Whole Brain Correlates of Individual Differences in Skin Conductance Responses during Human Fear Conditioning

Kevin Vinberg ^a, Jörgen Rosén ^{a, b}, Granit Kastrati ^{a, c}, Fredrik Åhs ^a

^a Department of Psychology and Social Work, Mid Sweden University, Östersund, Sweden

^b Department of Psychology, Uppsala University, Uppsala, Sweden. jorgen.rosen@psyk.uu.se

^c Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Abstract

Understanding the neural basis for individual differences in the conditioned skin conductance response (SCR) may inform on autonomic regulation in fear-related psychopathology. Previous region-of-interest (ROI) analyses have implicated the amygdala in regulating conditioned SCR, but whole brain analyses are lacking. This study examined correlations between individual differences in conditioned SCR and neural activity throughout the whole brain by using data from a large functional magnetic resonance imaging study ($N = 285$). Results show that conditioned SCR correlates with activity in the dorsal anterior cingulate cortex/anterior midcingulate cortex, anterior insula, bilateral temporoparietal junction, right frontal operculum, bilateral dorsal premotor cortex, right superior parietal lobe and midbrain. A ROI analysis replicated a correlation between amygdala activity and conditioned SCR, but amygdala contribution to SCR was modest compared with other regions. We suggest that implicated neural regions belong to a large-scale midcingulo-insular network related to salience detection and autonomic-interoceptive processing. Altered activity within this network may underlie individual differences in conditioned SCR and autonomic aspects of psychopathology.

Keywords: fear learning, autonomic activity, electrodermal activity, EDA, fMRI, salience network

1. Introduction

Differential fear conditioning is one of the most common methods for studying fear learning in laboratory settings (e.g. Fullana et al., 2016). It refers to the pairing of an initially neutral stimulus (CS+) with an aversive unconditioned stimulus (US), whereby the CS+ consequently comes to elicit increased autonomic responses relative to an unpaired neutral stimulus (CS-). Individual differences in differential fear conditioning have been linked to pathological fear and anxiety (Duits et al., 2015; Nees, Heinrich, & Flor, 2015; Lonsdorf & Merz, 2017) and are part of contemporary clinical models of these disorders (Mineka & Oehlberg, 2008; Bouton, Mineka, & Barlow, 2001; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). Understanding the neural basis of individual differences in differential fear conditioning may improve our understanding of autonomic regulation in pathological fear and anxiety (Lonsdorf & Merz, 2017), possibly providing us with biomarkers for pathology (e.g. MacNamara et al., 2015) and ultimately informing the development of more effective treatments (see e.g. Insel 2014). It has thus been argued that fear conditioning research should increasingly focus on understanding sources to individual differences and use large sample sizes to achieve this aim (Lonsdorf & Merz, 2017).

The most common way of measuring conditioned fear is the differential Skin Conductance Response (SCR; Lonsdorf et al., 2017), referring to changes in skin conductance induced by sympathetic activation (Dawson Schell & Fillion, 2007; Wallin et al. 1981). Previous studies considering the neural correlates of individual differences in magnitude of conditioned SCR, generally defined as the average CS+ SCR score subtracted from the average CS- SCR score, have implicated the amygdala (see e.g. Labar, Gatenby, Gore, LeDoux & Phelps, 1998; Phelps, Delgado, Nearing & LeDoux et al 2004; Dunsmoor, Prince, Murty, Kragel & Labar, 2011; Petrovic, Kalisch, Pessiglione, Singer & Dolan, 2008; MacNamara et al., 2015; Marin et al., 2019). This is in line with the general understanding of fear conditioning from animal models, where a neural circuitry centered on the amygdala has been demonstrated to be responsible for physiological and behavioral conditioning (LeDoux 2000; Davis 2000). It also connects with human lesion studies demonstrating diminished or absent conditioned SCR following amygdala damage (Labar et al. 1995; Bechara et al., 1995), as well as with human neuroimaging studies considering the neural correlates of within subject variation in conditioned SCR (Cheng, Knight, Smith, Stein & Helmstetter, 2003; Knight, Nguyen, & Bandettini, 2005; Cheng, Knight, Smith & Helmstetter, 2006).

1 However, these previous studies of the neural correlates of individual differences in conditioned SCR
2 have generally been based on small sample sizes ($N < 28$) and regions of interest analysis (ROIs)
3 targeting the amygdala. Such sample sizes fall below minimum requirements for correlation analysis in
4 fMRI research according to guidelines (Yarkoni, 2009; Yarkoni & Braver, 2010), that recommend a
5 sample size of at least $N = 40$ for determination of inter-individual correlations.

6 Furthermore, the previous focus on the amygdala excludes other regional activations potentially
7 important for explaining individual differences in conditioned SCR. In particular, the human fear
8 conditioning task is known to activate a large set of cortical, subcortical and brainstem areas other
9 than the amygdala (Fullana et al., 2016). In their meta-analysis, Fullana et al. (2016) proposed that
10 neural regions consistently activated during fear conditioning collectively constitute a large-scale
11 neural network, centered on the dorsal anterior cingulate cortex and anterior insula, that represents
12 autonomic-interoceptive processing in response to conditioned stimuli (Fullana et al. 2016; based on
13 findings by e.g. Cameron, 2009; Craig, 2009; Critchley & Harrison, 2013; Medford & Critchley, 2010).
14 Based on this proposal, one would expect individual differences in autonomic conditioned responding,
15 such as measured by SCR (Dawson Schell & Fillion, 2007), to correlate with altered activity within this
16 broader network.

17 Only two previous studies that investigated the association between individual differences in fear
18 conditioning and neural activity have met the minimum requirements for sample size suggested by
19 Yarkoni & Braver (2010): MacNamara et al (2015; $N = 49$) and Marin et al (2019; $N = 60$). Both these
20 studies implicated additional neural correlates besides the amygdala. In particular, the study by Marin
21 et al. (2019) implicated the dorsal anterior cingulate cortex and insula, two major nodes within the
22 neural network proposed to underlie fear conditioning by Fullana et al. (2016). MacNamara et al.
23 (2015) did not report correlations in these regions but found correlates of SCR in the supplementary
24 motor area, another area consistently activated during human fear conditioning (Fullana et al. 2016).
25 However, significant associations from both studies were still only reported using ROI analyses and
26 not whole brain analysis, meaning that neural correlates in areas outside the predefined ROIs may still
27 have gone unnoticed.

28 As previous studies have restricted analyses to predefined areas of the brain and/or have failed to
29 report significant whole brain correlations, we aimed to investigate the whole brain correlations of
30 individual differences in conditioned SCR by analyzing data from a large sample of participants
31 undergoing fear conditioning ($N = 285$). This sample size provided sufficient statistical power to detect
32 correlations with medium effect size and above ($r > .251$) throughout the whole brain (see the
33 Materials and Methods section for details). The use of a whole brain approach in establishing neural
34 correlates of individual differences in conditioned SCR has potential to identify novel brain regions
35 influencing fear conditioning and psychopathology. Besides the whole brain analysis of conditioned
36 SCR and neural activation, a second aim of our study was to replicate previous findings of an
37 association between individual differences in amygdala response and SCR using an ROI approach.
38 Finally, a third aim was to determine whether areas whose activity explained significant individual
39 variation in conditioned SCR did so independently of each other. A comparison of the relative
40 contributions of different brain areas to conditioned SCR has potential to inform on separate neural
41 pathways mediating individual differences in conditioned autonomic responding. Ultimately, such
42 findings may aid the understanding of autonomic regulation in pathological fear and anxiety.

