ARTICLE

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Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor

Kerry J. Ressler[1,2,4], Kristina B. Mercer[1], Bekh Bradley[2,3], Tanja Jovanovic[2], Amy Mahan[4], Kimberly Kerley[1], Seth D. Norrholm[2,3], Varun Kilaru[2], Alicia K. Smith[2], Amanda J. Myers[5], Manuel Ramirez[5], Anzhelika Engel[5], Sayamwong E. Hammack[6], Donna Toufexis[4,6], Karen M. Braas[7], Elisabeth B. Binder[2,8] & Victor May[7]

Pituitary adenylate cyclase-activating polypeptide (PACAP) is known to broadly regulate the cellular stress response. In contrast, it is unclear if the PACAP–PAC1 receptor pathway has a role in human psychological stress responses, such as post-traumatic stress disorder (PTSD). Here we find, in heavily traumatized subjects, a sex-specific association of PACAP blood levels with fear physiology, PTSD diagnosis and symptoms in females. We examined 44 single nucleotide polymorphisms (SNPs) spanning the PACAP (encoded by *ADCYAP1*) and PAC1 (encoded by *ADCYAP1R1*) genes, demonstrating a sex-specific association with PTSD. A single SNP in a putative oestrogen response element within *ADCYAP1R1*, rs2267735, predicts PTSD diagnosis and symptoms in females only. This SNP also associates with fear discrimination and with *ADCYAP1R1* messenger RNA expression in human brain. Methylation of *ADCYAP1R1* in peripheral blood is also associated with PTSD. Complementing these human data, *ADCYAP1R1* mRNA is induced with fear conditioning or oestrogen replacement in rodent models. These data suggest that perturbations in the PACAP–PAC1 pathway are involved in abnormal stress responses underlying PTSD. These sex-specific effects may occur via oestrogen regulation of *ADCYAP1R1*. PACAP levels and *ADCYAP1R1* SNPs may serve as useful biomarkers to further our mechanistic understanding of PTSD.

PACAP was first isolated from ovine hypothalamic extracts based on its ability to stimulate cyclic AMP production in anterior pituitary cells[1]. It is a highly conserved member of the VIP/secretin/glucagon peptide family, with pleiotropic functions in development, cell signalling, metabolism, homeostasis and cell protection[2–6]. Among these myriad functions, studies have demonstrated (1) high expression of PACAP peptide and its selective PAC1 receptor in hypothalamic and limbic structures, (2) PACAP regulation of corticotropin releasing hormone and autonomic function, (3) actions of PACAP in stress-related behaviour, (4) reduced anxiety-like phenotypes in PACAP and PAC1 receptor null mice, and (5) blunted corticosterone response in knockout animals after emotional stressors. Thus, PACAP–PAC1 receptor signalling is integrally involved in stress mechanisms[7,8]. We hypothesized that PACAPergic systems may be important mediators of abnormal stress responses following psychological trauma contributing to PTSD, which is an extreme maladaptive and debilitating psychiatric disorder affecting up to 40% of individuals over lifetime exposure to traumatic events[9,10].

Little is known about the biological processes regulating PTSD and other stress-related responses. To examine whether the PACAP–PAC1 pathway is associated with PTSD in a high risk, heavily traumatized population, we analysed blood levels of PACAP, and genetic variation and methylation of the PACAP (*ADCYAP1*) and PAC1 receptor (*ADCYAP1R1*) genes, in a cohort of more than 1,200 highly traumatized subjects with and without PTSD (see Supplementary Tables 1 and 2 for demographic information).

PACAP levels associated with PTSD in females

Using radioimmunoassay, we first examined PACAP peptide levels in peripheral blood samples from a previously described, highly traumatized, at risk population[11–13] that had been matched on age, sex, and trauma histories ($n = 64$, see Supplementary Tables 1–3 for demographics). We found that PTSD symptoms (PTSD symptom scale[14]) were significantly correlated with PACAP38 (PACAP peptide containing 38 residues) blood levels in females ($P < 0.005$, $r = 0.497$, Fig. 1a), but not in males ($P > 0.5$). Also in females, PTSD diagnosis was associated with PACAP38 levels ($P \leq 0.001$), with higher PACAP38 found in the PTSD cohort. Furthermore, PACAP levels (median split, low versus high) were differentially associated with PTSD symptoms in females (Fig. 1b). PACAP38 levels also predicted differential response on all three symptom clusters necessary to fulfil diagnostic criteria for PTSD (intrusive re-experiencing (for example, trauma flashbacks), avoidance (for example, avoidance of trauma reminders) and hyperarousal (for example, increased startle response)) in females but not males (Fig. 1c). These analyses were repeated in a second, all female cohort ($N = 74$) with similar findings (Fig. 1d; high versus low PACAP38 levels, controlling for age, substance abuse and total trauma exposure, one-tailed *t*-tests: total symptoms, $P \leq 0.05$, hyperarousal symptoms, $P \leq 0.001$; and percentage with clinically significant symptoms, $\chi^2 = 4.9$, $P < 0.05$). These observations were especially notable, as females may be at twice the risk for PTSD as compared to males[9,11], implicating roles for sex hormones, especially oestrogen, in the disorder[15–17]. When we controlled for

