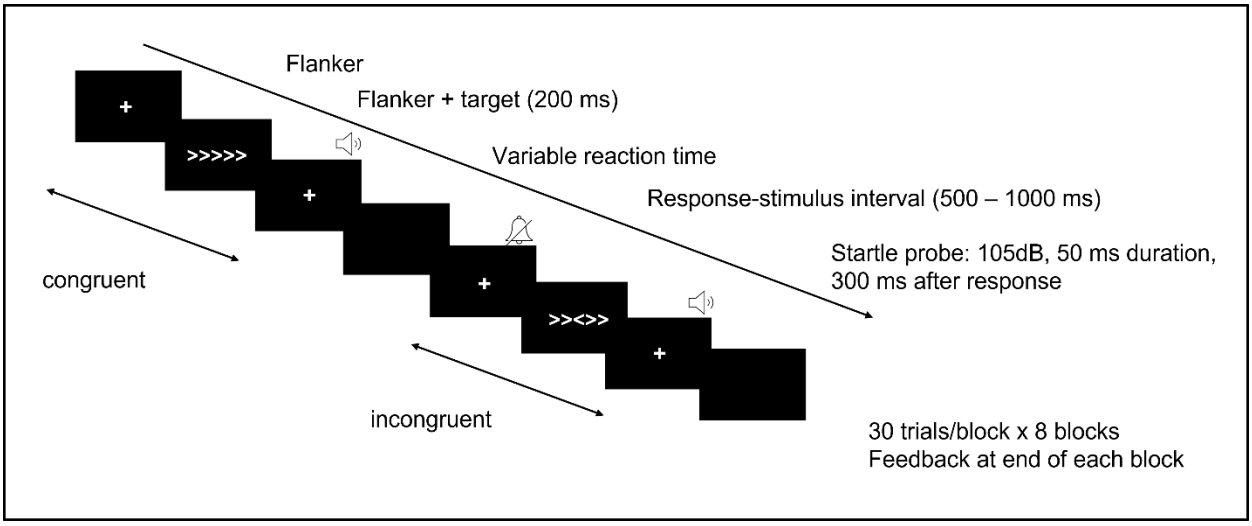
Upon completion, participants will be debriefed, compensated and released. In all, the task (including electrode preparation) should last about one hour to an hour and a half.

Experimental Paradigm

The experimental paradigm will closely follow the methodology of Hajcak and Foti (2008) where they used an arrowhead version (using < and > symbols) of the flanker task previously used in

(Hajcak et al., 2005). All participants will perform one practice block of 30 trials where the startle probe will be delivered on 10% of all trials. As described in Hajcak and Foti (2008), the task was presented as follows: On each trial, five horizontally aligned arrowheads were presented, and participants had to respond to the direction of the central arrowhead by pressing the left or right mouse button. Participants will be instructed to use their index and middle fingers on their dominant hand. The response window will be limited to 1800ms (or 2000ms from stimulus onset); if the participant does not respond within this window the response will be counted as incorrect. On compatible trials, all five arrowheads pointed in the same direction (either left or right), and on incompatible trials, the central arrowhead pointed in the direction opposite the direction of the flanking arrowheads. Compatible and incompatible trials were equally frequent, and all stimuli were presented for 200 ms with an intertrial interval that varied randomly from 500 to 1000 ms. Participants performed eight blocks of 30 trials. At the end of each block, performance from the block was calculated and participants received feedback designed to encourage fast and accurate responding. If performance was 75% correct or lower, the message “Please try to be more accurate” was displayed; performance above 90% correct was followed by “Please try to respond faster”; if performance was between these levels, the message “You're doing a great job” was displayed (Hajcak & Foti, 2008).

Figure 1: Task design including stimulus timing and startle probes



The experiment was originally programmed using Presentation (Neurobehavioral Systems, Inc., www.neurobs.com) which we have adapted for use with PsychoPy software v2023.2.3 (https://www.psychopy.org/) and available from the project repository on the Open Science Framework (https://osf.io/fbqtm/). Tasks will be translated into local languages when necessary (English, German, and French).