43 2. Results

44 2.1 SCR

45 Subjects displayed significantly larger SCR to the CS+ relative to the CS- ($t(284) = 23.28$; $p < .001$; d
46 $= 1.38$), indicating successful conditioning. An SCR difference score between the CS+ relative to the
47 CS- was calculated for each participant ($M = 0.64$, $SD = 0.47$) and examining the distribution of SCR
48 difference scores revealed substantial individual differences (see Supplementary Information 1).

49 2.2 Correlation between SCR difference scores and brain responses during fear conditioning

50 2.2.1 Whole brain analysis

1 Fear conditioning related brain responses (CS+>CS-) were correlated to SCR difference scores in the
2 dorsal anterior cingulate cortex/anterior midcingulate cortex, right anterior insula, right inferior frontal
3 gyrus/frontal operculum, bilateral temporoparietal junction/superior temporal gyrus, right superior
4 parietal lobe/postcentral gyrus, bilateral superior frontal gyri/dorsal premotor cortex, and a right-
5 lateralized midbrain region in areas consistent with periaqueductal gray and reticular formation. In
6 order to ensure the reliability of our findings and facilitate comparison with prior studies, we performed
7 the same analysis using average square root transformed raw value SCRs instead of Z transformed
8 SCRs. We also performed the same analysis without any participant exclusion (see the Materials and
9 Methods section regarding participant exclusion). Both these analyses resulted in a very similar
10 pattern of correlations (see Supplementary Information 3 and 4), showing that the results seem robust
11 to different types of normalization of SCR and to different choices regarding participant exclusion. For
12 a summary of results, see Figure 1 and Table 1. The effects of fear conditioning on fMRI responses
13 (CS+ > CS-) are described in Supplementary Information 6.
14

15
16 **Figure 1.** Correlation between individual differences in conditioned SCR and whole brain responses
17 during fear conditioning. A) Activation map of key implicated neural regions. Color-coded t values
18 ranges from $t = 3$ to $t = 6$. The statistical image was thresholded at $p < 0.05$ FWE-corrected and
19 displayed on an anatomical brain template. B) Scatter plots depicting correlation between SCR
20 difference scores and eigenvariates from significant whole brain clusters in the dorsal anterior
21 cingulate cortex (upper panel) and the temporoparietal junction (lower panel). R = Right. dACC =
22 dorsal Anterior Cingulate Cortex. TPJ = Temporoparietal Junction. IFG = Inferior Frontal Gyrus.
23 PAG/SC = Periaqueductal gray/Superior Colliculus. AI = Anterior Insula.

Table 1. Whole Brain Correlation to Conditioned SCR

Anatomical region	Hemisphere	Voxels	<i>t</i>	MNI Coordinates		
				<i>x</i>	<i>y</i>	<i>z</i>
Dorsal Anterior Cingulate Cortex/Anterior Midcingulate Cortex	N/A	50	4.79	6	8	40
Anterior Insula	Right	20	4.65	36	20	6
Inferior Frontal Gyrus/Frontal Operculum	Right	138	5.80	56	10	2
Temporoparietal Junction/Superior Temporal Gyrus	Right	81	5.66	64	-40	20
Superior Frontal Gyrus/Dorsal Premotor Cortex	Right	44	5.21	18	0	68
Midbrain	Right	59	5.22	10	-30	-12
Superior Parietal Lobe/Postcentral Gyrus	Right	3	4.52	22	-46	70
Superior Frontal Gyrus/Dorsal Premotor Cortex	Left	2	4.46	-14	-2	72
Temporoparietal Junction/Superior Temporal Gyrus	Left	1	4.37	-62	-36	22

Note. MNI coordinates and *t* values represent significant peak voxels of each cluster. Statistical significance was calculated using *t* tests implemented within the SPM software with an FWE corrected alpha level of $\alpha = .05$.

1 2.2.2 Correlation between individual differences in conditioned SCR and amygdala activation

2 While no correlation between individual differences in conditioned SCR and amygdala activation
3 survived the statistical threshold in the whole brain analysis, a ROI analysis targeting the bilateral
4 amygdala was performed. This analysis demonstrated significant correlations bilaterally in the
5 amygdala (right peak MNI coordinates: 20, -2, -14; cluster size = 38 voxels; *t* = 3.68; left peak MNI
6 coordinates: -22, -2, -16; cluster size = 8 voxels; *t* = 3.08). See figure 2.

7

1
2 **Figure 2.** Correlations between individual differences in conditioned SCR and amygdala activation. A)
3 Activation map depicting significant activation on coronal section at MNI Y-coordinate = -2. Color-
4 coded t values ranges from $t = 2.0$ to $t = 4.0$. The statistical image was thresholded at $p < 0.05$ FWE-
5 corrected. B) Scatter plot depicting correlation between SCR difference scores and eigenvariates from
6 the significant right amygdala cluster within the amygdala ROI.
7

8 2.2.3 Relative contribution of whole brain and amygdala correlates to individual differences in SCR

9 In order to examine the independent and/or shared contributions from implicated neural areas, we
10 extracted eigenvariates from all significant clusters in the whole brain analysis and the amygdala ROI
11 analysis and entered these as regressors in a hierarchical regression analysis. Together, regional
12 eigenvariates demonstrated a significant correlation to conditioned SCR with a moderate effect size
13 ($F(1, 284) = 4.23$; $r = .38$; $r^2 = .15$; $p < .001$). By including amygdala eigenvariates in the null model
14 and examining r^2 change by adding whole brain correlates, a significant change in r^2 was observed
15 ($F(1,284)$ change = 3.69; r^2 change = .10; $p < .001$). This meant that the whole brain correlates
16 explained additional variance beyond that shared with the amygdala. In contrast, including regional
17 activations from the whole brain analysis in the null model and examining r^2 change by adding
18 eigenvariates from the amygdala ROI did not result in any significant change ($F(1,284)$ change = 1.50;
19 r^2 change = .01; $p = .226$). This meant that no unique contribution from amygdala activity could be
20 demonstrated beyond that shared with other brain regions. When examining the beta weights of all
21 regressors in the regression model (see Supplementary Information 5), we noticed significant
22 contribution from right midbrain eigenvariates ($p = .031$). Also, when including all regional
23 eigenvariates except the midbrain in a null model and examining r^2 change by adding midbrain to the
24 model, a significant change in r^2 was observed ($F(1, 284)$ change = 4.70; r^2 change = .015; $p =$
25 .031). This meant that the midbrain demonstrated significant unique explained variance relative to all
26 other implicated regions. Notably, however, this unique contribution could not be observed in the
27 alternative model where the amygdala was excluded ($p = .096$; see Supplementary Information 5)
28 indicating that this finding should be interpreted with caution since it may not replicate.

