Amygdala functional connectivity mediates the association between catastrophizing and threat-safety learning in youth with chronic pain

Running title: Amygdala rsFC, catastrophizing and threat learning

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY RESULTS

Participants

Table S1: Participant characteristics of the sample included in analyses

	Patients Mean (SE)	Controls Mean (SE)	Statistics comparing the two groups
n	46	29	
Sex			X^2 (1, n=75) = 4.74, p = .03
male	3	8	
female	43	21	
Age	15.83 (0.35)	15.69 (0.69)	t(73)= -0.20, p = .85
Pain Details			
Pain Type	*n (%)*	n/a	
Neuropathic	10 (21.7%)		
Musculoskeletal	27 (58.7%)		
Visceral	9 (19.6%)		
Pain Duration	35.98 (6.18)	n/a	
Pain Intensity	6.13 (.24)	1.62 (0.29)	t(73)= -11.82, p < .001
Alertness Ratings			
Alertness Before RS	7.26 (0.31)	6.75 (0.39)	t(68) = -1.04, p = .30
Alertness After RS	6.67 (0.30)	6.48 (0.31)	t(72)= -.41, p = .68
Self-Report			
STAI-T	16.26 (1.19)	9.24 (1.25)	t(73)= -3.89, p < .001
FDI	17.76 (1.70)	1.41 (0.46)	t(73)= -7.50, p < .001
PCS	21.28 (1.48)	8.70 (1.19)	t(73)= -6.03, p < .001
*low PCS (≤ 14)**	24 %	86 %	
*moderate PCS (15-25)**	45 %	14 %	
*high PCS (≥ 26)**	31 %	0 %	
Post-Acquisition Ratings			
Fear rating CS+	5.49 (0.34)	5.03 (0.39)	t(73)= -.87, p = .39
Fear rating CS-	2.60 (0.22)	2.48 (0.26)	t(73)= -.34, p = .74
Differential fear rating CS+>CS-	2.89 (0.42)	2.55 (0.48)	t(73)= -.52, p = .60

* Clinical reference points as proposed by Pielich *et al.* [1]. SE = standard error; RS = resting state; STAI-T = State-Trait Anxiety Inventory for Children – Trait version; FDI = Functional Disability Index; PCS = Pain Catastrophizing Scale; CS+ = conditioned stimulus paired with aversive stimulus (threat stimulus); CS- = conditioned stimulus never paired with aversive stimulus (safe stimulus)

MRI data - comparing left and right amygdala seeds

Differences in rsFC profiles were inspected by directly contrasting left and right amygdala seeds. Several clusters survived initial cluster-defining threshold (CDT) of $p < .001$, and subsequent cluster-extent based FDR correction of p (cluster p-FDR) < .05. In brief, left amygdala rsFC was more pronounced overall, and was stronger compared to right amygdala rsFC to regions including frontal pole and frontal and central opercular regions. Right amygdala rsFC was stronger compared to left amygdala rsFC to regions including frontal pole, supramarginal and angular gyrus.

Contrasting amygdala rsFC across patients and controls

Table S2: Left amygdala rsFC separate for patients. Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. The clusters coincide with those visually presented in **Figure 3A** in the main text. Clusters are extracted using cluster defining threshold $p < .001$ and subsequent cluster-extent FDR-correction. Anatomical locations are derived from Harvard Oxford atlases. *FDRc* = cluster size threshold of *FDR-p* < .05.

Anatomical Location	**x**	**y**	**z**	**k**	**max stat**
Positive rsFC				***FDRc k*>131**	
Frontal lobe					
bilateral frontal medial cortex	6	44	-16	661	6.86
right orbitofrontal cortex	30	32	-16	181	5.67
right frontal pole	4	60	20	483	5.36
left precentral gyrus / supplementary motor area	-6	-18	48	142	4.4
left precentral gyrus (*more medial*)	-14	-22	68	243	4.19
Subcortical regions					
bilateral amygdala, extending to hippocampus, putamen, pallidum, insula, precentral, temporal cortex	-24	-4	-22	21204	27.1
Other					
right cerebellum, posterior lobe	18	-70	-42	131	4.27
Negative rsFC				***FDRc k*>70**	
Frontal lobe					
left frontal pole	-26	60	-6	237	4.41

