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Appendix 1: A negative association between brainstem pontine gray matter volume, wellbeing and resilience in healthy twins

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Supplementary Methods

Participants

1,669 twins were recruited from the Australian Twin Registry and completed the baseline study phase, with a sub-sample of 263 participants who also completed the MRI scanning session which were tested here. From initial screens conducted by the Australian Twin Registry, participants were excluded if they reported current or lifetime psychiatric illness, history of stroke or neurological disorder, genetic disorder, brain injury (causing loss of consciousness for more than 10 minutes), chronic and serious medical conditions (e.g., cancer or heart disease), blood-borne illnesses (e.g., HIV, hepatitis), drug/alcohol substance abuse, and sensory impairments to hearing, hand movement or vision not corrected by glasses/lenses. Of the participants who passed the screening criteria and participated in the study, 5.7% of the total sample (n=15) later reported a history of psychiatric illness in online questionnaires, and 11% (n=29) reported a family history of psychiatric disorders. Zygosity was originally measured using a 12-item questionnaire we developed[1] using validated items from previously established questionnaires[2,3] shown to have 95% convergence with DNA results[4], and then later confirmed using DNA.

Measures

Self-report Measures

Wellbeing was measured using the 26-item COMPAS-W scale of wellbeing[5]. The COMPAS-W scale is a composite index of subjective (hedonic) and psychological (eudaimonic) wellbeing and also provides scores for its subcomponents of Composure, Own-worth, Mastery, Positivity, Achievement and Satisfaction. Here we used standardized z-scores for total wellbeing in analyses, and tertile categories for 'flourishing', 'moderate mental health' and 'languishing' wellbeing groups when graphing group means (scoring validated using nonlinear canonical correlation analysis[5]).

Depression and anxiety mood symptoms were assessed using the DASS-42[6]. The DASS is a psychometrically validated measure of anxiety and depression symptoms in nonclinical and clinical populations[6-9]. Here we used total DASS-42 scores (log-transformed) as a measure of general depression/anxiety symptoms. These scores have been previously validated in the current sample using confirmatory factor analysis[10].

Early life stress (trauma) was measured using the 19-item Early Life Stress Questionnaire (ELSQ), which assesses the occurrence of specific early life stressors up to 18 years shown to have a psychological impact in childhood, including abuse, neglect, family conflict, illness/death, and natural disasters[11]. This scale is based on the Child Abuse and Trauma Scale which has strong reliability and validity and correlates with adult outcome and psychopathology[11].

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MRI Brain Volume

MRI data was acquired on a 3.0 Tesla GE Signa HDx scanner (GE Healthcare, Milwaukee, WI) using an eight-channel head coil. A T1 weighted high resolution SPGR scan with the following parameters was acquired: 180 slices, 1mm cubic voxels, 256x256 matrix: TR 8.3, TE 3.2, TI 500, and flip angle 11. Segmentation and spatial normalization of MRI data was performed using voxel based morphometry (VBM) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm), using a protocol described in detail elsewhere[12,13]. Images were smoothed using a FWHM 12mm kernel, spatially normalized by transforming each brain to a standardized stereotactic space based on the ICBM 152 template (Montreal Neurological Institute, Montreal, Quebec, Canada). Images were segmented into gray, white, CSF and non-brain portions based on a cluster analysis method to separate pixels based on intensity differences, together with a priori knowledge of spatial tissue distribution patterns in normal subjects[14]. Segmentation was confirmed with visual inspection. A correction was made to preserve quantitative tissue volumes following the normalization procedure[12]. The regions-of-interest (ROI) tested were those gray matter regions that underpin the affective circuits of reward, threat and cognitive control (amygdala/hippocampus, anterior cingulate, basal ganglia, brainstem, inferior parietal gyrus, insula, medial frontal gyrus, orbital frontal gyrus, and thalamus). These were determined a priori and were defined by standardized masks using the Automated Anatomical Labelling toolbox[15].

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Supplementary Results Table 1:
Gray matter volume regions-of-interest associations with wellbeing, resilience and depression/anxiety scores at uncorrected *p* levels < .005

Effect/Region	Side	Coordinates (MNI) x	y	z	Cluster size (mm^3)	*t* value	*p* (FDR-corrected)	*p* (uncorrected)
Wellbeing								
Negative Correlation								
Inferior Parietal Gyrus	Left	-29	-42	55	65	3.34	0.186	0.001
	Left	-66	-34	33	316	2.68	0.640	0.004
Medial Frontal Gyrus (MPFC)	Right	3	66	-2	61	2.71	0.798	0.004
Positive Correlation								
Medial Frontal Gyrus (VMPFC)	Right	2	14	-18	192	2.80	0.387	0.003
	Left	-2	12	-18	79	2.78	0.387	0.003
Resilience								
Negative Correlation								
Inferior Parietal Gyrus	Left	-29	-42	55	56	2.86	0.859	0.003
Positive Correlation								
Hippocampus	Left	-33	-34	-8	68	3.03	0.385	0.002
Anterior Cingulate	Right	11	30	12	682	3.07	0.286	0.001
Medial Frontal Gyrus (VMPFC)	Right	2	14	-18	292	3.44	0.225	<0.0001
	Left	-2	12	-20	92	3.19	0.225	0.001
Insula	Right	47	3	-5	1171	3.24	0.473	0.001
	Left	-45	-1	-6	761	2.81	0.473	0.003
Depression/Anxiety Scores								
Positive Correlation								
Anterior Cingulate	Right	5	8	28	137	2.89	0.374	0.002
	Left	-14	41	6	266	3.16	0.374	0.001
		-9	17	28	379	2.71	0.374	0.004
Basal Ganglia (Caudate)	Left	-12	5	22	288	3.03	0.422	0.001
Medial Frontal Gyrus (DMPFC)	Left	-9	-4	51	825	3.31	0.415	0.001
		-15	29	39	2065	2.97	0.415	0.002

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Note. Uncorrected peak-level *p* values at $p < .005$. MPFC: medial prefrontal cortex; VMPFC: ventromedial prefrontal cortex; DMPFC: dorsomedial prefrontal cortex. Analyses conducted in twin 1, controlling for age and sex. The wellbeing and depression/anxiety VBM analysis was conducted in all twin 1 participants (N=132). For the resilience VBM analysis, associations between gray matter volume and wellbeing scores were conducted in the early life trauma exposed group (N=97).

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