Startle probe

Startle will be elicited with a 105-dB burst of white noise, 50-ms in duration and near instantaneous rise time presented 300 ms after the response. Startle probe delivery will be performed through headphones worn by the participant during the task, which will be connected to a stimulation

computer. Loudness will be assessed using a sound meter. Startle probes will be presented on 50% of error trials, on 50% of correct trials that followed errors, and on a random 4% of other correct trials. This means that some startle probes are predictable (after both errors and correct trials that followed errors), whereas others are not (randomly selected correct trials). Accordingly, there are three trial types: *error trials*, *predictable correct trials* (i.e., correct trials on which the startle probe is predictable), and *unpredictable correct trials* (i.e., correct trials on which the startle probe is not predictable).

At a maximum of 105 dB, the intensity of this sound is safely below levels at which there might be any risk of pain or physical damage as established by OSHA (Occupational Safety and Health Administration) and NIOSH (National Institute for Occupational Safety and Health) guidelines. Specifically, risk associated with noise exposure is reduced in the current experiment by limiting noise intensity to 105 dB, limiting total noise exposure time to no more than 5 s, and using broad spectrum noise (i.e., white noise). The portion of the experiment in which white noise bursts will be delivered to participants will last approximately 10 minutes. During this period, participants will be exposed to no more than 100, 50-millisecond bursts of white noise for a total of 5 seconds of exposure. The OSHA recommended limit for noise exposure at 105 dB is no more than 1 hour/day (OSHA section 191095).

Neurophysiological Recordings

In Hajcak and Foti's (2008) study, continuous electroencephalographic (EEG) and electromyographic (EMG) activity was recorded using an ActiveTwo head cap and the ActiveTwo BioSemi system (BioSemi, Amsterdam, The Netherlands). Recordings were taken from 64 scalp electrodes based on the 10-20 system, as well as from two electrodes placed on the left and right mastoids. The replicating labs will be using one of the following EEG systems: (1) ANT Neuro eego mylab with 64-channel Waveguard Touch caps 2) Brain Products actiCHamp Plus, (3) Grael 4K-EEG Compumedics, or (4) Active two Biosemi (see Table 1 for details). Using elastic caps, all labs will record data with equivalent Cz, Fz, and Pz channels, and mastoids (TP9, TP10) positioned according to the extended 10/20 EEG system (Chatrian et al., 1985).

Hajcak and Foti's (2008) measured the startle response using standard procedures for assessing defensive reactivity (Bradley et al., 2005; Grillon et al., 1994). The electrooculogram (EOG) generated from blinks and eye movements was recorded from four facial electrodes: two approximately 1 cm above and below the participant's right eye, one approximately 1 cm to the left of the left eye, and one approximately 1 cm to the right of the right eye. The startle response was measured with two electrodes placed approximately 12 mm apart under the participant's left eye on the obicularis muscle. All labs will record EMG from the same positions and most labs will additionally record 2 bipolar channels of EOG (see Table 1).

Table 1. ***Overview of EEG set-up and recording details at each replicating lab***

Participating University	Amplifier System	Electrode/Cap Model, Number EEG + EOG	Sampling Rate	Reference, Ground	Online Filter	Screen Type, Size, Ratio, Refresh Rate	Stimulus Presentation, Language	Sound delivery system	EMG electrodes for fear potentiated startle (FPS)
Manchester Metropolitan University, United Kingdom	ANT Neuro eego mylab	64-channel Waveguard Touch caps + ref/gnd + 2 channels (4 electrodes of EOG	1000Hz	M1,M2	Anti-aliasing hardware filter	LED/IPS, 24 in, 1920x1080, 60 Hz	Psychopy, English	Headphones (Audio Technica ATH-M30x) and sound meter	External bipolar EMG channel
Oregon State University, USA	Brain Products actiCHamp plus	32 channel actiCAP slim/snap 6 electrodes from set diverted as external electrodes (26 cap; 6 external); 2 horizontal EOG, 2 vertical EOG, 2 mastoid	2500Hz	Left mastoid (online); linked mastoids (offline); dedicated ground	Brain Products anti-aliasing hardware filter	LED ASUS TUF 23.8" 1080P Monitor (VG249Q1R)	Presentation, English	Headphones (Sennheiser HD 280 Pro) and sound meter	EEG cap electrodes in bipolar montage
CORE Lab, Rennes, France	Grael, Compumedics	32 channel quik-cap (4 EOG)	512Hz	M1,M2	BP filter: 0.1-70 Hz	IIYAMA Prolite E2083 HSD LED,, 19 in, 1600x900 resolution	Eprime, French	Headphones (Sennheiser HD 25-1 II) and chronos device	External bipolar EMG channel
Ghent University, Belgium	Active two Biosemi	64-channel, electro-cap including CMS & DRL, six external electrodes (4 EOG)	512Hz	Online CMS & DRL; offline linked mastoids	LP filter: 102.4 Hz	19-inch CRT monitor (1600 x 1200 resolution at 75Hz)	Psychopy, English	Headphones and artificial ear (GRAS 43AG-S2, Ear and Cheek Simulator with Kemar Pinna)	External bipolar EMG channel
University of Michigan-Dearborn, USA	Active two Biosemi	64-channel, electro-cap including CMS & DRL, six external electrodes (4 EOG)	512 Hz	Online CMS & DRL; Offline linked mastoids	Anti-aliasing hardware and digital software filter	ASUS ROG Swift PG248Q 24-inch (1920x1080), 144Hz	Psychopy, English	Headphones (Sennheiser HD 25); Creative blaster AE-7 sound card	Biosemi EMG/ECG (external) active channels
University of Goettingen, Germany	Active two Biosemi	64-channels, electrode-caps including CMS & DRL, eight external electrodes (mastoids, vertical and horizontal EOG, EMG)	1024 Hz	Online CMS and DRL, offline linked mastoids	Upper hardware limit: ⅕th of sampling rate as bandwidth-filter (204.8 Hz)	Alienware 25 Gamingmonitor AW2523HF, 24.5", 1920 x 1080 resolution, 255 Hz	Psychopy, English	Bayerdynamic DT 770 PRO headphones	Biosemi EMG external active flat electrodes