29 Finally, in order to determine if neural correlates to differential SCR were driven more by CS+ or CS-
30 we correlated eigenvariates from regions that were correlated to SCR difference scores with average
31 raw value SCRs to the CS+ and CS- separately. Results demonstrated significant correlations
32 between all regional BOLD eigenvariates and CS+ SCR (p values $\leq .001$), except for right superior
33 parietal lobe ($p = .002$), left superior frontal gyrus ($p = .002$), right amygdala ($p = .006$) and left
34 amygdala ($p = .029$), where correlations did not survive the Bonferroni-corrected threshold ($\alpha =$
35 .00151; see the Materials and Methods section for details regarding the Bonferroni-correction).

1 However, no regional eigenvariates evidenced significant correlation to CS- SCR (p values $> .00151$)
 2 and all regional eigenvariates were significantly more correlated to CS+ SCR than CS- SCR (p values
 3 $< .00151$) except for right amygdala ($p = .018$) and left amygdala ($p = .011$). This indicated that neural
 4 correlations to differential SCR were mainly driven by increased responding to the CS+.

5 3. Discussion

6 Individual differences in conditioned SCR are common (Lonsdorf & Merz, 2017), stable (Torrents-
 7 Rodas et al. 2014; Fredrikson, Annas, Georgiades, Hursti & Tersman, 1993; Zeidan et al. 2012) and
 8 have been associated with psychopathology (Duits et al. 2015; Nees, Heinrich & Flor, 2015; Lonsdorf
 9 & Merz 2017). In this study we examined the whole brain correlates of such differences in a large
 10 sample ($N = 285$) using simultaneous SCR and fMRI recordings. The fear conditioning task produced
 11 significantly larger SCRs to the CS+ than to the CS-, indicating successful conditioning at the group
 12 level. However, in line with previous research (Lonsdorf & Merz, 2017), there were also substantial
 13 individual differences in magnitude of conditioned response to the CS+ relative to the CS-. The SCR
 14 difference scores (CS+ - CS-) were calculated and correlated with whole brain activation to the CS+
 15 relative to the CS- on a voxel-by-voxel basis. Results demonstrated that individuals showing
 16 heightened conditioned SCR to the CS+ relative the CS- also showed heightened neural activity to the
 17 CS+ relative to the CS- in dorsal anterior cingulate cortex (dACC)/anterior midcingulate cortex
 18 (aMCC), right anterior insula, bilateral temporoparietal junction/superior temporal gyri, right frontal
 19 operculum, bilateral dorsal premotor cortex (BA6), right superior parietal lobe/postcentral gyrus and a
 20 right-lateralized midbrain region in areas consistent with the periaqueductal gray and reticular
 21 formation. Furthermore, a ROI analysis indicated that responses in bilateral amygdala were correlated
 22 to conditioned SCR. Hierarchical regression analysis suggested, however, that cortical and midbrain
 23 regions contributed to individual differences in conditioned SCR beyond the amygdala. On the
 24 contrary, the amygdala did not show evidence of any unique contribution to SCR beyond the whole
 25 brain correlates. When examining the unique contributions of all neural correlates relative to each
 26 other, the right midbrain region showed a significant unique contribution, although this finding should
 27 be interpreted with caution since it did not replicate in a regression model excluding the amygdala. All
 28 regional activations demonstrated a stronger correlation with SCR to the CS+ compared to the CS-
 29 although some correlations did not survive Bonferroni correction. This indicated that neural activity
 30 was primarily associated with heightened conditioned SCR to the CS+.

31 As expected, our whole brain analysis replicated previously reported correlations between individual
 32 differences in conditioned SCR and brain activity in the dACC and insula (Marin et al., 2019) as well
 33 as the amygdala (Labar et al 1998; Phelps et al 2004; Petrovic et al. 2008; MacNamara et al. 2015;
 34 Marin et al. 2019). We also identified correlations in novel regions consisting of bilateral
 35 temporoparietal junction/superior temporal gyri, right frontal operculum, bilateral dorsal premotor
 36 cortex, right superior parietal lobe and right midbrain. Notably, all whole brain correlates found in the
 37 present study belong to the set of regions consistently activated during human fear conditioning
 38 (Fullana et al., 2016). This indicates that these regions not only activate to the CS+ relative to the CS-
 39 in general, but that the magnitude of this activation also co-varies with individual differences in the
 40 magnitude of conditioned responding indexed by SCR. This is consistent with the proposal by Fullana
 41 et al. (2016; based on findings by e.g. Cameron, 2009; Craig, 2009; Critchley & Harrison, 2013;
 42 Medford & Critchley, 2010) that these regions, especially the dACC and anterior insula, are part of a
 43 large-scale neural network regulating autonomic responding. Reasonably, increased autonomic
 44 activation would correlate with larger responses in neural regions regulating autonomic processing.

45 Notably, various research groups other than Fullana et al. (2016) have examined the functional
 46 connectivity and network-structure between the regions reported in the present study (see e.g. Uddin,
 47 Yeo & Spreng, 2019, for a review). While Fullana et al. (2016) emphasize their role in autonomic-
 48 interoceptive processing and conscious awareness, others have proposed a more general function of
 49 detecting and preparing response to salient events across homeostatic, affective and cognitive
 50 domains (which includes, but also extend beyond, autonomic-interoceptive processing and autonomic
 51 regulation; see e.g. Seeley et al., 2007; Menon & Uddin 2010; Uddin 2015; Menon 2015; Uddin et al.,
 52 2019). We follow guidelines by Uddin et al. (2019) and refer to this network as the 'midcingulo-insular
 53 network'. This name was chosen to reflect the anatomy and to avoid multiple functional labels
 54 dependent on context (e.g. 'salience network', Menon 2015; 'cingulo-opercular network', Dosenbach
 55 et al., 2008; 'ventral attention network', Corbetta, Patel & Shulman, 2008). Core regions of the
 56 midcingulo-insular network are the presently implicated dACC/aMCC (see Vogt, 2016, for a discussion

1 regarding the naming of this region) and anterior insula, which are proposed to constitute the major
2 input-output nodes within the midcingulo-insular network (Uddin et al., 2019; Menon, 2015).
3 Specifically, the insula is thought to integrate cognitive, affective, interoceptive and homeostatic
4 information while the dACC re-represents this summarized information in order to determine
5 autonomic, behavioral and cognitive responding (Menon, 2015; Medford & Critchley, 2010). Efferent
6 autonomic output from the dACC is proposed to be mediated by the periaqueductal gray (Menon,
7 2015) such as presently implicated (by overlap of the present midbrain activation cluster and
8 coordinates previously denoted as the periaqueductal gray, see Linnman, Moulton, Barmettler,
9 Becerra & Borsook, 2012). Notably, less well-characterized regions of the midcingulo-insular network
10 also include the presently implicated bilateral temporoparietal junction/superior temporal gyri (Uddin et
11 al., 2019; see e.g. Yeo et al. 2011; Seeley et al. 2007; Bzdok et al. 2013) and the presently implicated
12 right frontal operculum (as part of what has previously been called the “cingulo-opercular network” or
13 the “ventral attention network”; Uddin et al., 2019; see e.g. Corbetta et al. 2008). Right lateralized
14 temporoparietal junction and frontal operculum appear to be specifically recruited during exogenous
15 salience detection (Uddin et al., 2019), which was assumed in the current study. Finally, the presently
16 implicated amygdala is also proposed to constitute a major subcortical node within this network
17 (Menon 2015; Uddin et al., 2019). Thus, most of the regions implicated in the present analysis belong
18 to this same well-replicated network and are consistent with its role in regulating autonomic
19 responding. Exceptions to this proposal include bilateral dorsal premotor cortex. However, the
20 premotor cortex is known to constitute a major cortical elicitor of the SCR (Dawson et al., 2007;
21 Boucsein, 2012), making it a plausible link between midcingulo-insular activity and peripheral skin
22 conductance. Consequently, based on our findings and previous theory we propose that individual
23 differences in conditioned SCR may originate from altered activity within the midcingulo-insular
24 network (Uddin et al., 2019) in conjunction with dorsal premotor cortex.