right frontal pole	22	62	-6	479	6.28
left middle frontal gyrus	-38	24	44	379	5.54
left middle frontal gyrus (*more posterior*)	-30	8	56	371	6.29
right middle frontal gyrus	40	26	36	1836	6.13
right paracingulate / superior frontal gyrus	6	20	44	166	4.22
right paracingulate gyrus	10	32	26	70	4.05
Parietal lobe					
right supramarginal gyrus, anterior and posterior division / angular gyrus	52	-40	48	2260	6.4
left supramarginal gyrus, posterior division / angular gyrus	-44	-48	38	1206	6.29
bilateral precuneus	6	-66	48	3015	6.93
cingulate gyrus, posterior division	6	-30	44	498	4.39
Occipital lobe					
left lingual gyrus	-24	-64	0	121	4.79
right lingual gyrus / occipital fusiform gyrus	24	-70	0	151	4.77
Other					
brainstem	22	-28	-42	99	4.74
left cerebellum	-48	-66	-50	377	6.01
left cerebral white matter	-20	-12	32	183	6.09
right cerebral white matter	18	-22	30	156	5.54

Table S3: Left amygdala rsFC separate for controls. Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. The clusters coincide with those visually presented in **Figure 3B** in the main text. Clusters are extracted using cluster defining threshold $p < .001$ and subsequent cluster-extent FDR-correction. Anatomical locations are derived from Harvard Oxford atlases. *FDRc* = cluster size threshold of *FDR-p* < .05.

Anatomical Location	**x**	**y**	**z**	**k**	**max stat**
Positive rsFC				***FDRc k*>76**	
Frontal lobe					
left frontal pole	-6	54	44	457	5.64
bilateral cingulate gyrus, anterior division	-2	30	2	114	4.36
bilateral cingulate gyrus, anterior division (*mid-cingulate*) / supplementary motor area	0	0	42	1627	6.16
right inferior frontal gyrus, pars triangularis	48	30	8	133	4.65

left precentral gyrus	-42	-12	54	217	4.79
Occipital cortex					
left lateral occipital cortex, superior division	-30	-76	54	104	4.87
Subcortical regions					
bilateral amygdala, extending to hippocampus, putamen, pallidum, insula, precentral, temporal cortex	-24	-4	-22	34265	34.99
Other					
brainstem	-6	-36	-48	85	4.29
brainstem / cerebellum	10	-48	-40	477	5.56
cerebellum	12	-58	-22	76	4.86
Negative rsFC				***FDRc k*>86**	
Frontal lobe					
left frontal pole	-26	62	2	679	5.34
right frontal pole	24	48	26	86	3.82
left middle frontal gyrus	-36	14	42	687	5.65
right middle frontal gyrus	40	18	48	4083	6.5
Parietal lobe					
left supramarginal gyrus, anterior and posterior division / angular gyrus	-44	-48	36	4586	6.51
right supramarginal gyrus, posterior division / angular gyrus	52	-46	38	1533	7.65
Temporal lobe					
left inferior temporal gyrus, posterior division	-48	-30	-16	140	4.4
right inferior temporal gyrus, posterior division	52	-34	-16	107	4.75
Occipital lobe					
left intracalcerine / lateral occipital cortex	-26	-66	8	311	4.89
Other					
left cerebellum	-48	-54	-48	548	6.32
left cerebral white matter	-20	-24	26	176	4.68
left cerebral white matter	-24	-48	26	88	4.19
right cerebral white matter	36	-42	0	370	4.84
right cerebral white matter	34	-24	36	159	4.55

Table S4: Right amygdala rsFC separate for patients. Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. The clusters coincide with those visually presented in **Figure 3A**. Clusters are extracted using cluster defining threshold $p < .001$ and subsequent cluster-extent FDR-correction. Anatomical locations are derived from Harvard Oxford atlases. *FDRc* = cluster size threshold of *FDR-p* < .05.