University of Bristol, United Kingdom	Brain Products actiCHamp plus	64 channels, acticap snap including CMS & DRL, 6 electrodes from set diverted as external electrodes (58 cap; 6 external); 2 horizontal EOG, 2 vertical EOG, 2 mastoid	1000 Hz	Online CMS and DRL, offline linked mastoids	Brain Products antialising filter	Asus ROG PG248Q, 24-inch, (1920x1080), resolution at 60 Hz	Psychopy, English	Headphones (Sony WH-CH500)	EEG cap electrodes
University of Liverpool, Uk	Active two Biosemi	64-channels, electrode-caps plus CMS & DRL, 8 external electrodes (mastoids, vertical and horizontal EOG)	512 Hz	Online CMS and DRL, offline linked mastoids	Upper hardware limit: ⅕th of sampling rate as bandwidth-filter (approximatel y 104 Hz)	HP E233 LED backlit monitor, with 60Hz refresh rate	Psychopy, English	Headphones (Audio Technica ATH-M30x) and sound meter	Biosemi EMG external active flat electrodes

EEG data processing

All EEG data will be analyzed in EEGLAB (Delorme & Makeig, 2004) and processed using the pipeline that follows the original study as closely as possible (see Hajcak and Foti, 2008) and also the pipeline that follows the current standard in the field. Before implementing both pipelines, we will downsample EEG data to a common denominator of 250 Hz, making data analysis more efficient without compromising the quality of results.

In the pipeline closely following the original study, EEG data will be re-referenced to the numeric mean of the mastoids and band-pass filtered with cutoffs of 0.1 and 30 Hz. The EEG will be segmented for each trial, beginning 200 ms before the response and continuing for 800 ms. The EEG will be corrected for blinks and eye movements using the method developed by Gratton et al. (1983). Specific intervals for individual channels will be rejected in each trial using a semiautomated procedure, with physiological artifacts identified by the following criteria: a voltage step of more than 50.0 μV between sample points, a voltage difference of more than 300.0 μV within a trial, and a maximum voltage difference of less than 0.50 μV within a 100-ms interval.

We will complement this direct replication with a modern preprocessing approach optimized for measuring ERN (Clayson et al., 2021) to test the robustness of the reported effects. If the modern pipeline provides evidence for a similar pattern of results as the original, the effect will be considered not only replicated but also robust and, to some extent, independent of analytical choices. If the direct replication fails - expected effects fail to reach significance - we will conclude that the replicability of the effect depends on analytical choices and is not robust.