25 Within the midcingulo-insular network, the amygdala has been proposed to contribute to the cortical
26 processing of salience through biasing cortical input based on context-specific negatively valenced
27 cues (Menon et al., 2015). This is consistent with its role in learning, storing and responding to threat-
28 conditioned cues, such as demonstrated in animal models (Ledoux 2000; Davis 2000). However, the
29 midcingulo-insular network is also proposed to base salience processing on integrated cognitive
30 information and subjective experience (Uddin et al. 2019; Menon 2015), such as processed in the
31 anterior insula (see e.g. Medford & Critchley 2010 for a more detailed account), and such processes
32 have been dissociated from the amygdala (e.g. Bechara et al., 1995; Tabbert, Stark, Kirsch, & Vaitl,
33 2006; for a discussion see e.g. LeDoux & Pine, 2016; LeDoux & Brown, 2017). This implies that
34 amygdala activity should only have a partial influence on overall midcingulo-insular activity and
35 consequent behavioral, autonomic and cognitive responding. The present finding that a number of
36 midcingulo-insular regions explained significant individual variation in conditioned SCR beyond the
37 amygdala is consistent with this model. Specifically, amygdala activity may serve as one among
38 several inputs in determining the overall salience of a conditioned stimulus, while another important
39 input is explicit cortical processing beyond the amygdala. Based on the overall input of salience
40 information, integrated in the anterior insula, the strength of autonomic output may then be
41 determined.

42 It has been suggested that understanding sources of individual differences in fear conditioning may
43 uncover individual risk/resilience factors with respect to fear and anxiety that may ultimately aid the
44 understanding and treatment of fear-related psychopathology (Lonsdorf & Merz, 2017). Our proposal
45 that individual differences in conditioned SCR covary with activity in a large-scale neural network
46 substantiating autonomic-interoceptive processing and salience detection may highlight such a
47 source. Indeed, overactivity within the midcingulo-insular network has previously been suggested to
48 underlie pathological anxiety (Menon, 2011; Menon 2015), as patients with anxiety disorders show
49 altered functional connectivity within this network (Peterson, Thome, Frewen & Lanius 2014) as well
50 as hyperactivity within the anterior insula (Paulus & Stein, 2006; Stein, Simmons, Feinstein & Paulus,
51 2007) and amygdala (Etkin & Wager, 2007) nodes of the network. Based on these and similar
52 findings, it has been suggested that increases in anxiety and neuroticism may result from excessive
53 processing of emotion-related salient cues (Menon, 2011) and/or alterations in interoceptive-
54 autonomic processing (e.g. Paulus & Stein 2006; Medford & Critchley, 2010). As pathological fear and
55 anxiety has also been associated with altered SCR discrimination during fear conditioning (Duits et al.
56 2015; Nees, Heinrich & Flor 2015) and as our results indicate that SCR discrimination covary with
57 midcingulo-insular activity, our results are consistent with this anxiety model. Specifically, altered
58 midcingulo-insular activity in response to threat-related cues may constitute a risk factor, biomarker or

possibly even a cause of pathological fear and anxiety. We recommend future studies to continue examining this possibility, as it may have implications for the understanding and treatment of fear-related psychopathology.

One limitation of the present study is the use of social stimuli as CSs. While the general CS+>CS-BOLD contrast analyzed in the present study largely demonstrated a pattern of activation typical to fear conditioning (Fullana et al., 2016; see Supplementary Information 6), the potential influence of social threat processing cannot be entirely ruled out. Therefore, we recommend replicating our study using non-social stimuli as CSs.

In summary, the present study reports the largest to date study on individual differences in autonomic fear conditioning and brain activation. We are reporting several novel areas whose activation predict individual differences in conditioned SCR as well as replicating previous findings from smaller studies. Our results are consistent with the activation of a large-scale midcingulo-insular network substantiating autonomic-interoceptive processing and salience detection. We propose that altered activity within this network underlie individual differences in conditioned SCR and possibly autonomic regulation in pathological fear and anxiety. Future research should continue investigating this network as well as its possible relationship to fear and anxiety. Ultimately, such efforts may uncover the mechanisms of fear-related psychopathology and aid its treatment.

4. Materials and Methods

4.1 Subjects

This study was part of a twin study of genetic influences on fear related brain functions. Results describing genetic influences on SCR and fMRI responses during fear conditioning will be published elsewhere. 311 adult mono- and dizygotic twin volunteers were recruited from the Swedish Twin Registry (Svenska Tvillingregistret). Six participants were unable to participate in the fMRI-procedure and were excluded before data collection. After data collection, another twenty participants were excluded from analysis due to one or several of the following reasons: unsuccessful recording of skin conductance responses (2 participants); loss of brain imaging data due to excessive head-movement (5 participants); participant failure to comply with task instruction regarding button presses in at least 80% of trials (11 participants; see section 4.3.1); participant use of psychotropic medication (7 participants). Thus, 285 participants (female = 167, mean age = 33.92 years, $SD = 10.11$ years) were included in the analyses below. All participants passed the following exclusion criteria: pregnancy, inability to lie still for a 1 h duration, intolerance of tight confinements, ongoing psychological treatment, use of psychotropic medications, metal objects in the body (due to surgery, fragmentation etc.), current alcohol or drug related problems. However, in order to ensure the reliability of our findings we also performed an additional supplementary analysis where all participants with fMRI and SCR data were included ($N = 303$; see Supplementary Information 4). Participants provided written informed consent in accordance with guidelines from the Regional Ethics Review Board in Uppsala and received SEK 1000 as reimbursement for their participation. The study protocol was approved by the Regional Ethics Review Board in Uppsala.

4.2 Materials

4.2.1 Stimuli and Contexts

For the fear conditioning task, two male three-dimensional virtual humanoid characters and a virtual environment (Figure 3) were created in Unity (version 5.2.3, Unity Technologies, San Francisco, CA). The virtual environment consisted of a room with four red colored brick walls, grey concrete roof and a wooden floor.

1
2 *Figure 3.* Experimental setup. Two male characters were displayed in the scanner during the fear
3 conditioning task (top left, bottom right). One character predicted an electric shock (CS+) whereas the
4 other served as a control stimulus and was never followed by shock (CS-). Between character
5 presentations participants viewed the empty virtual environment (top right, bottom left).

6 4.2.2 Stimulus Presentation Software

7 Contexts and stimuli were presented on a flat screen in the MR scanner with the help of a projector
8 (Epson EX5260). The computer running the stimulus presentation used a custom version of Unity
9 (version 5.2.3, Unity Technologies, San Francisco, CA) and communicated with BIOPAC (BIOPAC
10 Systems, Goleta, CA) through a parallel port interface. The software for the parallel port interface was
11 custom made and used standard .NET serial communication libraries by Microsoft (Microsoft
12 Corporation, Albuquerque, New Mexico).