Anatomical Location	**x**	**y**	**z**	**k**	**max stat**
Positive rsFC				***FDRc k*>142**	
Frontal lobe					
bilateral frontal pole	4	60	20	595	5.95
bilateral frontal medial cortex	6	48	-16	754	5.91
Subcortical regions					
bilateral amygdala, extending to hippocampus, putamen, pallidum, insula, precentral, temporal cortex	24	-6	-18	22421	28.76
Other					
cerebellum / brainstem	12	-46	-40	142	4.4
Negative rsFC				***FDRc k*>107**	
Frontal lobe					
left frontal pole	-24	56	-4	859	5.5
right frontal pole	24	60	-16	305	5.48
left middle frontal gyrus	-30	8	56	497	5.44
left middle frontal gyrus (more inferior)	-42	18	38	388	4.62
right middle frontal gyrus	40	24	42	320	4.06
right superior frontal gyrus	18	18	56	592	4.47
Parietal lobe					
left angular gyrus / supramarginal gyrus, posterior division	-48	-52	42	1184	6.7
right supramarginal gyrus, posterior division / angular gyrus	48	-42	54	1093	6.25
bilateral precuneous cortex	-8	-64	32	2332	6.48
bilateral cingulate gyrus, posterior division	4	-30	24	596	5.16
Occipital lobe					
right lingual gyrus / occipital fusiform gyrus	22	-64	-10	222	4.08
Other					
left cerebellum	-48	-64	-52	246	4.69
left cerebellum (more superior)	-38	-58	-34	107	4.57

Table S5: Right amygdala rsFC separate for controls. Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. The clusters coincide with those visually presented in **Figure 3B**. Clusters are extracted using cluster defining threshold $p < .001$ and subsequent cluster-extent FDR-correction. Anatomical locations are derived from Harvard Oxford atlases. *FDRc* = cluster size threshold of *FDR-p* < .05.

Anatomical Location	**x**	**y**	**z**	**k**	**max stat**
Positive rsFC				***FDRc k*>83**	
Frontal lobe					
right inferior frontal gyrus, pars triangularis	48	30	12	176	5.16
bilateral supplementary motor area / cingulate gyrus, anterior division	10	0	62	1323	5.45
Temporal lobe					
left planum temporale / parietal operculum	-38	-36	14	83	4.31
Subcortical regions					
bilateral amygdala, extending to hippocampus, putamen, pallidum, insula, precentral, temporal cortex	24	-4	-18	35928	37.03
Negative rsFC				***FDRc k*>78**	
Frontal lobe					
left frontal pole	-24	62	2	1396	6.93
right frontal pole	16	54	-16	664	4.98
left middle frontal gyrus	-36	14	42	676	5.74
right middle frontal gyrus	40	20	42	172	4.16
bilateral superior frontal gyrus / paracingulate gyrus	-2	24	54	1199	5.38
left inferior frontal gyrus, pars opercularis	-50	14	6	87	4.1
bilateral cingulate gyrus, anterior / posterior division	6	-12	30	122	4.33
Parietal lobe					
left precuneous cortex / angular gyrus / supramarginal gyrus, posterior division	-6	-64	48	4599	7.3
right supramarginal gyrus, posterior division / angular gyrus	52	-42	38	530	5.49
Temporal lobe					
left middle temporal gyrus, posterior division	-44	-34	-12	182	4.48
Occipital lobe					
right occipital fusiform gyrus	24	-76	0	202	5.22
Subcortical regions					

right hippocampus / cerebral white matter	36	-42	0	78	5.55
Other					
left cerebellum	-44	-58	-52	378	5.74
right cerebellum	28	-82	-52	876	5.66
left cerebral white matter	-24	-24	26	304	5.65
right cerebral white matter	34	-24	36	285	5.38

Table S6: Group differences in left amygdala rsFC. Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. The clusters coincide with those visually presented in **Figure 3C** in the main text. Clusters are extracted using cluster defining threshold $p < .001$ and subsequent cluster-extent FDR-correction. Anatomical locations are derived from Harvard Oxford atlases. *FDRc* = cluster size threshold of *FDR-p* < .05.

Anatomical Location	**x**	**y**	**z**	***k***	**max stat**
patients > controls				***FDRc k*>76**	
Parietal lobe					
right supramarginal gyrus, anterior division	64	-28	48	133	4.18
controls > patients					
no significant clusters					

Relation between pain catastrophizing and threat-safety learning (total effect - path *c*)

The two experimental CS ratings of anxiousness and unpleasantness were combined into one 'fear' rating. When taking the ratings separately, similar correlations were observed. In patients, there was also a moderate, positive correlation between pain catastrophizing and self-reported differential anxiousness (CS+ > CS-; $r_p = .53$, $p < .001$), and between pain catastrophizing and self-reported differential unpleasantness (CS+ > CS-; $r_p = .35$, $p = .02$) (**Figure S1**), with elevated pain catastrophizing being associated with increased differential fear (i.e., the total effect, path *c*). In controls, these correlations were not significant.

