SUPPLEMENTARY INFORMATION

Mineralocorticoid Receptor Iso/Val (rs5522) Genotype Moderates the Association between Prior Childhood Emotional Neglect and Amygdala Reactivity

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Amygdala reactivity task details

Participants completed 4 blocks of a perceptual face processing task in which they viewed a trio of faces (expressing either anger of fear) and selected which 1 of 2 faces (displayed on the bottom) was identical to the target stimulus (displayed on top). Each block consisted of 6 images derived from a standard set of facial affect pictures (1). Faces were balanced for gender and target affect. Each of the 6 face trios was presented for 4 seconds with a variable inter-stimulus interval of 2-6 seconds, for a total block length of 48 seconds. Interleaved between these blocks, participants completed 5 blocks of a sensorimotor control task during which they viewed a trio of geometric shapes (circles, horizontal ellipses, vertical ellipses) and selected which 1 of 2 shapes (displayed on the bottom) was identical to the target shape (displayed on top). Each sensorimotor task consisted of 6 different shape trios. Each of the 6 different shape trios was presented for 4 seconds with a fixed inter-stimulus interval of 2 seconds, for a total block length of 36 seconds. The total paradigm length was 390 seconds. Reaction times and accuracy were recorded through an MR-compatible button-box.

Image preprocessing

The general linear model of SPM8 was used for whole-brain image analysis. Individual subject data were realigned to the first volume in the time series to correct for head motion before being spatially normalized into the standard stereotactic space of the Montreal Neurological Institute template using a 12-parameter affine model. Next, data were smoothed to minimize noise and residual differences in individual anatomy with a 6mm FWHM Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Next, the ARtifact detection Tool (ART) was used to generate regressors to account for images due to

large motion (i.e., >0.6mm relative to the previous time frame) or spiking artifacts (i.e., global mean intensity 2.5 standard deviations from the entire time series). To ensure that an adequate signal from the amygdala was obtained, we used our ROI mask to ensure that all participants had more than 85% coverage of the amygdala.

Illumina genotyping

Consistent with the manufacturer's protocol (Illumina, San Diego, CA, USA), approximately 200 ng of DNA was used to genotype each subject sample. A BeadArray scanner detected each specifically hybridized DNA that was fluorescently labeled by a single base extension reaction which were then stained and imaged on an Illumina Bead Array Reader. Next, Illumina BeadStudio software produced SNP genotypes from fluorescent intensities using default cluster settings.

Analyses with ancestry informative principal components and in Caucasians only

After pruning regions in high linkage disequilibrium (LD), 110,317 SNPs were used to generate ancestry informative principal components (PC). Tracy-Widom (TW) statistics showed that individuals differed significantly on the first 8 components which were subsequently included in the first step of the regression in the entire sample (n = 208; sample loss due to participants who did not fit components). Analyses were also conducted within Caucasians only (n = 158). In both analyses, the main findings were generally reproduced: PC: $F(13,194) = 2.10$, $p = 0.02$, $R^2 = 0.12$; Caucasians only: $F(5,152) = 3.12$, $p = 0.01$, $R^2 = 0.09$. Childhood emotional neglect and MR val carrier status predicted heightened threat-related amygdala reactivity in each model (emotional neglect: PC: $b = 0.08$, $SE = 0.03$, $t = 3.00$, $p = 0.003$; Caucasians only: $b = 0.07$, $SE = 0.03$, $t = 2.61$, $p = 0.01$; MR val carrier status: PC: $b = 0.34$, $SE = 0.19$, $t = 1.79$, $p = 0.07$;

Caucasians only b = 0.45, SE = 0.20, t = 2.23, p = 0.03). Notably, the main effect of val carrier status was reduced to a trend in the PC model. The interaction in both remained significant after accounting for main effects in each model (PC: b = -0.15, SE = .06, t = -2.36, p = 0.02; ΔF = 5.58, ΔR^2 = 0.03, p = 0.02; Caucasians only: b = -0.16, SE = .07, t = -2.16, p = 0.03; ΔF = 4.65, ΔR^2 = 0.03, p = 0.03) with simple slope analyses reproducing the findings with the entire sample; there was a significant positive association between childhood emotional neglect and threat-related amygdala reactivity in iso homozygotes (PC: b = 0.11, SE = 0.03, t = 3.79, p = 0.0002; Caucasians only: b = 0.08, SE = 0.02, t = 3.64, p = 0.0003) but not val carriers (PC: b = -0.04, SE = 0.05, t = -0.64, p = 0.52; Caucasians only: b = -0.02, SE = 0.04, t = -0.49, p = 0.62).