[1]Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA. [2]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30322, USA. [3]Atlanta VA Medical Center, Atlanta, Georgia 30033, USA. [4]Yerkes National Primate Research Center, Atlanta, Georgia 30329, USA. [5]University of Miami, Miller School of Medicine, Miami, Florida 33136, USA. [6]Department of Psychology, University of Vermont, Burlington, Vermont 05401, USA. [7]Departments of Anatomy and Neurobiology and Pharmacology, University of Vermont College of Medicine, Burlington, Vermont 05401, USA. [8]Max Planck Institute of Psychiatry, Munich 80804, Germany.

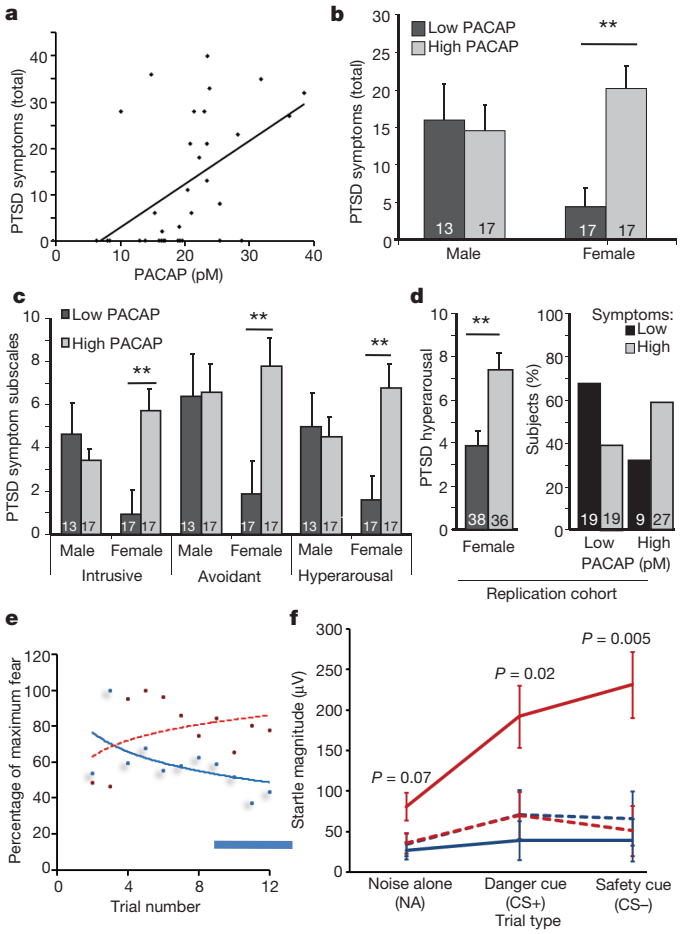


Figure 1 | PACAP blood levels predict PTSD symptoms in females. **a**, PTSD symptoms (scale range 0–51), relative to plasma PACAP38 blood levels (pM); ($N = 34$ females; $r = 0.497$, $P \leq 0.005$). **b**, Total PTSD symptoms plotted relative to sex and levels of plasma PACAP38 ($N = 64$, low: <20 pM, high: >20 pM); females with high PACAP blood levels have increased symptoms (**$P < 0.0005$). **c**, PACAP levels (low versus high) are also differentially associated with PTSD intrusive, avoidance and hyperarousal symptoms in females ($N = 64$,**$P < 0.005$). **d**, PACAP levels (low versus high) were examined in a replication sample of highly traumatized women, with differential association in hyperarousal symptoms (left, $N = 74$,**$P = 0.002$) and in the percentage of subjects with significant symptoms (right, $\chi^2 = 4.9$, $P < 0.05$). **e**, Acoustic startle reflex (EMG) relative to the fear conditioning trial in subjects without PTSD (blue) versus with PTSD (red). Habituation is seen in non-PTSD subjects during late acquisition (bar). **f**, Startle magnitude during the late acquisition period versus trial type (noise alone, CS+ and CS−), showing that females with high PACAP levels show enhanced startle responses to both fear cues (CS+, $P = 0.02$) and safety cues (CS−, $P = 0.005$) ($N = 27$; 16 male, 11 female). Dashed lines, low PACAP; solid lines, high PACAP; blue, male; red, female. Bars represent mean ± s.e.m., N values for each group at bottom of bar graphs.

common stress-related phenomena (depression and history of substance abuse), the effect of PACAP level on PTSD remained ($P < 0.05$). In contrast, there was no effect of PACAP level on depression symptoms or substance abuse when controlling for PTSD.