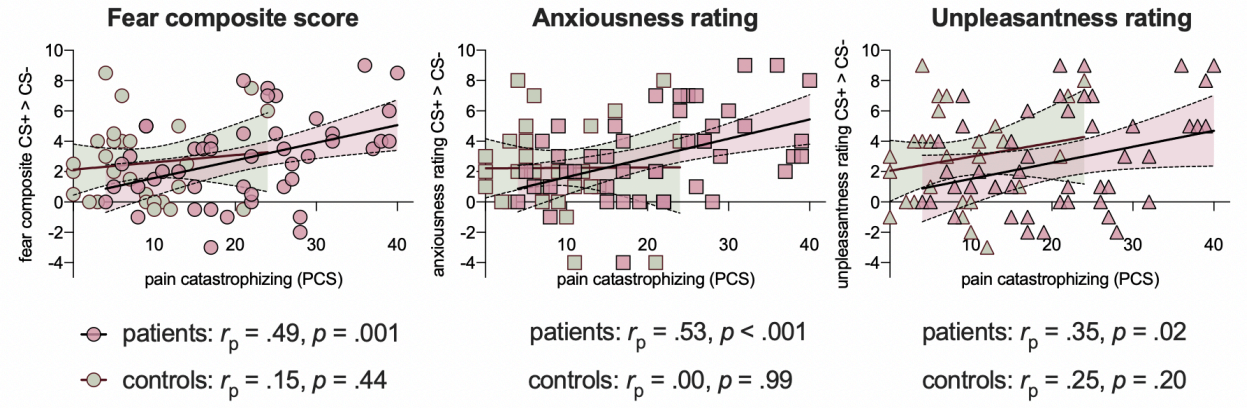

Figure S1. Association between pain catastrophizing and either the composite or separate indices of threat-safety learning (path *c*) in patients with chronic pain (pink) and controls (green). In patients, all three indices show a positive, moderate correlation with differential threat-safety learning indices. In controls, these associations were not significant.

Relation between pain catastrophizing and amygdala rsFC (path *a*)

Table S7: Left amygdala rsFC in relation to pain catastrophizing (X-M, path *a*). Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. While only clusters are included that survive $p < .001$, clusters were extracted using the thresholds $p < .05$ and $k > 50$ for visualization purposes (the clusters reflect those visually presented in **Figure 5** in the main text). Anatomical locations are derived from Harvard Oxford atlases.

Anatomical Location	x	y	z	*k*	max stat
Positive Correlations					
Frontal lobe					
left frontal pole / orbital cortex	-24	42	-12	202	8.28
right frontal pole	8	68	0	213	10.57
left middle frontal gyrus	-34	12	38	60	7.64
right superior frontal gyrus / paracingulate gyrus	6	24	48	141	7.87
bilateral precentral gyrus	4	-24	68	1051	10.64
Parietal lobe					
right parietal operculum	32	-28	20	66	7.62
right angular gyrus	34	-56	32	50	7.96

Temporal lobe					
left temporal pole	-34	10	-38	126	8.46
right temporal fusiform cortex	-38	-14	-32	105	6.93
Occipital lobe					
right lateral occipital cortex, superior division	44	-70	34	303	8.3
right occipital pole	16	-96	-22	54	7.42
Subcortical regions					
left amygdala / hippocampus / putamen	-20	-6	-18	424	10.73
bilateral caudate / thalamus	0	6	6	768	9.34
right thalamus	4	-24	4	131	7.02
Other					
brainstem	4	-40	-66	82	8.21
left cerebellum, anterior lobe	-4	-44	-28	253	7.97
right cerebral white matter	42	-36	-4	75	7.02
Negative Correlations					
Frontal lobe					
left frontal pole	-22	48	22	870	7.04
right frontal pole	28	58	-18	57	7.2
bilateral paracingulate gyrus	14	32	30	1873	8.22
Parietal lobe					
left supramarginal gyrus, anterior and posterior division / parietal operculum	-56	-34	38	943	7.98
right supramarginal gyrus, anterior and posterior division / parietal operculum	58	-32	34	1537	8.43
left precuneous cortex	-16	-66	24	63	7.71
right precuneous cortex	10	-64	22	67	7.46
Temporal lobe					
left middle temporal gyrus, superior division	-56	-24	-8	91	6.93
Occipital lobe					
right cuneal cortex	12	-84	22	225	7.17
Subcortical regions					
right putamen	34	6	0	161	7.79
Other					
left cerebellum, anterior lobe	-18	-58	-20	480	7.66
left cerebellum	-18	-70	-56	93	7.05
right cerebellum	24	-62	-60	146	8.23
right cerebellum, posterior lobe	16	-68	-20	609	7.94
right cerebellum, posterior lobe	34	-80	-26	139	7.1
right cerebellum, posterior lobe	32	-80	-42	129	7.18
right cerebellum, posterior lobe	50	-50	-38	73	6.95