Analyses by MR genotype group (iso/iso, iso/val, val/val)

A multiple regression using MR genotype (i.e., iso/iso, iso/val, val/val) as opposed to MR val carrier status produces a significant overall model for right amygdala reactivity in the entire sample, F(5,273) = 4.17, p = .001. Elevated childhood emotional neglect (*b* = 0.05, SE = 0.02, t = 2.76, p = 0.006) and more val alleles (*b* = 0.22, SE = 0.13, t = 1.74, p = 0.08) independently predicted heightened threat-related amygdala reactivity. Importantly, the MR genotype x childhood emotional neglect interaction significantly contributed to the model after accounting for these main effects (b = -0.10, SE = 0.05, t = -2.26, p = 0.02; ΔF = 5.60, ΔR^2 = 0.054, p = 0.02; Figure S1). Simple slope analyses indicated that there was a significant positive association between childhood emotional neglect and threat-related amygdala reactivity in individuals with the iso/iso (b = .11, SE = .03, t = 3.80, p = .0002) and iso/val (b = .05, SE = .02, t = 2.21, p = .0003) genotypes but not val homozygotes (b = -0.01, SE = .04, t = -0.34, p = .73).

Analyses in Ventral and Dorsal Amygdala Regions

The ventral amygdala ROI was anchored by MNI coordinates x = ±21, y = -3, z = -23, with widths of 14 mm, 6 mm, and 6 mm along the x-, y-, and z-axes, respectively. The total volume of the ventral amygdala was 1024 mm^3 in each hemisphere. The dorsal amygdala ROI was anchored by the MNI coordinates x = ±21, y = -4, z = -13, with widths of 14 mm, 8 mm and 10 mm along the x-, y-, and z-axes, respectively. The total volume of the dorsal amygdala was 1920 mm^3 in each hemisphere. The reported widths reflect the total for the ROI along each axis and are centered on the MNI coordinate anchoring each axis (i.e., with x = 21 and width = 14 mm, the range of coordinates included along that axis of the ROI are from x = 14 to x = 28). We defined a larger volume for the dorsal amygdala to encompass the extended projection of the central nucleus to the substantia innominata and nucleus basalis of Meynert, cholinergic projection fields providing glutamatergic innervation of the forebrain (P.J. Whalen, personal communication). The posterior extent of both the dorsal and ventral amygdala were carefully defined to exclude the hippocampus. As in our analyses for the whole amygdala, we extracted BOLD parameter estimates from functional clusters exhibiting signal from the Faces > Shapes contrast at a combined voxel-level threshold of $p < 0.05$, FWE-corrected whole-brain, and cluster threshold of ≥ 10 contiguous voxels.

As in analyses with the whole amygdala, the overall model was significant for the dorsal and ventral regions in the right (dorsal: $F(5,273) = 3.22$, $p = 0.008$, $R^2 = 0.06$; ventral: $F(5,273) = 3.23$, $p = 0.007$, $R^2 = 0.06$), but not left (both $Fs(5,273) < 1.44$, both $ps > .21$) amygdala. In the right amygdala, elevated childhood emotional neglect (dorsal: $b = 0.06$, SE = 0.02, $t = 2.79$, $p = 0.006$; ventral: $b = 0.06$, SE = 0.02, $t = 2.35$, $p = 0.02$) independently predicted heightened

threat-related amygdala reactivity in both dorsal and ventral regions. The main effect of MR genotype approached a trend in the right dorsal amygdala (*b* = 0.28, SE = 0.15, t = 1.87, p = 0.06) but was not significant in the right ventral amygdala (*b* = 0.25, SE = 0.17, t = 1.42, p = 0.16). The MR genotype x childhood emotional neglect interaction reached a trend in the right dorsal and ventral amygdala (dorsal: *b* = -0.09, SE = 0.05, t = -1.85, $\Delta F = 3.44$, $\Delta R^2 = 0.012$, p = 0.06; ventral: *b* = -0.10, SE = 0.06, t = -1.76, $\Delta F = 3.11$, $\Delta R^2 = 0.011$, p = 0.08).