The modern approach (Clayson et al., 2021) involves: (1) re-referencing to the numeric mean of the mastoids, (2) implementing a bandpass filter ranging from 0.1 to 30Hz; (2) employing a notch filter set at 50/60Hz (depending on the lab) to eliminate any electrical noise; (3) performing spherical interpolation of channel activity that remains constant or significantly deviates from the

activity of other channels, determined through visual inspection of data and plotting channel spectra maps; (4) removal of extremely noisy data segments (for improving Independent Component Analysis (ICA) performance) as detected by EEGLAB's clean_artifacts.m function, which uses the artifact subspace reconstruction (ASR) algorithm; (5) cleaning the data of ocular, muscular, or 'bad' channel artifacts with ICA (using the function 'runica' implemented in EEGLAB) and using SASICA (Semi-Automated Selection of Independent Components of the electroencephalogram for Artifact correction) plugin in EEGLAB (Chaumon, Bishop, & Busch, 2015). The following options will be enabled in SASICA: 'Autocorrelation' to differentiate muscle components (components reflecting brain data are known to be strongly autocorrelated), 'Focal components' (to determine bad channels), 'Signal to noise ratio' (to reject components with a low signal to noise ratio), and ADJUST (for detection of eyeblinks, and vertical and horizontal eye movements). The decision to reject or retain components will follow SASICA's recommendations, for reproducibility and consistency across analysts; (6) rejecting outlier epochs in terms of kurtosis, joint probability, or power spectrum (Tabachnick & Fidell, 2007). Specifically, kurtosis and joint probability are computed over time for each trial and each electrode using EEGLAB's pop_rejkurt.m and pop_jointprob.m functions, respectively, after which these values are normalized by subtracting the trial-average and dividing by the standard deviation over trials. Trials with a normalized kurtosis or joint probability higher than 3.29 are considered outliers and rejected. Similarly, power spectra in the frequency band from 1 to 30 Hz are computed per trial and per electrode using EEGLAB's pop_rejspec.m function, after which these are dB normalized and the trial-average is subtracted. Trials with an absolute average power value of more than 90 dB are considered outliers and rejected. The same epoching and baseline correction measures will be applied here as in the original pipeline.

To determine whether the magnitude of the ERN predicts the increase in startle following errors, we will define startle potentiation for each participant as the startle magnitude after errors minus the average of startle responses on predictable correct and unpredictable correct trials. The ERN will be defined as the average activity at electrode FCz as used in Hajcak and Foti (2008) or Cz if FCz is not available, as the ERN can be captured at Cz as well (Sandre et al., 2020), in the 0- to 100-ms time window following response onset in error trials, after applying single-trial baseline normalization using the -100 to 0 ms window before response onset as baseline.

The original study did not specify participant exclusion criteria based on the number of trials remaining after artifact rejection. In both pipelines, to ensure sufficient data quality, we will exclude participants for whom more than 75% of trials are rejected overall or fewer than six trials per condition remain. The latter criterion is based on evidence that the ERN demonstrates sufficient reliability with a minimum of six trials (Olvet and Hajcak, 2009). The feedback text written in between each block, will encourage the participant to make faster responses, and therefore is intended to encourage errors. We will report the number of participants excluded from analysis.

EMG data processing

For the startle data, EMG activity will be band-pass filtered (28–512 Hz; 24 dB/octave roll-off), rectified, then low-pass filtered at 30 Hz (24 dB/octave) and baseline-corrected (-50 to 0 ms time window). Individual trials will be examined and rejected if the startle reflex began less than 20 ms following probe onset. Startle response magnitudes and latencies will be quantified in terms of the peak in the 20- to 120-ms window after the presentation of the startle probe.

As well as adhering closely to the data preprocessing protocol outlined in Hajcak and Foti (2008), in addition, we will also implement a more recent pipeline optimized for measuring EMG startle (Bradford et al., 2014). This involves: (1) high-pass filtering (4th order 28 Hz Butterworth filter, zero phase shift), (2) epoching from 50 ms pre-probe to 250 ms post-probe onset, (3) rectifying and smoothing (2nd order 30 Hz Butterworth low-pass filter, zero phase shift) the data. We will reject trials with values greater than +/- 20 μV in the 50 ms pre-probe to 10 ms post-probe window as artifact (i.e., unstable baseline). We will reject trials with mean amplitude less than -10 μV in the 100-250 ms post-probe window as artifact (i.e., movement artifact and baseline over-correction). Next, we will visually inspect figures of epochs of all processed startle data (all trials vs. accepted trials vs. algorithm rejected trials) and manually reject trials with excessive deflection in the baseline or post-probe windows not detected by automatic artifact detection. We will exclude participants who have lost >30 % of trials due to artifact rejection.