In addition to the psychological symptoms that define the syndrome, subjects with PTSD have been found to have abnormally high conditioned fear responses. This high level of fear may result from a combination of an inability to habituate to aversive stimuli, a decreased ability to extinguish (learn to inhibit) fear memories, and possibly an over-consolidation of the original fear memory[18–22]. Hence, we determined the physiological (electromyographic) levels of conditioned fear for 27 participants (16 male, 11 female) with PACAP blood levels. Fear potentiation was determined by measuring the acoustic startle reflex in the presence of startle cues alone, or startle cues combined with stimuli paired (conditioned stimulus, CS+) or unpaired (CS−) with an aversive airblast. Female (but not male) subjects with high PACAP38 levels demonstrated markedly increased startle reflex responses to both CS+ ($P = 0.02$) and CS− ($P = 0.005$) cues. This was particularly pronounced during the late acquisition phase when normal subjects had habituated to the fearful stimuli (Fig. 1e, f). In aggregate, these data suggest that PACAP38 peptide is strongly associated with the psychological and physiological symptoms of PTSD in women with a history of trauma.

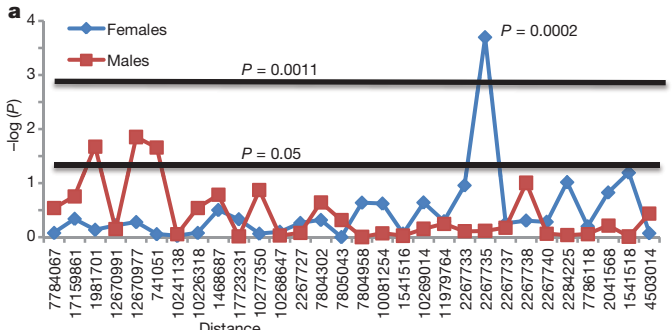
ADCYAP1R1 associated with PTSD in females

To assess whether there may be a genetic association of PTSD with polymorphisms in either the PACAP (*ADCYAP1*) or PAC1 receptor (*ADCYAP1R1*) locus, we performed a tag-SNP analysis ($r^2 = 0.8$; minor allele frequency (MAF) = 0.1) across both genes with a total of 44 SNPs (14 *ADCYAP1* and 30 *ADCYAP1R1* SNPs). Using logistic regression, we examined whether each SNP was associated with PTSD diagnosis in this cohort of highly traumatized urban civilian subjects ($n = 798$)[11,12,23], in total, or stratified by gender (females: $n = 503$; males: $n = 295$). Only the *ADCYAP1R1* receptor SNP rs2267735 ($P = 0.0002$ in females; NS in males) remained significant after experiment-wide multiple correction for sex and 44 independent tests (Fig. 2a and b, Supplementary Fig. 1). No SNPs in the peptide *ADCYAP1* gene met experiment-wide criteria for association (Supplementary Fig. 2). Given these striking gender differences and recent data demonstrating that *ADCYAP1R1* gene expression may be dynamically modulated by oestrogen[24], the distribution of oestrogen response elements (EREs) within the *ADCYAP1R1* gene was examined (Supplementary Table 4). We found that rs2267735 was within a predicted ERE (Fig. 2c, Genomatix; matrix similarity = 0.877, core similarity = 1.0). Because rs2267735 is positioned within the central variable region of the consensus sequence, *in silico* analyses do not currently allow us to predict how the 'C' versus 'G' allele may differentially alter ERE function, and further *in vitro* analyses are warranted.

We next determined if the association between rs2267735 and PTSD diagnosis could be replicated in an additional 439 subjects. These subjects were from the same overall study, but were interviewed and had DNA collected after the original discovery population. Thus they served as a replication source from the same population but distinct in time and with different interviewing staff. The table in Fig. 3a shows the logistic regression results for males and females separately in the initial population described in the tag-SNP analysis, the replication sample from the same population, and the combined sample of 1,237 individuals. The main effect of the SNP on PTSD diagnosis could be replicated in women ($P < 0.05$) and combining both samples increased the significance of the association ($N = 763$, $P < 0.00002$). As in the discovery sample, no effects were observed in males (male combined sample $N = 474$, $P = 0.7$).