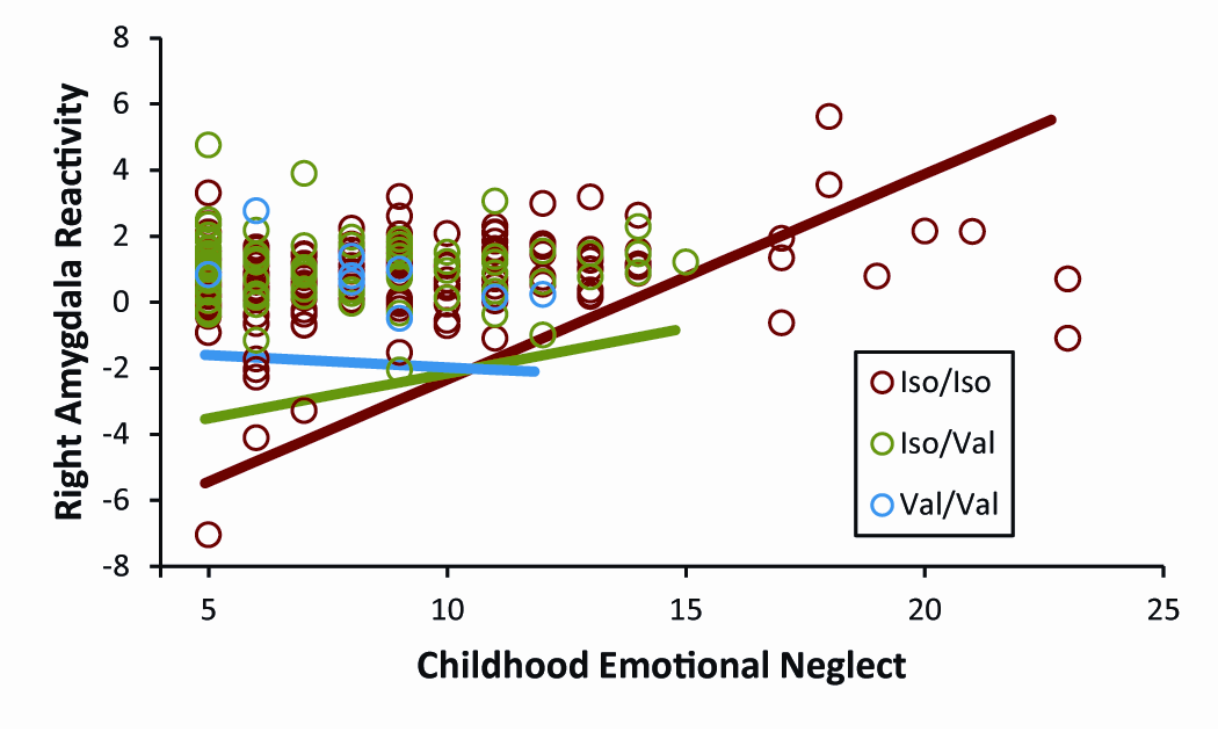
Supplemental References

1. Ekman P, Friesen WV: Pictures of Facial Affect. Palo Alto, CA: Consultin Psychologists Press, 1976

Supplemental Figure Legend

Figure S1. MR Genotype Group x Emotional Neglect Interaction. Data plotted for all three genotype groups (val/val, iso/val, iso/iso).

Figure S1.


Right Amygdala Reactivity
8
6
4
2
0
-2
-4
-6
-8
5
10
15
20
25
Childhood Emotional Neglect
Iso/Iso
Iso/Val
Val/Val