Expression of the PPM1F gene is regulated by stress and associated with anxiety and depression

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Abstract

Background—Molecular mechanisms underlying psychological sequelae of exposure to stressful experiences, such as Post-Traumatic Stress Disorder (PTSD) and depression, are not well understood.

Methods—Using convergent evidence from animal and human transcriptomic and genomic studies, we aimed to identify genetic mechanisms underlying depression and anxiety after traumatic experiences.

Results—From a transcriptome-wide analysis in mice, we found the *Ppm1f* gene to be differentially expressed in the amygdala and medial prefrontal cortex (mPFC) a week after immobilization stress. Next, we found that *PPM1F* mRNA levels in human blood were down-regulated in cases with symptoms of comorbid PTSD and depression (PTSD&Dep), and consistently in cases with anxiety symptoms in a separate human dataset. Furthermore, we showed that a genetic variant of *PPM1F*, rs17759843, was associated with PTSD&Dep and with *PPM1F* expression in both human brain and blood. Given prior reported mechanistic links between *PPM1F* and *CAMK2*, we examined blood mRNA level of *CAMK2G* in human and found it to be

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lower in PTSD&Dep. We also found that Ppm1f protein levels and its colocalization with Camk2G were altered in the amygdala and mPFC of male mice. Additionally, we found that a systemic dose of corticosterone blocked the depressive-like phenotype elicited by stress in female mice. Lastly, corticosterone rescued the anxiety-like phenotype and mRNA levels of *Ppm1f* in the amygdala and mPFC in male mice and in the mPFC of female mice.

Discussion—Taken together, our data suggest a mechanistic pathway involving *PPM1F* and *CAMK2G* in stress and trauma-related manifestation of anxiety and depression across species.

Keywords

Stress; Anxiety; Depression; PPM1F; Camk2g; PTSD

Introduction

Exposure to stressful or traumatic life experiences increases risks of developing depressive disorders, anxiety disorders, or post-traumatic stress disorder (PTSD) (1). Anxiety and depressive disorders rank at the top of disabling mental illnesses causing tremendous economic costs to society (2). However, effectiveness of pharmacological treatments for anxiety and depressive disorders still remains rather limited with small effect sizes (3, 4). To facilitate efforts in developing novel treatments for these disorders, better insights into molecular mechanisms underlying anxiety and depression are needed.

With the goal of elucidating molecular mechanisms underlying anxiety and depression after traumatic experiences, we used convergent evidence from both animal and human studies. We note that among susceptible individuals, PTSD and depression are often comorbid (PTSD&Dep) (5). The shared symptoms and relatively high prevalence of PTSD and depression comorbidity suggest that examining their comorbidity in the aftermath of trauma may be a more powerful approach than examining either outcome alone. In addition, animal stress models leading to similar PTSD-like or depression-like behaviors can facilitate our efforts in uncovering their neurobiological mechanisms (6, 7).

Methods

Animals

All experiments were performed on adult wild-type C57BL/6J mice obtained from Jackson Labs. Male and female mice were group-housed in a temperature-controlled vivarium, with ad libitum access to food and water. See detailed methods for ethics protocols. Female mice were used only when explicitly stated, otherwise only male mice were used.

Immobilization Stress

Immobilization Stress (IMO) procedures, as previously described (8–10), were conducted in a room separate from housing and behavioral paradigms (11). All mice were habituated to the test chambers before the IMO stress and received exposure to handling and the training context completely separate and distinct from the IMO context (9). We chose the 6–8 days post-IMO time point because we wanted to compare these results with our previous findings studying gene regulation at this same time point (8,9,11,12).

Microarray hybridization and analysis

Mice were sacrificed under basal conditions (Home Cage *Control Group*) at 6 or 8 days after a 2 hour immobilization (IMO *Stress Group*). The reason for having two amygdala microarrays was to replicate the findings on day 6 with those on day 8 and get more robust results. Amygdala and medial prefrontal cortex (mPFC) tissue from both hemispheres was extracted by 1mm micropunch. Total RNA was extracted from the tissue and purified with the RNeasy Mini Kit catalog #74106 (Qiagen) following the manufacturer's instructions. Illumina Mouse WG-6 v2 Expression BeadChip microarray was assayed for 45,281 transcripts. These microarray are available at GEO #GSE100086. Plots of principal variance component analysis and volcano plots are in Supplemental Figure S1.