To further examine *ADCYAP1R1* rs2267735 SNP associations with continuous PTSD symptom levels in females, we analysed both an additive and a dominant model with total PTSD symptoms and symptom subscales using the combined samples (Fig. 3b–e). The 'CC' genotype was most robustly associated with total PTSD symptoms, and among subscales, hyperarousal symptoms were the most strongly associated with rs2267735. Notably, even after controlling for childhood trauma history and adult trauma, age and race (which slightly reduces total N owing to missing data), the rs2267735 'CC' genotype was associated with higher levels of PTSD hyperarousal symptoms compared to 'G' carriers in women ($P = 0.0008$, Fig. 3e), but not men ($P = 0.51$).

We repeated the above analyses with Beck depression inventory (BDI) symptoms and history of life-time substance abuse, and found no associations with these measures and rs2267735 (Supplementary Fig. 3), suggesting that this association may be relatively specific to PTSD. To address whether rs2267735 might be associated with other severe psychiatric illnesses, we performed analyses using bipolar



b

SNP	Chr. 7 position	Distance between SNPs (bp)	*N* (females)	*P*-value (females)	*N* (males)	*P*-value (males)
rs7784067	31084099		502	0.834	297	0.291
rs17159861	31085162	1,063	491	0.457	293	0.176
rs1981701	31089060	3,898	496	0.729	290	*0.021
rs12670991	31089447	387	508	0.603	297	0.71
rs12670977	31089583	136	503	0.525	299	*0.014
rs741051	31093792	4,209	492	0.879	289	*0.022
rs10241138	31096363	2,571	499	0.948	295	0.883
rs10226318	31097459	1,096	495	0.834	293	0.165
rs1468687	31105948	8,489	497	0.314	297	0.973
rs17723231	31107448	11,085	489	0.466	294	0.288
rs10277350	31109768	2,320	495	0.86	297	0.135
rs10268647	31114877	5,109	494	0.794	292	0.935
rs2267727	31116266	1,389	499	0.546	292	0.834
rs7804302	31117646	1,380	506	0.48	299	0.226
rs7805043	31118094	448	498	0.995	300	0.483
rs7804958	31118186	92	498	0.23	293	0.992
rs10081254	31121671	3,485	503	0.239	295	0.845
rs1541516	31122542	871	494	0.858	287	0.941
rs10269014	31128737	6,195	498	0.228	296	0.701
rs11979764	31131353	2,616	507	0.508	297	0.565
rs2267733	31134809	3,456	505	0.11	295	0.78
rs2267735	31135504	695	506	**0.0002	297	0.763
rs2267737	31136610	1,106	508	0.557	298	0.669
rs2267738	31136824	214	502	0.493	294	0.099
rs2267740	31137003	179	499	0.52	295	0.864
rs2284225	31138503	1,500	495	0.097	280	0.875
rs7786118	31139148	2,145	508	0.63	297	0.918
rs2041568	31141418	2,270	502	0.149	294	0.611
rs1541518	31148279	6,861	507	0.064	297	0.974
rs4503014	31149140	861	505	0.843	297	0.366

*$P < 0.05$; **$P < 0.0005$

c

Canonical ERE cnaGGTCAngGTGaCCt
rs2267735 tcaGGTCAgcagaGgaCg

Figure 2 | Genetic association of PAC1 receptor (*ADCYAP1R1*) with PTSD. **a**, 30 SNPs spanning the *ADCYAP1R1* gene (*x* axis), with the –log(*P*-value) of logistic regression for each SNP association with PTSD (diagnosis based on DSM-IV criteria from PTSD Symptom Scale). Subjects were analysed with logistic regression in females only ($N = 503$) or males only ($N = 295$). Horizontal lines represent the nominal $P = 0.05$ or the corrected *P*-value, $P = 0.0011$ (44 SNPs, correcting for 30 *ADCYAP1R1* SNPs and 14 *ADCYAP1* SNPS; Supplementary Fig. 1). rs2267735 is the only SNP remaining significant after multiple corrections ($P = 0.0002$). **b**, Table of *P*-values resulting from the association of each genotyped, *ADCYAP1R1* SNP with PTSD diagnosis (by gender). The location on chromosome 7 for each SNP including the distance (bp) between the SNPs is given. The average distance between SNPs is 2.5 kb. SNP rs2267735 is located in an intron of *ADCYAP1R1*, and is not in linkage disequilibrium with other SNPS (for African Americans in our population, data not shown). **c**, rs2267735 (C/G), in red, is located within a canonical oestrogen response element (ERE) binding site (capital letters, conserved canonical ERE nucleotides; blue letters, mismatches with the *ADCYAP1R1* gene and canonical ERE; reverse strand shown).