Statistical analyses

To test H.1, we will use Pearson's correlation between the ERN and startle response. To test H.2, we will run 2 x 2 ANOVA of startle magnitude with factors predictability (predictable, unpredictable) and trial type (i.e., correct or error). The effect of trial type will be used to make the conclusion of whether startle responses are larger after errors than after correct responses. The ERN and startle responses will be statistically evaluated with R. Greenhouse-Geisser correction will be applied to *p* values associated with multiple-*df*, repeated measures comparisons.

Evaluation of the Replication of Effects

Replication success was defined in the #EEGManyLabs protocol (Pavlov et al., 2021). For each hypothesis separately, we will, first, compute effect sizes (H.1: Pearson’s r Fisher’s z-transformed, H.2: Cohen’s dz) for each individual lab and then combine all datasets in a random-effects meta-analysis (with labs as a random effect) using the REML estimator for random-effects variance. Employing a random-effects meta-analysis will address and help in estimating the effect of heterogeneity in EEG devices and samples between labs. The replication will be considered successful if a statistically significant meta-analytic estimate ($p < .02$) across replicating labs is observed and if the effect is in the expected direction. We will report distribution of the weighted effect sizes, their 95% confidence intervals, heterogeneity ($\tau 2$). The metafor package (Viechtbauer, 2010) for R will be used for the meta-analyses.

If either H.1 or H.2 results in statistically non-significant effects, we will estimate evidence for the null hypothesis using a random-effects Bayesian meta-analysis. For this, we will use JASP (Love et al., 2019) with default priors (Cauchy prior with a scale of 0.707).

References

Åkerstedt, T., & Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience*, *52*(1–2), 29–37.

Bernstein, P. S., Scheffers, M. K., & Coles, M. G. H. (1995). " Where did I go wrong?" A psychophysiological analysis of error detection. *Journal of Experimental Psychology: Human Perception and Performance*, *21*(6), 1312.

Bonini, F., Burle, B., Liégeois-Chauvel, C., Régis, J., Chauvel, P., & Vidal, F. (2014). Action monitoring and medial frontal cortex: leading role of supplementary motor area. *Science*, *343*(6173), 888-891.

Botvinick, M. M., Braver, T. S., Barch, D. M., Carter, C. S., & Cohen, J. D. (2001). Conflict monitoring and cognitive control. *Psychological Review*, *108*(3), 624.

Bradford, D. E., DeFalco, A., Perkins, E. R., Carbajal, I., Kwasa, J., Goodman, F. R., Jackson, F., Richardson, L. N. S., Woodley, N., & Neuberger, L. (2022). Whose signals are being amplified? Toward a more equitable clinical psychophysiology. *Clinical Psychological Science*, 21677026221112116.

Bradley, M. M., Moulder, B., & Lang, P. J. (2005). When good things go bad: the reflex physiology of defense. *Psychological Science*, *16*(6), 468–473.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. *Journal of Personality and Social Psychology*, *67*(2), 319.

Clayson, P. E., Baldwin, S. A., Rocha, H. A., & Larson, M. J. (2021). The data-processing multiverse of event-related potentials (ERPs): A roadmap for the optimization and standardization of ERP processing and reduction pipelines. *NeuroImage*, *245*, 118712.

Clayson, P. E. (2024). Beyond single paradigms, pipelines, and outcomes: Embracing multiverse analyses in psychophysiology. *International Journal of Psychophysiology*, 112311.

Dehaene, S., Artiges, E., Naccache, L., Martelli, C., Viard, A., Schürhoff, F., Recasens, C., Martinot, M. L. P., Leboyer, M., & Martinot, J.-L. (2003). Conscious and subliminal conflicts in normal subjects and patients with schizophrenia: the role of the anterior cingulate. *Proceedings of the National Academy of Sciences*, *100*(23), 13722–13727.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21.

Dien, J. (2010). The ERP PCA Toolkit: An open source program for advanced statistical analysis of event-related potential data. *Journal of Neuroscience Methods*, *187*(1), 138–145.

Falkenstein, M., Hielscher, H., Dziobek, I., Schwarzenau, P., Hoormann, J., Sundermann, B., & Hohnsbein, J. (2001). Action monitoring, error detection, and the basal ganglia: an ERP study. *Neuroreport*, *12*(1), 157–161.