Bioinformatics of the microarray analysis

The top genes regulated after stress in the Amygdala and mPFC were further analyzed with DAVID Bioinformatics 6.8 to understand their functions. Note that *Ppm1f* was the only top gene present in all the microarrays (Figure 1A–E).

Reverse Transcription and PCR Quantification

Protocol was followed as previously described (8).

RT-PCR arrays

RNA was isolated and reverse transcribed as outlined in the section above. We used a custom plate format 24×4 catalog #CAPM10412 (Applied Biosystems, Carlsbad, CA).

Elevated plus maze

The elevated plus maze was performed as previously described (12).

Tail suspension test

Mice were suspended with duct tape ~2 cm at the end of the tail. The session lasted for 6 minutes. Behavior was recorded by a video camera and a researcher blind to the experimental groups analyzed the immobility time.

Mouse fear conditioning

Mice were fear conditioned in standard rodent modular test chambers (ENV-008-VP; Med Associates Inc, St. Albans, VT) with an inside area of 30.5cm (L) × 24.1cm (W) × 21.0cm (H). Mice received 5 trials of a conditioned stimulus (CS; 30 second tone, 6 kHz, 70 dB) co-terminating with a footshock (500ms, 0.6mA) unconditioned stimulus (US).

Gene expression profiles in human GTP participants

Participants were recruited from Atlanta inner-city residents by the Grady Trauma Project (GTP) (13–15). This study was approved by the Institutional Review Board of Emory University School of Medicine and Grady Memorial Hospital.

PTSD symptoms were assessed with the modified PTSD Symptom Scale (PSS) (16). Depressive symptoms were measured with the Beck Depression Inventory (BDI)(17). Cases

with current symptoms of comorbid PTSD and depression (PTSD&Dep) were defined as having PSS 14 and BDI 14. Controls were defined as not having either PTSD or depressive symptoms, as reflected by a PSS 7 and BDI 7, despite being exposed to trauma. See Supplementary Methods for rationale behind these cutoff scores. Of note, these participants were not part of a rigorously assessed clinical sample.

RNA was extracted from blood collected in the morning. All samples had RNA Integrity Number (RIN) 6. Raw probe intensities were generated on Illumina HumanHT-12 v3 or v4 BeadChip arrays. We performed normalization of probe intensities using the Supervised Normalization of Microarray algorithm (18), removing the effects of batch and RIN. Association between mRNA level of a gene of interest and PTSD&Dep status was examined using multiple linear regression adjusting for gender, age, and population substructure.

Center for Health Discovery and Well Being (CHDWB) replication sample and gene expression profiles

The CHDWB project evaluated effectiveness of a health- and prevention-focused approach (19). Anxiety symptoms were assessed with the GAD-7 scale (20), a well-validated and efficient tool for screening anxiety symptoms. A score of 5–9 on the GAD-7 indicates mild anxiety, 10–14 moderate, and 15–21 severe anxiety symptoms (20). We categorized participants with GAD-7 scores of 5 as having anxiety symptoms and those with GAD-7 scores 1 as controls.

RNA was extracted from whole blood and all samples had RIN >7. Probe intensities were generated on Illumina HT12 v3 or v4 beadchip arrays. We performed normalization using the Supervised Normalization of Microarrays (18). Human *PPM1F* microarrays from CHWDB are available at GEO #GSE61672.

PPM1F genotypes and principal components among GTP participants

DNA was extracted from saliva or blood, and genotyping was conducted using Illumina's HumanOmni1-Quad. Standard quality control of the genotyping was performed using PLINK (21), removing individuals with >2% missing data and removing one in each pair of related individuals with an identity by descent proportion >0.12 (indicating cousins or a closer relation). We used principal component analysis to infer axes of ancestry and remove outlier subjects following methods previously described (22). Association between *PPM1F* single nucleotide polymorphisms (SNPs) and PTSD&Dep was examined using PLINK, adjusting for gender and 10 PCs. Multiple testing was addressed with permutation (23).

eQTL analysis

Association between rs17759843 and blood mRNA level of *PPM1F* was provided by the *Blood eQTL browser* (genenetwork.nl/bloodeqtlbrowser) (24). Quality control for this dataset was described in detail by Westra and colleagues (24).