Relation between amygdala rsFC and threat-safety learning (path *b*)

Table S8: Left amygdala rsFC in relation to threat-safety learning, while controlling for pain catastrophizing (M-Y, path *b*). Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. While only clusters are included that survive $p < .001$, clusters were extracted using the thresholds $p < .05$ and $k > 50$ for visualization purposes (the clusters reflect those visually presented in **Figure 6** in the main text). Anatomical locations are derived from Harvard Oxford atlases.

Anatomical Location	x	y	z	*k*	max stat
Positive Correlations					
Frontal lobe					
left frontal pole / frontal orbital cortex	-20	36	-10	149	7.01
right frontal pole	36	50	-14	260	6.95
right inferior frontal gyrus, pars opercularis and pars triangularus	50	26	10	1112	7.65
Parietal lobe					
right postcentral / precentral gyrus	40	-24	50	341	7.09
Temporal lobe					
left middle temporal gyrus, temporooccipital part	-62	-50	0	175	8.04
right middle temporal gyrus, temporooccipital part	60	-40	2	271	7.88
Occipital lobe					
right lateral occipital cortex, inferior division	30	-86	-2	1015	7.3
Subcortical regions					
right thalamus	6	-24	16	167	8.18
Other					
left cerebral white matter	-24	-38	8	53	8.46
Negative Correlations					
Frontal lobe					
left frontal pole	-38	44	22	211	7.9
right superior frontal gyrus	10	4	70	103	7.57
cingulate gyrus, anterior division / paracingulate gyrus	-2	38	22	2989	13.21
subcallosal cortex	0	10	-14	51	11.58
Parietal lobe					
right postcentral gyrus	14	-38	58	718	13
right parietal operculum / supramarginal gyrus, anterior division	52	-26	28	167	10.95
left angular gyrus	-48	-58	22	480	9.11
bilateral precuneous / supracalcarine cortex	0	-60	10	10490	11.95

Temporal lobe					
right temporal pole	52	12	-38	56	8.7
left inferior temporal gyrus, temporooccipital part	-48	-46	-30	75	8.16
Occipital lobe					
right lateral occipital cortex, superior division	46	-76	20	252	9.97
Subcortical regions					
left thalamus	-16	-8	14	93	9.26
left thalamus (*more ventral*)	-18	-22	-2	90	7.19
left putamen / pallidum	-24	-2	-2	88	8.64
Other					
cerebellum	-4	-52	-48	1386	10.66

Brain mediators of the relation between pain catastrophizing and threat-safety learning (indirect effect - path *a x b*)

Table S9: Left amygdala circuit mediators of relation between pain catastrophizing and threat-safety learning (path X-M-Y). Presented are anatomical locations, corresponding MNI coordinates, cluster size *k* and max (peak) statistic of the clusters. While only clusters are included that survive $p < .001$, clusters were extracted using the thresholds $p < .05$ and $k > 50$ for visualization purposes (the clusters reflect those visually presented in **Figure 7** in the main text). Anatomical locations are derived from Harvard Oxford atlases.

Anatomical Location	**x**	**y**	**z**	***k***	**max stat**
Positive Mediation					
Parietal lobe					
right supramarginal gyrus, anterior division / parietal operculum	54	-26	30	109	7.71
Other					
brainstem	12	-46	-48	67	8.06
Negative Mediation					
no significant clusters					

Post-hoc, we evaluated whether amygdala-SMG rsFC would also mediate the relation between pain catastrophizing and the separate indices of threat-safety learning (i.e., anxiousness and unpleasantness). **Figure S2** shows the findings remain significant and are similar in strength.