Fu, Z., Wu, D. A. J., Ross, I., Chung, J. M., Mamelak, A. N., Adolphs, R., & Rutishauser, U. (2019). Single-neuron correlates of error monitoring and post-error adjustments in human medial frontal cortex. *Neuron*, *101*(1), 165-177.

Garrett-Ruffin, S., Hindash, A. C., Kaczkurkin, A. N., Mears, R. P., Morales, S., Paul, K., Pavlov, Y. G., & Keil, A. (2021). Open science in psychophysiology: An overview of challenges and emerging solutions. *International Journal of Psychophysiology*, *162*, 69–78.

Gehring, W. J., Goss, B., Coles, M. G. H., Meyer, D. E., & Donchin, E. (2018). The Error-Related Negativity. *Perspectives on Psychological Science*, *13*(2), 200–204. https://doi.org/10.1177/1745691617715310

Gehring, W. J., Himle, J., & Nisenson, L. G. (2000). Action-monitoring dysfunction in obsessive-compulsive disorder. *Psychological Science*, *11*(1), 1–6.

Gerlitz, J.-Y., & Schupp, J. (2005). Zur Erhebung der Big-Five-basierten persoenlichkeitsmerkmale im SOEP. *DIW Research Notes*, *4*(10.1016).

Gratton, G., Coles, M. G. H., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalography and Clinical Neurophysiology*, *55*(4), 468–484.

Grillon, C., Ameli, R., Goddard, A., Woods, S. W., & Davis, M. (1994). Baseline and fear-potentiated startle in panic disorder patients. *Biological Psychiatry*, *35*(7), 431–439.

Hajcak, G., & Foti, D. (2008). Errors Are Aversive: Defensive Motivation and the Error-Related Negativity. *Psychological Science*, *19*(2), 103–108. https://doi.org/10.1111/j.1467-9280.2008.02053.x

Hajcak, G., McDonald, N., & Simons, R. F. (2003). Anxiety and error-related brain activity. *Biological Psychology*, *64*(1–2), 77–90.

Hajcak, G., McDonald, N., & Simons, R. F. (2004). Error-related psychophysiology and negative affect. *Brain and Cognition*, *56*(2), 189–197.

Hajcak, G., Moser, J. S., Yeung, N., & Simons, R. F. (2005). On the ERN and the significance of errors. *Psychophysiology*, *42*(2), 151–160. https://doi.org/https://doi.org/10.1111/j.1469-8986.2005.00270.x

Holroyd, C. B., Dien, J., & Coles, M. G. H. (1998). Error-related scalp potentials elicited by hand and foot movements: evidence for an output-independent error-processing system in humans. *Neuroscience Letters*, *242*(2), 65–68.

Holroyd, C. B., Nieuwenhuis, S., Yeung, N., & Cohen, J. D. (2003). Errors in reward prediction are reflected in the event-related brain potential. *Neuroreport*, *14*(18), 2481–2484.

Iannaccone, R., Hauser, T. U., Staempfli, P., Walitza, S., Brandeis, D., & Brem, S. (2015). Conflict monitoring and error processing: new insights from simultaneous EEG–fMRI. *Neuroimage*, *105*, 395-407.

Kang, H. (2021). Sample size determination and power analysis using the G* Power software. *Journal of educational evaluation for health professions*, *18*.

Ledgerwood, A., Hudson, S. T. J., Lewis Jr, N. A., Maddox, K. B., Pickett, C. L., Remedios, J. D., Cheryan, S., Diekman, A. B., Dutra, N. B., & Goh, J. X. (2022). The pandemic as a portal: Reimagining psychological science as truly open and inclusive. *Perspectives on Psychological Science*, *17*(4), 937–959.

Lo, S. L., Schroder, H. S., Moran, T. P., Durbin, C. E., & Moser, J. S. (2015). Neurophysiological evidence of an association between cognitive control and defensive reactivity processes in young children. *Developmental Cognitive Neuroscience*, *15*, 35-47.

Luu, P., Collins, P., & Tucker, D. M. (2000). Mood, personality, and self-monitoring: negative affect and emotionality in relation to frontal lobe mechanisms of error monitoring. *Journal of Experimental Psychology: General*, *129*(1), 43.

Meyer, A., Hajcak, G., Glenn, C. R., Kujawa, A. J., & Klein, D. N. (2017). Error-related brain activity is related to aversive potentiation of the startle response in children, but only the ERN is associated with anxiety disorders. *Emotion*, *17*(3), 487.