13 4.2.3 Brain imaging

14 Imaging data were acquired using a 3.0 T scanner (Discovery MR750, GE Healthcare) and an 8-
15 channel head-coil. Foam wedges, earplugs and headphones were used to reduce head motion and
16 scanner noise. We acquired T1-weighted structural images with whole-head coverage, TR = 6400 ms,
17 TE = 28 ms, acquisition time 6.04 min and flip angle 11 (degrees). Functional images were acquired
18 using gradient echo-planar-imaging (EPI), TR = 2400 ms, TE = 28 ms, flip angle = 80 (degrees), slice
19 thickness 3.0 mm with no spacing, axial orientation, frequency direction R/L, interleaved bottom up.
20 Higher order shimming was performed and the number of dummy scans before the experiment was 5.
21 Number of slices were 47 and voxel size 3.0 mm³.

22 4.2.4 Skin conductance responses

23 Skin conductance recording was controlled with the MP-150 BIOPAC system (BIOPAC Systems,
24 Goleta, CA). Radio-translucent disposable dry electrodes (EL509, BIOPAC Systems, Goleta, CA)
25 were coated with isotonic gel (GEL101, BIOPAC Systems, Goleta, CA) and placed on the palmar
26 surface of the left hand. The signal was high-pass filtered at 0.05Hz and SCRs were scored using
27 Ledalab software package (Benedek & Kaernbach, 2010) implemented in Matlab (Mathworks, Inc.,
28 Natick, MA). SCR was analyzed using the maximum phasic driver amplitude 1-4 seconds after CS
29 presentation for each participant.

30 Unlike previous studies considering the neural correlates of CS+>CS- SCR (Labar et al 1998; Phelps
31 et al 2004; Petrovic et al. 2008; MacNamara et al. 2015; Marin et al. 2019), SCRs in the present study

1 were range-corrected by Z transformation within individuals (Ben-Shakar, 1985). Z transformation
2 increases sensitivity to conditioning related effects and prevents confounding by non-conditioning
3 related individual differences in general SCR magnitude (Ben-Shakar, 1985). For comparison,
4 however, neural correlations based on raw SCR scores were also examined and can be found in
5 Supplementary Information 3.

6 Skin conductance was recorded without a low-pass filter. By using this recording procedure, we
7 noticed that a small number of trials produced unreasonably high SCR values (e.g. 17 mS
8 responding), likely due to electrode movement. As such extreme values may skew correlations even
9 using standard scores, we excluded from all analysis trials with a raw value SCR score > 3 mS. This
10 was based on previous research indicating a general maximum SCR between 2 and 3 mS using
11 similar methodology as the one used in this paper (Boucsein, 2012). Using this criterion 97/12200
12 trials were excluded from analysis (0.795% of all trials).

13 4.3 Procedure

14 4.3.1 Fear conditioning task

15 Two virtual characters served as CSs, one as a threat cue (CS+) predicting the US and the other as a
16 control cue (CS-). CSs were presented on a screen in the MR scanner at a distance of 2.7 m in the
17 virtual environment. The relatively long distance of 2.7 m was selected in order for the effect of
18 conditioning on SCR not to be occluded by proximal threat effects on SCR, as was observed in two
19 previous studies by Rosén et al. (Rosén, Kastrati & Åhs, 2017; Rosén, Kastrati, Reppling, Bergkvist &
20 Åhs, 2019). Participants were told prior to the experiment that they could learn to predict the US but
21 were not told which character served as the CS+. Participants were furthermore instructed to select
22 either “yes” or “no” with the click of a button immediately following each CS presentation, indicating
23 which character would be followed by the US. The inclusion of the button presses was to assure
24 participants’ attention during the task and data from these responses were not subjected to further
25 analysis. Which of the two characters that served as CS+ and CS- was counterbalanced across
26 participants using four different stimulus presentation orders. Each CS presentation lasted for 6 s
27 followed by an inter-stimulus interval of 8-12 s with no CS being present, but the context still being
28 displayed.

29 A habituation phase preceded the fear conditioning, where each CS was presented four times without
30 reinforcement for a total of eight CS presentations. This was followed by the fear conditioning phase,
31 during which each CS type (CS+ and CS-) was presented 16 times. The experimental task thus
32 consisted of 40 CS presentations in total, with 8 for habituation and 32 for conditioning. During
33 conditioning, eight of the CS+ presentations co-terminated with a presentation of the US (50% partial
34 reinforcement schedule; in accordance with guidelines for increasing sensitivity to inter-individual
35 differences in fear conditioning, see Lonsdorf & Merz, 2017). Total time for the fear conditioning task
36 was 9 min and 47 s.

37 The US consisted of an electric shock delivered to the subjects’ wrist via radio-translucent dry
38 electrodes (EL509, BIOPAC Systems, Goleta, CA). Prior to the experiment, the shock was calibrated
39 using an ascending staircase procedure where shock intensity is increased until rated by participants
40 as “uncomfortable” but not “painful” (Åhs, Kragel, Zielinski, Brady, & LaBar, 2015). US duration was 18
41 ms and controlled using the STM100C module connected to the STM200 constant voltage stimulator
42 (BIOPAC Systems, Goleta, CA).

43 4.3.2 Analysis of SCR data

44 SCR Z scores were averaged separately across CS+ and CS- trials within each participant. A paired
45 samples t test was performed comparing the average CS+ SCRs to the average CS- SCRs at a level
46 of significance of $\alpha = .05$ using JASP software (JASP Team (2020). JASP (Version 0.14.1) [Computer
47 software]). This allowed us to determine whether the fear conditioning task was successful in evoking
48 greater SCR to the CS+ than to the CS-. Secondly, in order to examine the correlations between
49 conditioned SCR and fMRI responses during fear conditioning, an SCR difference score was
50 calculated for each participant by subtracting the average SCR to CS+ presentations from the average
51 SCR to CS- presentations. The distribution of SCR difference scores was examined to ensure the

1 validity and sensitivity of neural regression analyses (see Supplementary Information 1 regarding
2 methodology and results of SCR distribution analysis).

3 4.3.3 Analysis of fMRI data

4 Analyses of fMRI data were performed using SPM12 (Wellcome Department of Cognitive Neurology,
5 University College, London). Preprocessing of images was performed using interleaved slice time
6 correction, realignment and co-registration to acquired T1 images. Quality of images was controlled
7 using MRIQC (mask validation matching; Esteban et al. 2017) and movement parameters. Images
8 were normalized to Montreal Neurological Institute (MNI) standard space. 8 mm FWHM Gaussian
9 smoothing was applied to ameliorate differences in inter-subject localization.