disorder, schizophrenia, and Alzheimer's disease samples. From the data of the Genetic Association Information Network (GAIN) publicly accessible database (http://www.genome.gov/19518664), we analysed the association of rs2267735 (included on the Affymetrix 6.0 SNP array) with bipolar disorder as well as schizophrenia. We did not observe a significant association of this SNP with these two disorders in subjects with African American (954 cases, 1,195 controls) or

a

rs2267735–PTSD	*N* (1,237)	*Wald* χ^2	OR (CI)	*P*-value
Male original	295	0.036	1.03 (0.71–1.49)	0.85
Male replication	179	0.57	0.83 (0.52–1.33)	0.45
Male combined	474	0.123	0.95 (0.71–1.27)	0.73
Female original	503	13.7	1.72 (1.29–2.28)	**0.00021**
Female replication	260	4.8	1.54 (1.04–2.29)	**0.029**
Female combined	763	18.4	1.66 (1.32–2.09)	**0.000018**

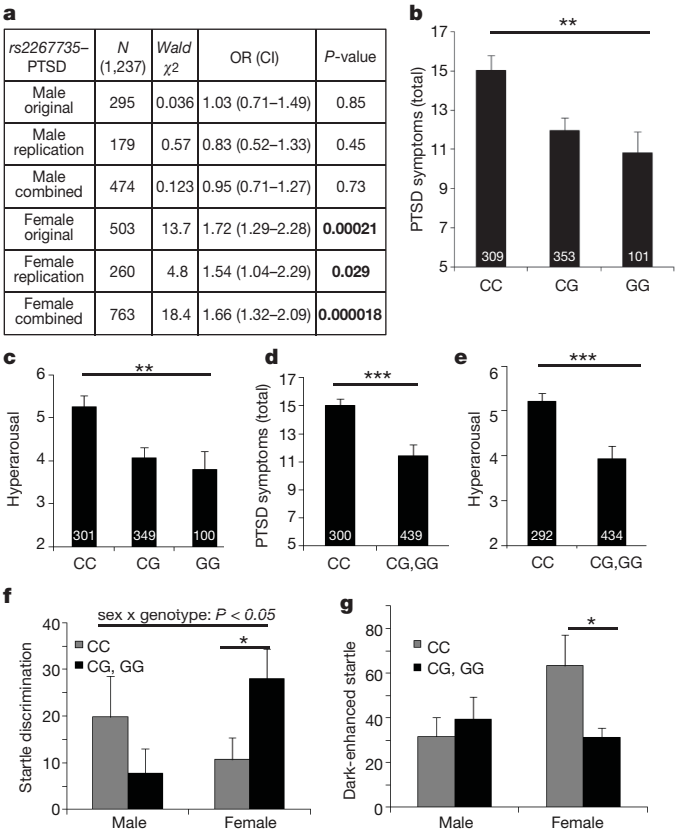


Figure 3 | Association of *ADCYAP1R1* rs2267735 with PTSD symptoms and physiological fear responses. **a**, Table demonstrating the *N*, Wald χ^2; odds ratio (OR) for C as risk allele and *P*-value, in males and females, in the original, replication and combined samples for logistic regression of rs2267735 with PTSD diagnosis. CI, confidence interval. **b**, Total PTSD symptoms are differentially associated with rs2267735 genotype in females ($P \leq 0.001$). **c**, Hyperarousal is the most robustly associated symptom with rs2267735 genotype ($P = 0.0009$). **d**, In a dominant/recessive model, even after controlling for childhood trauma, adult trauma and age, genotype predicts total PTSD symptoms ($P \leq 0.001$) and **e**, hyperarousal symptoms ($P \leq 0.0001$). **f**, Fear discrimination, measured with potentiated startle (CS+ startle minus CS− startle) is impaired in females with rs2267735 'CC' genotype. **g**, Dark-enhanced startle ($\text{startle}^{\text{dark}} - \text{startle}^{\text{light}}$) is significantly increased in females with rs2267735 'CC' genotype. *N* values are shown at base of each bar, bars represent mean ± s.e.m. *N* values are slightly different across analyses owing to differences in number of subjects across measures. *$P < 0.05$; **$P < 0.001$; ***$P < 0.0002$.

European (1,378 cases, 1,351 controls) ancestry. Specifically, we found that all pre-computed *P*-values for associations of rs2267735 with schizophrenia or bipolar disorder were higher than *P* uncorrected = 0.01, indicating no major contribution of this variant.

Additionally, we examined the association of rs2267735 and Alzheimer's in a previously characterized Alzheimer's disease sample[25]. In this cohort of 342 subjects, we found no association with rs226735 and Alzheimer's disease diagnosis using either the additive genetic model ($P = 0.19$) or the dominant/recessive model ($P = 0.89$). These data suggest that we find robust associations with rs2267735 in women, but not men, with PTSD. In contrast, we find no association with depression symptoms, substance abuse, Alzheimer's disease, bipolar disorder, or schizophrenia across different samples. Note that for all of these negative results, owing to the limited sample sizes, we cannot rule out the possibility that rs2267735 may be associated with PTSD in men or with other disorders with a smaller effect size than we see with PTSD in women.