Postmortem eQTL Analysis

Association between rs17759843 and brain mRNA level of *PPM1F* was provided by Brain Cloud, which has gene expression profiles from the dorsolateral prefrontal cortex of 269 subjects without neuropathological and neuropsychiatric diagnosis (25).

Results

Behavior and gene expression profiles in Amygdala and mPFC of stressed mice

We performed transcriptome-wide differential expression analysis of genes in the Amygdala and mPFC in mice 6 and 8 days after immobilization (IMO) stress versus controls. We examined gene expression profiles in the Amygdala at 6 days and 8 days after IMO stress to determine if the long-term gene expression changes found at 6 days were also sustained at 8 days. Figures 1A–D show the heatmaps revealing differences in the gene expression in stressed versus control mice groups. Of the probes that were differentially expressed at unadjusted p<0.05 in the Amygdala, we used the following criteria to select probes for follow-up: a) probe differentially expressed at unadjusted p-value <0.05 at both 6 days and 8 days after stress; b) probe with >1.5 fold change between cases and controls; c) probe is expressed at moderate to high levels in the Amygdala (through search in the Allen Brain Atlas). Supplemental Table S1 lists the 24 probes that met these criteria. Of note, without adjusting for multiple testing, there is an increased risk of false positives. Therefore, we performed replication of these probes in different mouse cohorts to determine if they were differentially expressed between cases and controls using RT-PCR. Of these 24 probes, 21 had available primers for the RT-PCR replication (Supplemental Table S2). Supplemental Table S3 lists the results of the PCR replication in the Amygdala. We found five probes whose altered mRNA levels were replicated with PCR; *Ppm1f* was one of them. Along with our replication studies, we showed that these gene expression changes, which occurred 6 to 8 days after IMO, may need an incubation period because no significant changes in gene expression were found 1 day after exposure to stress (Supplemental Table S3). Interestingly, although *Ppm1f* is significantly regulated 6–8 days post-stress, it is not regulated in the Amygdala 30 minutes or 2 hours after fear conditioning (FC) nor in the mPFC 2 hours after FC (Supplemental Figure S2). Thus, *Ppm1f* appears to have a dynamic expression after stress in the brain. Moreover, only five of these genes were significantly regulated after stress - *Hbp1, Ssbp4, Fbxl5, Ppm1f* and *Fhl1* – at three different time points: 6, 7 and 8 days after IMO (Supplemental Table S3). Thus, we examined the expression of these five genes in human. Among them, only *PPM1F* was differentially expressed between cases with symptoms of PTSD&Dep versus controls (Supplemental Table S4; more detailed description to follow).

We followed similar criteria to select probes with unadjusted p-value <0.05 from the mPFC transcriptome-wide differential analysis for further replication in a mouse microarray (more details in Supplemental Methods). See Supplemental Table S5 for the list of genes that survived the filtering criteria and were followed up with RT-PCR replication. The number of top-regulated genes within these microarrays and their overlap are represented in Figure 1E. *Ppm1f* was the only gene highly regulated in all the three mouse microarray conditions.

Behaviorally, two groups of mice were exposed to IMO stress and 6 days later they were tested in the elevated plus maze (EPM, anxiety-like behavior) or the tail suspension test (TST, depressive-like behavior). The EPM results showed that stress reduced the time mice spent in the open arms (Figure 1F top, t=4.597 df=14, p=0.0004). Moreover, the TST analysis revealed that stress increased the time mice spent in immobility (Figure 1F down, f=2.280, df=7, p=0.0361).

Brain functions altered after acute stress, network analysis and predicted interactions with drugs

We performed bioinformatics analyses of the shared genes that had altered expression in the microarray. The list of the genes studied with DAVID are in Supplemental Tables S1 and S5 and Supplemental Figure S3. We found that the top three functions altered in both the Amygdala and mPFC were phosphoproteins (including *Ppm1f*), alternative splicing, and splice variants (Figure 2A). A more detailed functional analysis with IPA showed the biological pathways that were most different between stress and control animals included cell morphology, gene expression, and cellular development (Figure 2B). Supplemental Figure S4 shows that PPM1F has direct relationships with the CaMK2 family and indirect relationships with brain-derived neurotrophic factor (BDNF), cAMP response element-binding (CREB), extracellular signal-regulated kinase 1/2 (Erk 1/2) and (Mitogen-activated protein kinase kinase) Map2K 1/2.