10 First-level analysis used event-related modeling including regressors for CS+ habituation trials, CS-
11 habituation trials, CS+ acquisition trials, CS- acquisition trials and US delivery in a general linear
12 model (GLM). Regressors mapped to the intervals of 6 s where each type of stimulus was displayed in
13 the scanner and was convolved with the hemodynamic response function to predict the fMRI time
14 course (for the brief US, the regressor still mapped to the 6 seconds following US delivery). Also, 6
15 movement parameter regressors, derived from the image realignment, and a mean value intercept
16 regressor were included in the model. At the second level, we first examined the overall whole brain
17 CS+>CS- BOLD contrast to assess conditioning related effects. Voxel-wise statistical significance was
18 calculated using *t* tests implemented in the SPM12 software with an alpha level of $\alpha = .05$ using family-
19 wise error (FWE) correction. As results regarding this contrast is not central to the present study they
20 are published in the Supplementary Information 6. However, it should be noted that these results were
21 typical for fear conditioning studies in general (see e.g. Fullana et al. 2016). In the present analysis,
22 we proceeded to examine regional activity within the whole brain CS+>CS- BOLD contrast that
23 covaried with individual differences in the SCR difference score. This was done by entering each
24 individuals previously obtained differential SCR score (CS+ minus CS-) as a second level between-
25 subjects regressor of their average CS+>CS- BOLD activation, effectively correlating the CS+>CS-
26 SCR difference with the CS+>CS- BOLD difference. Voxel-wise statistical significance was again
27 calculated using *t*-tests implemented in the SPM12 software with an FWE corrected alpha level of $\alpha =$
28 $.05$. Notably, this provided a local maxima height threshold of $t = 4.36$ given our sample size ($N =$
29 285). This roughly corresponds to a correlation coefficient of $r = .25$ given the conversion formula $r =$
30 $\sqrt{t^2 / (t^2 + DF)}$, thus corresponding to an effect size within the medium range according to
31 guidelines by Cohen (Cohen 1988; Cohen 1992).

32 Secondly, we aimed to replicate previous findings of an association between individual differences in
33 amygdala response and SCR using region-of-interest (ROI) analysis. The amygdala ROI was defined
34 using the automated anatomical labeling (AAL) library (Maldjian, Laurienti, Kraft, & Burdette, 2003)
35 and included both right and left amygdala. Analysis was performed the same way as the previous
36 whole brain correlation only this time individual SCR scores were correlated exclusively to contrast
37 values within the amygdala ROI, thus increasing sensitivity within this theoretically implicated region.

38 Third, in order to compare the independent contributions to explaining individual differences in
39 conditioned SCR of clusters of voxels that showed significant correlations, we extracted eigenvariates
40 from whole brain significant clusters and the amygdala ROI. Extracted eigenvariates for each cluster
41 were then entered as regressors in a hierarchical regression implemented in the JASP software. The
42 unique contribution to SCR of a specific neural region could then be calculated by adding all other
43 regional eigenvariates to the null model and examining significant ($p < .05$) *r*-squared change as a
44 result of including the specific neural region's eigenvariates in the model.

45 Finally, in order to determine if obtained neural correlates to SCR difference scores was driven more
46 by SCR to CS+ or CS-, we correlated eigenvariates extracted from implicated neural regions and
47 average SCRs to the CS+ and CS- separately. In this analysis we used square root transformed raw
48 value SCRs in order to obtain roughly normalized SCRs without confounding of CS+ and CS-
49 response magnitude such as when using Z transformation. As the distributions of square root
50 transformed raw value SCRs to the CS+ and CS- did not meet criteria for normality (by visual
51 inspection of histograms, QQ-plot and Shapiro-Wilke's test showing $p < .001$; see Supplementary
52 Information 2), we used Spearman's *Rho* instead of Pearson's *r* correlations. To compare the
53 difference between CS+ and CS- correlations we used the Steiger (1980) direct comparison of
54 dependent correlation coefficients such as implemented in free automated software by Lee and

1 Preacher (2013), as this is the most robust way of testing the difference between dependent
2 Spearman coefficients (Myers & Sirois, 2006). To compensate for multiple testing during correlation
3 comparisons, we used a Bonferroni corrected alpha level of $\alpha = .05/33 = .00151$. This compensated
4 for a total of 33 tests, reflecting 11 implicated neural regions each being correlated to CS+ and CS-
5 separately, as well as each having these correlations compared in an additional test.

6 Acknowledgements

7 This research was supported by grants from the Swedish Research Council (2014-01160, 2018-
8 01322) and the Bank of Sweden Tercentenary Foundation (P20-0125).

9 **Declaration of Interest:** The authors declare no conflict of interest.

10 References

- 11 Åhs, F., Kragel, P. A., Zielinski, D. J., Brady, R., & LaBar, K. S. (2015). Medial prefrontal pathways for
12 the contextual regulation of extinguished fear in humans. *NeuroImage*, 122, 262–271.
13 <https://doi.org/10.1016/j.neuroimage.2015.07.051>
- 14 Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. (1995). Double
15 dissociation of conditioning and declarative knowledge relative to the amygdala and
16 hippocampus in humans. *Science*, 269(5227), 1115–1118.
17 <https://doi.org/10.1126/science.7652558>
- 18 Benedek, M., & Kaernbach, C. (2010). A continuous measure of phasic electrodermal activity. *Journal*
19 *of Neuroscience Methods*, 190(1), 80–91. <https://doi.org/10.1016/j.jneumeth.2010.04.028>
- 20 Ben-Shakhar, G. (1985). Standardization Within Individuals: A Simple Method to Neutralize Individual
21 Differences in Skin Conductance. *Psychophysiology*, 22(3), 292–299.
22 <https://doi.org/10.1111/j.1469-8986.1985.tb01603.x>
- 23 Boucsein, W. (2012). *Electrodermal activity* (2nd ed.). Springer Science + Business
24 Media. <https://doi.org/10.1007/978-1-4614-1126-0>
- 25 Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the
26 etiology of panic disorder. *Psychological Review*, 108(1), 4–32. <https://doi.org/10.1037/0033-295x.108.1.4>
- 28 Bzdok, D., Langner, R., Schilbach, L., Jakobs, O., Roski, C., Caspers, S., Laird, A. R., Fox, P. T.,
29 Zilles, K., & Eickhoff, S. B. (2013). Characterization of the temporo-parietal junction by
30 combining data-driven parcellation, complementary connectivity analyses, and functional
31 decoding. *NeuroImage*, 81, 381–392. <https://doi.org/10.1016/j.neuroimage.2013.05.046>
- 32 Cameron, O. G. (2009). Visceral brain-body information transfer. *NeuroImage*, 47(3), 787–794.
33 <https://doi.org/10.1016/j.neuroimage.2009.05.010>
- 34 Cheng, D. T., Knight, D. C., Smith, C. N., & Helmstetter, F. J. (2006). Human amygdala activity during
35 the expression of fear responses. *Behavioral Neuroscience*, 120(6), 1187–1195.
36 <https://doi.org/10.1037/0735-7044.120.5.1187>
- 37 Cheng, D. T., Knight, D. C., Smith, C. N., Stein, E. A., & Helmstetter, F. J. (2003). Functional MRI of
38 human amygdala activity during Pavlovian fear conditioning: Stimulus processing versus
39 response expression. *Behavioral Neuroscience*, 117(1), 3–10. <https://doi.org/10.1037/0735-7044.117.1.3>
- 41 Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences (2nd Edition)* (2nd ed.).
42 Routledge.
- 43 Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112(1), 155–159.
44 <https://doi.org/10.1037/0033-2909.112.1.155>
- 45 Corbetta, M., Patel, G., & Shulman, G. L. (2008). The Reorienting System of the Human Brain: From
46 Environment to Theory of Mind. *Neuron*, 58(3), 306–324.
47 <https://doi.org/10.1016/j.neuron.2008.04.017>
- 48 (Bud) Craig, A. D. (2009). How do you feel — now? The anterior insula and human awareness. *Nature*