To parallel our results with plasma PACAP38 levels, we next examined whether physiological measures of fear are differentially associated with the *ADCYAP1R1* rs2267735 SNP. In PTSD, but not depression[18], fear response to an inhibitory CS−, or 'safety signal', is

exaggerated. The discrimination between CS+ and CS− improves across the training procedure in controls, but not in those with PTSD. We examined whether rs2267735 was associated with impaired fear discrimination late in conditioned acquisition, during the same period noted in Fig. 1e. Notably, females with the 'CC' genotype were significantly less able to discriminate CS+ from CS− signals (Fig. 3f, sex × genotype interaction, $P < 0.05$, and 'CC' versus 'G' carriers in females, $P < 0.05$).

We next examined whether a difference in dark-enhanced startle, a measure of increased anxiety in humans that is similar to light-enhanced startle in rodents[26–28], was differentially associated with rs2267735. Again, we found that females, but not males, with the 'CC' genotype showed significantly more startle in the dark compared to the light (Fig. 3g, males, $N = 35$, $P = 0.71$; females, $N = 53$, $P = 0.02$). Together, these data suggest that the *ADCYAP1R1* rs2267735 SNP may be relatively specific in its association with PTSD psychological and physiological phenotypes. Further, the robust association of rs2267735 with hyperarousal symptoms suggests that the role of PACAP–PAC1 may be specifically involved in the normalization of the stress response, a process which is particularly dysregulated in PTSD.

ADCYAP1R1 methylation and mRNA expression

Environmental, genetic and epigenetic mechanisms are likely to moderate the long-term effects of trauma exposure. Using the Illumina HumanMethylation27 BeadChip, we interrogated methylation in DNA extracted from peripheral blood at the first site within the *ADCYAP1R1* CpG island (Supplementary Fig. 2). Methylation at this site was significantly associated with total PTSD symptoms (Fig. 4a, $N = 94$, $r = 0.354$, $P < 0.0005$) in a sex-independent manner. Further, CpG methylation level (median split) was associated with PTSD diagnosis (Fig. 4b, $\chi^2 = 8.1$, $P < 0.005$), but not depression ($P > 0.05$, Supplementary Fig. 3e). There was no significant association between methylation of *ADCYAP1* and PTSD symptoms. These data suggest that *ADCYAP1R1* is regulated, in part, through epigenetic mechanisms that contribute to differential function of the PAC1 receptor in PTSD.

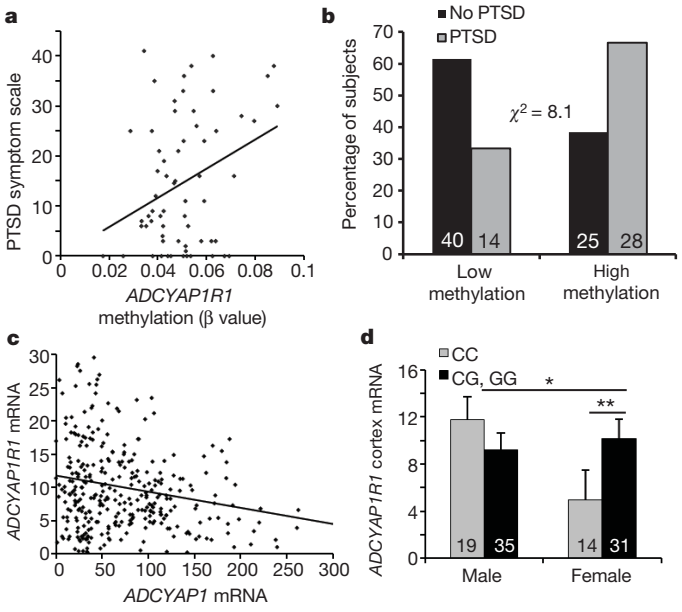


Figure 4 | *ADCYAP1R1* methylation and mRNA expression. **a**, Methylation within the first CpG island of *ADCYAP1R1* (β value, Illumina cg27076139) is positively correlated with total PTSD symptoms (both sexes; $N = 107$; $r = 0.354$, $P < 0.0005$). **b**, Subjects with PTSD have higher levels of *ADCYAP1R1* methylation (median split, $N = 107$; χ^2 analyses, $P < 0.005$). **c**, *ADCYAP1* mRNA levels are inversely correlated with *ADCYAP1R1* mRNA levels in cortex (from prior data set[13])($r = -0.219$; $P < 0.001$). **d**, *ADCYAP1R1* mRNA levels are differentially expressed in females compared to males based on imputed *ADCYAP1R1* rs2267735 genotype (from prior data set[13]) (*$P < 0.05$ male versus female CC carriers, **$P < 0.05$, one-tailed, CC versus G-carriers). Bars represent mean ± s.e.m. *N* values for each group at bottom of each graph.