Miltner, W. H. R., Braun, C. H., & Coles, M. G. H. (1997). Event-related brain potentials following incorrect feedback in a time-estimation task: evidence for a “generic” neural system for error detection. *Journal of Cognitive Neuroscience*, *9*(6), 788–798.

Moser, J. S., Moran, T. P., Kneip, C., Schroder, H. S., & Larson, M. J. (2016). Sex moderates the association between symptoms of anxiety, but not obsessive compulsive disorder, and error-monitoring brain activity: A meta-analytic review. *Psychophysiology*, *53*(1), 21-29.

Moser, J. S., Moran, T. P., Schroder, H. S., Donnellan, M. B., & Yeung, N. (2013). On the relationship between anxiety and error monitoring: a meta-analysis and conceptual framework. *Frontiers in human neuroscience*, *7*, 466.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113.

Olvet, D.M. and Hajcak, G. (2009), The stability of error-related brain activity with increasing trials. Psychophysiology, 46: 957-961. https://doi.org/10.1111/j.1469-8986.2009.00848.x

Pasion, R., & Barbosa, F. (2019). ERN as a transdiagnostic marker of the internalizing-externalizing spectrum: A dissociable meta-analytic effect. *Neuroscience & Biobehavioral Reviews*, *103*, 133-149.

Pavlov, Y. G., Adamian, N., Appelhoff, S., Arvaneh, M., Benwell, C. S. Y., Beste, C., Bland, A. R., Bradford, D. E., Bublatzky, F., Busch, N. A., Clayson, P. E., Cruse, D., Czeszumski, A., Dreber, A., Dumas, G., Ehinger, B., Ganis, G., He, X., Hinojosa, J. A., … Mushtaq, F. (2021). #EEGManyLabs: Investigating the replicability of influential EEG experiments. *Cortex*, *144*, 213–229. https://doi.org/https://doi.org/10.1016/j.cortex.2021.03.013

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the general population. *Applied psychological measurement*, *1*(3), 385-401.

Riesel, A., Weinberg, A., Endrass, T., Meyer, A., & Hajcak, G. (2013). The ERN is the ERN is the ERN? Convergent validity of error-related brain activity across different tasks. *Biological Psychology*, *93*(3), 377–385.

Riesel, A., Weinberg, A., Moran, T., & Hajcak, G. (2013). Time course of error-potentiated startle and its relationship to error-related brain activity. *Journal of Psychophysiology*.

Jackson F, Nelson BD, Meyer A, Hajcak G. Pubertal development and anxiety risk independently relate to startle habituation during fear conditioning in 8-14 year-old females. Dev Psychobiol. 2017 May;59(4):436-448. doi: 10.1002/dev.21506. Epub 2017 Apr 6. PMID: 28383759; PMCID: PMC5458749.

Sandre, A., Banica, I., Riesel, A., Flake, J., Klawohn, J., & Weinberg, A. (2020). Comparing the effects of different methodological decisions on the error-related negativity and its association with behaviour and gender. *International Journal of Psychophysiology*, *156*, 18-39.

Saunders, B., & Inzlicht, M. (2020). Assessing and adjusting for publication bias in the relationship between anxiety and the error-related negativity. *International Journal of Psychophysiology*, *155*, 87-98.

Simmons, J. P., Nelson, L. D., & Simonsohn, U. (2011). False-positive psychology: Undisclosed flexibility in data collection and analysis allows presenting anything as significant. *Psychological science*, *22*(11), 1359-1366.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*.

Tabachnick, B. G., & Fidell, L. S. (2007). *Experimental designs using ANOVA* (Vol. 724). Thomson/Brooks/Cole Belmont, CA.

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, *36*, 1–48.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*, *54*(6), 1063.

Weinberg, A., Meyer, A., Hale-Rude, E., Perlman, G., Kotov, R., Klein, D. N., & Hajcak, G. (2016). Error-related negativity (ERN) and sustained threat: Conceptual framework and empirical evaluation in an adolescent sample. *Psychophysiology*, *53*(3), 372–385. https://doi.org/https://doi.org/10.1111/psyp.12538

Weinberg, A., Riesel, A., & Hajcak, G. (2012). Integrating multiple perspectives on error-related brain activity: The ERN as a neural indicator of trait defensive reactivity. *Motivation and Emotion*, *36*, 84–100.

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