- 1 *Reviews Neuroscience*, 10(1), 59–70. <https://doi.org/10.1038/nrn2555>
- 2 Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure
3 therapy: An inhibitory learning approach. *Behaviour Research and Therapy*, 58, 10–23.
4 <https://doi.org/10.1016/j.brat.2014.04.006>
- 5 Critchley, H. D., & Harrison, N. A. (2013). Visceral Influences on Brain and Behavior. *Neuron*, 77(4),
6 624–638. <https://doi.org/10.1016/j.neuron.2013.02.008>
- 7 Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In:
8 J.P. Aggleton (Ed.), *The Amygdala* (pp. 213-288). Oxford University Press.
- 9 Dawson, M.E., Schell, A.M., Filion, D.L. (2007). The electrodermal system. In: Cacioppo, John T., Tas-
10 sinary, Louis G., Berntson, Gary G. (Eds.), *Handbook of Psychophysiology*. Cambridge Uni-
11 versity Press Cambridge, UK, pp. 159–181.
- 12 Dosenbach, N. U., Fair, D. A., Cohen, A. L., Schlaggar, B. L., & Petersen, S. E. (2008). A dual-net-
13 works architecture of top-down control. *Trends in Cognitive Sciences*, 12(3), 99–105.
14 <https://doi.org/10.1016/j.tics.2008.01.001>
- 15 Duits, P., Cath, D. C., Lissek, S., Hox, J. J., Hamm, A. O., Engelhard, I. M., ... Baas, J. M. P. (2015).
16 Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depression and*
17 *Anxiety*, 32(4), 239–253. <https://doi.org/10.1002/da.22353>
- 18 Dunsmoor, J. E., Prince, S. E., Murty, V. P., Kragel, P. A., & LaBar, K. S. (2011). Neurobehavioral
19 mechanisms of human fear generalization. *NeuroImage*, 55(4), 1878–1888.
20 <https://doi.org/10.1016/j.neuroimage.2011.01.041>
- 21 Etkin, A., & Wager, T. D. (2007). Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional
22 Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. *American Journal of*
23 *Psychiatry*, 164(10), 1476–1488. <https://doi.org/10.1176/appi.ajp.2007.07030504>
- 24 Esteban, O., Birman, D., Schaer, M., Koyejo, O. O., Poldrack, R. A., & Gorgolewski, K. J. (2017).
25 MRIQC: Advancing the automatic prediction of image quality in MRI from unseen sites. *PLOS*
26 *ONE*, 12(9), e0184661. <https://doi.org/10.1371/journal.pone.0184661>
- 27 Fox, J. (2015). *Applied Regression Analysis and Generalized Linear Models* (Third ed.). SAGE
28 Publications, Inc.
- 29 Fredrikson, M., Annas, P., Georgiades, A., Hursti, T., & Tersman, Z. (1993). Internal consistency and
30 temporal stability of classically conditioned skin conductance responses. *Biological*
31 *Psychology*, 35(2), 153–163. [https://doi.org/10.1016/0301-0511\(93\)90011-v](https://doi.org/10.1016/0301-0511(93)90011-v)
- 32 Fullana, M. A., Harrison, B. J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., & Radua,
33 J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis
34 of fMRI studies. *Molecular Psychiatry*, 21(4), 500–508. <https://doi.org/10.1038/mp.2015.88>
- 35 Insel, T. R. (2014). The NIMH Research Domain Criteria (RDoC) Project: Precision Medicine for
36 Psychiatry. *American Journal of Psychiatry*, 171(4), 395–397.
37 <https://doi.org/10.1176/appi.ajp.2014.14020138>
- 38 Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2005). *The role of the human amygdala in the*
39 *production of conditioned fear responses*. 26, 1193–1200.
40 <https://doi.org/10.1016/j.neuroimage.2005.03.020>
- 41 LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human Amygdala
42 Activation during Conditioned Fear Acquisition and Extinction: a Mixed-Trial fMRI Study.
43 *Neuron*, 20(5), 937–945. [https://doi.org/10.1016/s0896-6273\(00\)80475-4](https://doi.org/10.1016/s0896-6273(00)80475-4)
- 44 LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning
45 following unilateral temporal lobectomy in humans. *The Journal of Neuroscience*, 15(10),
46 6846–6855. <https://doi.org/10.1523/jneurosci.15-10-06846.1995>
- 47 LeDoux, J. E. (2000). Emotion Circuits in the Brain. *Annual Review of Neuroscience*, 23(1), 155–184.
48 <https://doi.org/10.1146/annurev.neuro.23.1.155>
- 49 LeDoux, J. E., & Brown, R. (2017). A higher-order theory of emotional consciousness. *Proceedings of*
50 *the National Academy of Sciences*, 114(10), E2016–E2025.

- 1 <https://doi.org/10.1073/pnas.1619316114>
- 2 LeDoux, J. E., & Pine, D. S. (2016). Using Neuroscience to Help Understand Fear and Anxiety: A
3 Two-System Framework. *American Journal of Psychiatry*, 173(11), 1083–1093.
4 <https://doi.org/10.1176/appi.ajp.2016.16030353>
- 5 Lee, I. A., & Preacher, K. J. (2013, September). Calculation for the test of the difference between two
6 dependent correlations with one variable in common [Computer software]. Available
7 from <http://quantpsy.org>.
- 8 Lonsdorf, T. B., & Merz, C. J. (2017). More than just noise: Inter-individual differences in fear
9 acquisition, extinction and return of fear in humans - Biological, experiential, temperamental
10 factors, and methodological pitfalls. *Neuroscience and Biobehavioral Reviews*, 80(April), 703–
11 728. <https://doi.org/10.1016/j.neubiorev.2017.07.007>
- 12 Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., ... Merz, C. J.
13 (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis
14 of studies on human fear acquisition, extinction, and return of fear. *Neuroscience and*
15 *Biobehavioral Reviews*, 77, 247–285. <https://doi.org/10.1016/j.neubiorev.2017.02.026>
- 16 MacNamara, A., Rabinak, C. A., Fitzgerald, D. A., Zhou, X. J., Shankman, S. A., Milad, M. R., & Phan,
17 K. L. (2015). Neural correlates of individual differences in fear learning. *Behavioural Brain*
18 *Research*, 287, 34–41. <https://doi.org/10.1016/j.bbr.2015.03.035>
- 19 Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for
20 neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*,
21 19(3), 1233–1239. [https://doi.org/10.1016/s1053-8119\(03\)00169-1](https://doi.org/10.1016/s1053-8119(03)00169-1)
- 22 Marin, M. F., Barbey, F., Rosenbaum, B. L., Hammoud, M. Z., Orr, S. P., & Milad, M. R. (2019).
23 Absence of conditioned responding in humans: A bad measure or individual differences?
24 *Psychophysiology*, 13350. <https://doi.org/10.1111/psyp.13350>
- 25 Medford, N., & Critchley, H. D. (2010). Conjoint activity of anterior insular and anterior cingulate
26 cortex: awareness and response. *Brain Structure & Function*, 214(5–6), 535–549.
27 <https://doi.org/10.1007/s00429-010-0265-x>
- 28 Menon V. (2015) Salience Network. In: Arthur W. Toga, editor. *Brain Mapping: An Encyclopedic*
29 *Reference*, vol. 2, pp. 597-611. Academic Press: Elsevier.
- 30 Menon, V. (2011). Large-scale brain networks and psychopathology: a unifying triple network model.
31 *Trends in Cognitive Sciences*, 1–24. <https://doi.org/10.1016/j.tics.2011.08.003>
- 32 Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to
33 understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica*, 127(3),
34 567–580. <https://doi.org/10.1016/j.actpsy.2007.11.007>
- 35 Myers, L., & Sirois, M. J. (2006). Spearman correlation coefficients, differences between. In: Kotz, S.,
36 Balakrishnan, N., Read, C. B., Vidakovic, B. (Eds.), *Encyclopedia of Statistical Sciences* (pp.
37 7901–7903). New York, NY: John Wiley and Sons.
38 <https://doi.org/10.1002/0471667196.ess5050.pub2>
- 39 Nees, F., Heinrich, A., & Flor, H. (2015). A mechanism-oriented approach to psychopathology : The
40 role of Pavlovian conditioning. *International Journal of Psychophysiology*, 98(2), 351–364.
41 <https://doi.org/10.1016/j.ijpsycho.2015.05.005>
- 42 Paulus, M. P., & Stein, M. B. (2006). An Insular View of Anxiety. *Biological Psychiatry*, 60(4), 383–387.
43 <https://doi.org/10.1016/j.biopsycho.2006.03.042>
- 44 Peterson, A., Thome, J., Frewen, P., & Lanius, R. A. (2014). Resting-State Neuroimaging Studies: A
45 New Way of Identifying Differences and Similarities among the Anxiety Disorders? *The*