To examine the potential relationship of genotype and brain mRNA expression as described previously[29], we used a brain mRNA expression data set[30] to test whether *ADCYAP1R1* rs2267735 is associated with differential gene expression. We first examined whether cortical *ADCYAP1R1* and *ADCYAP1* mRNA levels were correlated. As shown in Fig. 4c, these mRNA levels were significantly inversely correlated ($r = -0.219$, $P < 0.001$, including males and females), suggesting that brain levels of PACAP peptide and PAC1 mRNA are tightly regulated.

We next used a previously analysed data set with combined genome-wide association and brain mRNA expression data[30] to examine whether *ADCYAP1R1* rs2267735 imputed genotypes were associated with differential *ADCYAP1R1* expression in brain. We found a sex × genotype effect (Fig. 4d, $F(3,99) = 4.3$, $P < 0.05$) with females with the 'CC' genotype expressing significantly less *ADCYAP1R1* mRNA than males ($F(1,33) = 5.5$, $P < 0.05$) or than females who are 'G' carriers (one-tailed, $F(1, 45) = 2.87$, $P < 0.05$). Thus, mRNA encoding the PACAP peptide and PAC1 receptor appeared to be tightly regulated within the human cortex, and *ADCYAP1R1* mRNA levels were associated with the *ADCYAP1R1* rs2267735 SNP.

Fear induces *Adcyap1r1* in mouse amygdala

Despite prior studies examining PACAP–PAC1 receptor function in central/peripheral nervous system development, endocrine homeostasis, metabolism, cellular protection/regeneration and chronic stress responses[2,3,6,31–34], a role for PACAP signalling in fear conditioning has not been evaluated. Given our data implicating PACAP in PTSD, we wondered if *Adcyap1r1* mRNA was differentially regulated in mice using Pavlovian fear conditioning[35–40], a means of studying acute fear and trauma responses that has been proposed to model PTSD[19,22]. We performed classical fear conditioning experiments using male mice, in which a previously neutral tone CS (6 kHz) was paired with 10 foot-shocks (1 mA, 0.5 s; Fig. 5a). This conditioning paradigm consistently provides robust fear learning in mice leading to changes in gene expression within the amygdala, a region critical for fear learning and expression. Quantitative PCR analyses shows that amygdala *Adcyap1r1* mRNA increased ~1.5-fold during the consolidation of fear (Fig. 5b, $P < 0.05$), with a similar trend within the medial prefrontal cortex (mPFC). When peak freezing was compared with brain mRNA levels, we find a significant correlation between fear learning and *Adcyap1r1* mRNA (Fig. 5c, $r^2 = 0.49$, $P < 0.05$).

Oestrogen induces *Adcyap1r1* in rat BNST

To further establish the relationship between PACAP–PAC1 receptors and oestrogen in a validated model of sex hormone regulation, we examined oestrogen-induced changes in *Adcyap1* and *Adcyap1r1* transcripts in the bed nucleus of stria terminalis (BNST) in female rats. The BNST is a component of the extended amygdala that is subject to significant gonadal hormonal control[7,27,28]. In rodents, it is critical for emotional behaviour, mediating stress responses and the light-enhanced startle response. We examined gene expression in the BNST in ovariectomized female rats following 21-day implantation of continuous release oestrogen pellets. Compared to control implants, oestradiol increased *Adcyap1* transcripts in the dorsal and ventral BNST 2.1- and 3.4-fold, respectively ($P \leq 0.01$, Fig. 5d). Additionally, oestradiol increased *Adcyap1r1* mRNA 1.5-fold in the dorsal BNST samples ($P < 0.05$, Fig. 5e), and future studies should also examine oestradiol sensitivity of these genes in amygdala. While these rodent studies are complex and have differing experimental designs, our data clearly illustrate dynamic PACAP–PAC1 receptor regulation within central areas mediating fear, stress and oestrogen responsiveness.