Examine expression of the above five genes in comorbid PTSD & Depression

We next examined expression of the five genes identified to be significantly differentially regulated in mouse Amygdala after exposure to stress in 230 GTP human participants who were exposed to at least one traumatic experience. Their sociodemographic characteristics are presented in Supplemental Table S6. Of these participants, 142 were cases with PTSD&Dep symptoms and 88 were controls with no PTSD and no depressive symptoms. Among these five genes, *PPM1F* (ILMN_2059535) was significantly associated with PTSD&Dep after adjusting for gender, age, and population substructure (β=0.135; p=0.005; Bonferroni adjusted p=0.025; N=230). Cases with PTSD&Dep symptoms had lower *PPM1F* expression than controls (Figure 3A). Notably, *PPM1F* is highly expressed in the brain in both mice and humans (Supplemental Figure S5).

PPM1F in anxiety in a replication sample

Given that PTSD patients present with anxiety symptoms, we examined blood mRNA of *PPM1F* (ILMN_2059535) in anxiety in the CHWDB cohort, which has 151 cases with anxiety symptoms and 165 controls (N=316). Their characteristics are presented in Supplemental Table S7. We found that *PPM1F* was significantly down-regulated in patients with anxious symptomatology versus controls after adjusting for gender and ethnicity (p=0.044, N=316, Figure 3B, Supplemental Table S7), consistent with the association found in the GTP sample.

Association between PPM1F SNPs and PTSD&Dep

Having identified *PPM1F* mRNA expression level to be significantly associated with PTSD, depression and anxiety, we next investigated whether certain *PPM1F* SNPs influence this association. To this end, we examined the association between *PPM1F* SNPs and PTSD&Dep, and whether the identified significant SNPs influenced *PPM1F* expression level in blood and brain. In the GTP human dataset, there was a total of 14 tagging SNPs for *PPM1F*. Among these SNPs, rs17759843, located in the 3′UTR of *PPM1F*, was significantly associated with PTSD&Dep after adjusting for sex and 10 genetic principal components (OR=0.53; p=0.0046; permuted p=0.0463; N=2361; Figure 4). The minor allele of rs17759843 was associated with lower odds for comorbid PTSD&Dep (Figure 4). rs17759843 has a MAF of 0.018 and *p*-value for departure from HWE of 0.6 in the unaffected population and 1 in affected population.

PPM1F rs17759843 influences expression of PPM1F in human blood

Since we found rs17759843 significantly associated with PTSD&Dep, we examined whether this SNP is associated with human blood *PPM1F* mRNA level in 358 GTP participants. There was no significant association between this SNP and *PPM1F* mRNA level after adjusting for sex, age, and population substructure (p=0.635, N=358). However, when we looked at the Blood eQTL browser (24) of 5300 human samples, there was a highly significant association between rs17759843 and *PPM1F* (p=6.1×10^{-25}, FDR=0.00; effect size Z-score = 10.31). Specifically, the minor allele of rs17759843 was associated with higher expression of *PPM1F*. This directionality is consistent with our genetic and gene expression findings in the GTP sample, in which the minor allele of rs17759843 was significantly associated with lower probability of having PTSD&Dep, and that controls had higher expression of *PPM1F* relative to that of PTSD&Dep cases. With a larger sample size and thus more power, we conclude that rs17759843 is indeed a blood cis-eQTL for *PPM1F mRNA* expression in humans.

rs17759843 influences expression of PPM1F in human brain

Since *Ppm1f* mRNA level was upregulated in the amygdala and downregulated in the mPFC of stressed mice, which is consistent with another published study (26), we examined whether rs17759843 is associated with brain mRNA level of *PPM1F* in the publicly available BrainCloud dataset, which has human gene expression profiles of the prefrontal cortex. There was a total of three probes for *PPM1F* in this dataset. Of these, two probes had significant association with *PPM1F*: Probe 8896: p=0.0032, Bonferroni-adjusted p=0.0097; and Probe 16009: p=0.0004, Bonferroni-adjusted p=0.0012. In sum, rs17759843 is significantly associated with *PPM1F* mRNA level in human prefrontal cortex.