- 1 *Canadian Journal of Psychiatry*, 59(6), 294–300.
- 2 <https://doi.org/10.1177/070674371405900602>
- 3 Petrovic, P., Kalisch, R., Pessiglione, M., Singer, T., & Dolan, R. J. (2008). Learning affective values
- 4 for faces is expressed in amygdala and fusiform gyrus. *Social Cognitive and Affective*
- 5 *Neuroscience*, 3(2), 109–118. <https://doi.org/10.1093/scan/nsn002>
- 6 Phelps, E. A., Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction Learning in Humans:
- 7 Role of the amygdala and vmPFC. *Neuron*, 43(6), 897–905.
- 8 <https://doi.org/10.1016/j.neuron.2004.08.042>
- 9 Razali, N. M., & Wah, Y. B. (2011). Power Comparisons of Shapiro-Wilk, Kolmogorov-Smirnov,
- 10 Lilliefors and Anderson-Darling Tests. *Journal of Statistical Modeling and Analytics*, 2, 21–33.
- 11 Rosén, J., Kastrati, G., & Åhs, F. (2017). Social, proximal and conditioned threat. *Neurobiology of*
- 12 *Learning and Memory*, 142, 236–243. <https://doi.org/10.1016/j.nlm.2017.05.014>
- 13 Rosén, J., Kastrati, G., Reppling, A., Bergkvist, K., Åhs, F. (2019). The effect of immersive virtual
- 14 reality on proximal and conditioned threat. *Scientific Reports*. 2019 Nov 22;9(1):17407. doi:
- 15 10.1038/s41598-019-53971-z. PMID: 31758051; PMCID: PMC6874534.
- 16 Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., ... Greicius, M. D.
- 17 (2007). *Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive*
- 18 *Control*. 27(9), 2349–2356. <https://doi.org/10.1523/JNEUROSCI.5587-06.2007>
- 19 Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples).
- 20 *Biometrika*, 52(3–4), 591–611. <https://doi.org/10.1093/biomet/52.3-4.591>
- 21 Steiger, J. H. (1980). Tests for comparing elements of a correlation matrix. *Psychological Bulletin*,
- 22 87(2), 245–251. <https://doi.org/10.1037/0033-2909.87.2.245>
- 23 Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased Amygdala and Insula
- 24 Activation During Emotion Processing in Anxiety-Prone Subjects. *American Journal of*
- 25 *Psychiatry*, 164(2), 318–327. <https://doi.org/10.1176/ajp.2007.164.2.318>
- 26 Torrents-Rodas, D., Fullana, M. A., Bonillo, A., Andiñón, O., Molinuevo, B., Caseras, X., & Torrubia, R.
- 27 (2014). Testing the temporal stability of individual differences in the acquisition and
- 28 generalization of fear. *Psychophysiology*, 51(7), 697–705. <https://doi.org/10.1111/psyp.12213>
- 29 Uddin, L. Q., Yeo, B. T. T., & Spreng, R. N. (2019). Towards a Universal Taxonomy of Macro - scale
- 30 Functional Human Brain Networks How Many Functional Brain Networks Are A fundamental
- 31 construct in neuroscience is the definition. *Brain Topography*, 32(6), 926–942.
- 32 <https://doi.org/10.1007/s10548-019-00744-6>
- 33 Wallin, B. G. (1981). Sympathetic Nerve Activity Underlying Electrodermal and Cardiovascular
- 34 Reactions in Man. *Psychophysiology*, 18(4), 470–476. <https://doi.org/10.1111/j.1469-8986.1981.tb02483.x>
- 35
- 36 Vogt, B. A. (2016). Midcingulate cortex: Structure, connections, homologies, functions and diseases.
- 37 *Journal of Chemical Neuroanatomy*, 74, 28–46. <https://doi.org/10.1016/j.jchemneu.2016.01.010>
- 38 Yarkoni, T. (2009). Big Correlations in Little Studies: Inflated fMRI Correlations Reflect Low Statistical
- 39 Power—Commentary on Vul, E., Harris, C., Winkielman, P. & Pashler, H. (2009).
- 40 *Perspectives on Psychological Science*, 4(3), 294–298. [https://doi.org/10.1111/j.1745-](https://doi.org/10.1111/j.1745-6924.2009.01127.x)
- 41 [6924.2009.01127.x](https://doi.org/10.1111/j.1745-6924.2009.01127.x)
- 42 Yarkoni, T., Braver, T.S. (2010). Cognitive neuroscience approaches to individual differences in
- 43 working memory and executive control: conceptual and methodological issues. In: Gruszka,
- 44 A., Matthews, G., Szymura, B. (Eds.) *Handbook of individual differences in cognition* (pp. 87–
- 45 107). New York: Springer.
- 46 Thomas Yeo, B. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M.,
- 47 Roffman, J. L., Smoller, J. W., Zöllei, L., Polimeni, J. R., Fischl, B., Liu, H., & Buckner, R. L.
- 48 (2011). The organization of the human cerebral cortex estimated by intrinsic functional
- 49 connectivity. *Journal of Neurophysiology*, 106(3), 1125–1165.
- 50 <https://doi.org/10.1152/jn.00338.2011>

- 1 Zeidan, M. A., Lebron-Milad, K., Thompson-Hollands, J., Im, J. J. Y., Dougherty, D. D., Holt, D. J., Orr,
2 S. P., & Milad, M. R. (2011). Test–Retest Reliability during Fear Acquisition and Fear
3 Extinction in Humans. *CNS Neuroscience & Therapeutics*, 18(4), 313–317.
4 <https://doi.org/10.1111/j.1755-5949.2011.00238.x>