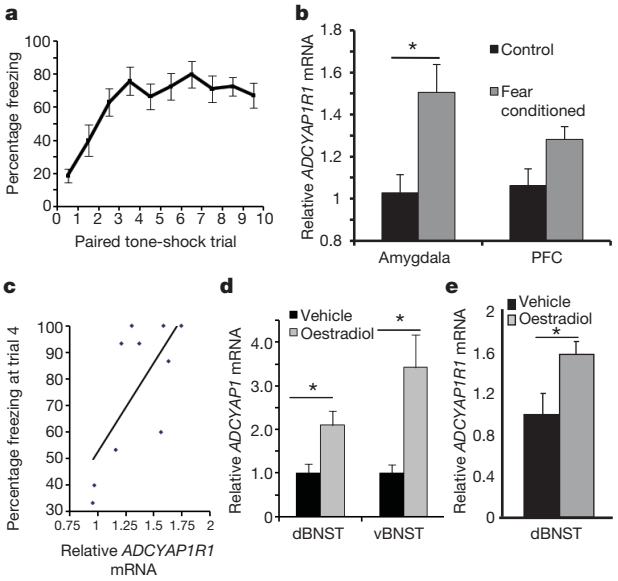


Figure 5 | Regulation of *Adcyap1r1* and *Adcyap1* mRNA in rodent models. **a**, Percentage of time freezing, in mice, to the conditioned tone (CS+) following tone-shock pairings during the conditioned fear trials. **b**, RT–PCR analyses of mRNA levels within mouse amygdala and mPFC 2 h after fear conditioning or in control handling conditions, showing a significant increase in amygdala *Adcyap1r1* mRNA ($N = 15$, 1.47 fold, $P < 0.05$) and a non-significant trend in mPFC (1.19 fold change). **c**, Correlation between average amygdala and PFC *Adcyap1r1* mRNA and percentage freezing at trial 4, demonstrating an association between *Adcyap1r1* mRNA with rate of fear learning ($r^2 = 0.49$, $P < 0.05$). **d**, *Adcyap1* mRNA in rat BNST in female rats ($N = 12$ per group) following ovarectomy and oestradiol implant versus vehicle replacements. *Adcyap1* mRNA is increased in both dorsal BNST (dBNST, 2.1-fold) and ventral BNST (vBNST, 3.4-fold) after oestradiol implantation. **e**, *Adcyap1r1* transcripts are also increased in dorsal BNST (1.6-fold, $N = 4$ per group). Bars represent mean ± s.e.m. $^*P < 0.05$.

Discussion

Since its identification more than 20 years ago, PACAP has been increasingly implicated in diverse cellular stress response pathways and neurotrophic function. However, the organizational role of the PACAP system in orchestrating behavioural stress responses has yet to be clarified. Our data suggest that PACAP–PAC1 receptor expression and signalling may be integrally involved in regulating the psychological and physiological responses to traumatic stress. Further, we report an association of an ERE-embedded *ADCYAP1R1* SNP with PTSD, and we demonstrate fear- and oestrogen-dependent regulation of PACAP systems within stress-responsive regions of the brain. These data may begin to explain sex-specific differences in PTSD diagnosis, symptoms and fear physiology. Future work targeting the PACAP–PAC1 receptor system may lead to novel and robust biomarkers; it may also further our understanding of the neural mechanisms underlying pathological responses to stress, and help identify potential therapeutic targets for the prevalent and debilitating syndrome of PTSD.

METHODS SUMMARY

This highly traumatized, civilian, cross-sectional cohort has been previously described in candidate gene-association studies of PTSD and depression[11–13]. Research interviews, salivary DNA and blood samples were collected from patients receiving services in the primary care clinics at Grady Memorial Hospital (Atlanta, Georgia, USA). All study procedures have been reviewed and approved by the Emory Institutional Review Board and the Grady Hospital Research Oversight Committee. PTSD measures in this manuscript are based on the PTSD symptom scale[14], which has been validated within this population using the Clinician Administered PTSD Scale. PACAP38 radioimmunoassay (1:30,000, Peninsula Laboratories) was performed at University of Vermont, using double antibody immunoprecipitation as described[41]. For genotyping, pairwise tagging ($r^2 > 0.8$, MAF > 0.1) was used to choose tag-SNPs for both *ADCYAP1* and *ADCYAP1R1*. The coordinates were chr. 18 885000–906000 and chr. 7 31048667–31117836 for *ADCYAP1* and *ADCYAP1R1*, respectively (NCBI B36), which includes approximately 10 kilobases (kb) upstream and 5 kb downstream of the coding regions for both genes. Genotypes for the tag-SNPs were generated using Sequenom iPlex with follow-up analyses using Taqman. For methylation analyses, bisulphite-converted DNA was whole-genome amplified, fragmented, and hybridized to the HumanMethylation27 BeadChip (Illumina). Individual samples were stratified to separate BeadChips according to PTSD status to limit bias. The BeadChips were scanned using a BeadStation 500GX, and the methylation level (β value) was calculated using the Methylation Module of the BeadStudio software. The eyeblink component of the acoustic startle response was measured by electromyographic recordings of the right orbicularis oculi muscle with two 5-mm Ag/AgCl electrodes filled with electrolyte gel, as described[18,19]. The mouse fear conditioning and rat oestrogen replacement studies are described in detail in Supplementary Methods.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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