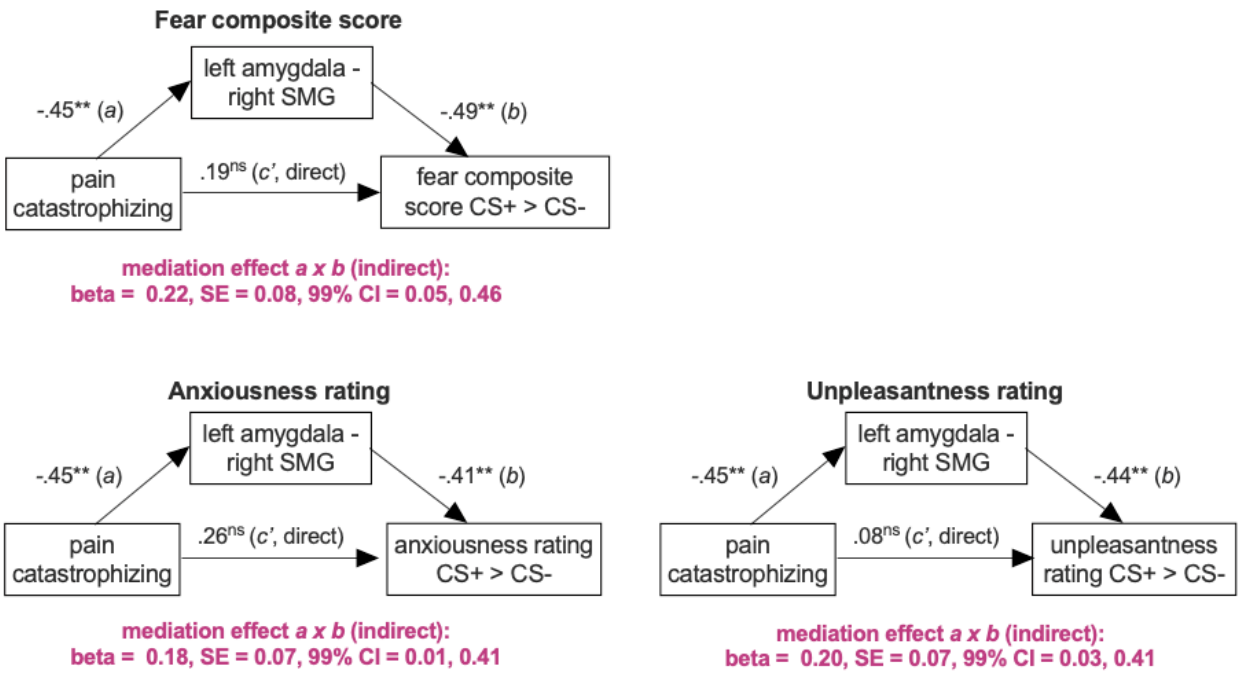

Figure S2. The path diagrams and standardized coefficients for all three outcome models (fear composite score, anxiousness rating and unpleasantness rating) are presented for descriptive purposes, as calculated offline (post-hoc).

Comparing key constructs across the different pain types

We inspected whether the key constructs in this study differed across patients with neuropathic, musculoskeletal or visceral pain using a one-way ANOVA. No main effects of group were observed in pain catastrophizing ($F_{2,43}$ = 1.10, p = .34), differential fear ratings ($F_{2,43}$ = 0.76, p = .48), amygdala-SMG group difference cluster rsFC ($F_{2,43}$ = 1.82, p = .18) or amygdala-SMG/PO brain mediator cluster rsFC ($F_{2,43}$ = 0.86, p = .43).

Comparing the group difference and brain mediator clusters

First, there was a moderate correlation between amygdala rsFC with the group difference SMG cluster and brain mediator SMG/PO cluster in patients (r = .48, p = .001), which was similar in controls (r = .47, p = .01). Second, while amygdala rsFC with the group difference SMG cluster differed between patients and controls (see manuscript), amygdala rsFC with the brain mediator

SMG/PO cluster did not ($F_{1,73}$ = 2.38, p = .13). Third, while amygdala rsFC with both clusters correlated with pain catastrophizing in the patient group, the correlation was stronger for the brain mediator SMG/PO cluster (group difference amygdala-SMG cluster: r = -.33, p = .03; brain mediator SMG/PO cluster: r = -.46, p = .001). Fourth, while amygdala rsFC with the brain mediator SMG/PO cluster correlated with threat-safety discrimination indices (differential fear CS+>CS-: r = -.58, p < .001), amygdala rsFC with the group difference SMG cluster did not (r = -.14, p = .37).

REFERENCE

[1] Pielech M, Ryan M, Logan D, Kaczynski K, White MT, Simons LE. Pain catastrophizing in children with chronic pain and their parents: proposed clinical reference points and reexamination of the Pain Catastrophizing Scale measure. Pain 2014;155(11):2360-2367.