CAMK2

Since PPM1F regulates calcium/calmodulin-dependent protein kinase 2 (CaMK2) in neuronal cells (27, 28) and fibroblasts (29), we examined blood mRNA level of *CaMK2* in PTSD&Dep. After QC, the human dataset had three probes for *CaMK2* (one for *CaMK2D* and two for *CaMK2G*; none for *CaMK2A* or *CaMK2B*). Of these three probes, the *CaMK2G* probe had significantly lower expression in cases with PTSD&Dep symptoms

versus controls after adjusting for gender, age, and population substructure (β=0.072; p=0.0158; Bonferroni corrected p = 0.0474, N=230; Supplemental Figure S6A–B).

We analyzed *Camk2g* in the mouse brain because it was the CaMK2 isoform that was most significantly regulated in our human dataset. After stress *Camk2g* mRNA was marginally upregulated in the mouse amygdala 6 days after IMO (t=2.213, df=8, p=0.058, Supplemental Figure S6C). However, *Camk2g* expression was not regulated in mouse amygdala 2 hours after FC in mice with or without a previous exposure to stress (Supplemental Figure S6D).

Immunohistochemistry of Ppm1f and Camk2g

Ppm1f protein levels were upregulated in the Central amygdala (CeA), Basolateral amygdala (BLA), the summatory of CeA+BLA (Total Amygdala) and the mPFC in male mice 6 days after stress IMO (Supplemental Figure S7). Moreover, Ppm1f levels in the BLA were also upregulated in female mice 6 days after IMO (Supplemental Figure S8). Camk2g levels were upregulated in the CeA, BLA, Total Amygdala and mPFC in male mice 6 days after IMO (Supplemental Figure S9). However, there were no changes in Camk2g levels 6 days after IMO in female mice (Supplemental Figure S10). Figure 5 shows that IMO enhances the colocalization of Ppm1f and Camk2g in the Prelimbic cortex (PrL) (t= −2.308, df=12, p=0.046), Total Amygdala (t= −2.598, df=24, p=0.017) and the mPFC in male mice (t= −2.814, df=41, p=0.008) (Figure 5). See Figure 6 for representative images of these immunohistochemistry studies.

Corticosterone and Ppm1f

Vehicle or corticosterone was given 1 hour after IMO in male and female mice. 6 days later TST, EPM and mRNA levels were evaluated. In males, there was a significant increase in total immobility as measured by TST in IMO+Veh (t=−2.328, df=14, p=0.037, Figure 7A) and IMO+CORT (t=−2.45, df=14, p=0.029, Figure 7B) groups compared to Control+Veh group. In females, there was a significant increase in total immobility as measured by a TST in IMO+Veh (t=−2.394, df=8, p=0.044, Figure 7C] compared to Control+Veh group. Analysis in males of the EPM showed significant differences in the time in open arms [F(2,21)=10.358; p=0.001, post-hoc IMO-Veh p=0.001 vs the other groups], entries to open arms [F(2,21)=11.902; p=0.000, post-hoc p=0.0001 vs the other groups], time in closed arms [F(2,21)=9.691; p=0.001, post-hoc p=0.001 vs the other groups] and entries to closed arms [F(2,21)=5.762; p=0.010, post-hoc p=0.033 vs the other groups]. The EPM results in females showed a significant effect for treatment on the total number of entries to closed arms [F(2,21)=6.879; p=0.005]. Post-hoc comparisons revealed an increased number of entries to closed arms in IMO+Veh (p=0.023) and IMO+Cort group (p=0.002) compared to Control+Veh group. Analysis of the mRNA levels in males showed that the IMO+Veh group presented enhanced levels of *Ppm1f* compared to Control group (t=2.383, df=13, p=0.0331, Figure 7M) in the Amygdala and decreased levels in the mPFC (t=2.321, df=13, p=0.0371, Figure 7N). The mRNA levels in females were upregulated in mPFC in the group IMO+Veh vs Control+Veh (t= 3,248 df=13 p=0.0064, Figure 7P) and also in the group IMO+Cort vs Control+Veh in the Amygdala (t=2.579, df=14, p=0.0218, Figure 7S).

Discussion

Here, we found that *Ppm1f* mRNA levels were significantly altered in the amygdala and mPFC after stress exposure in a mouse model. Furthermore, we demonstrate that the human blood mRNA level of *PPM1F* was significantly down-regulated in PTSD&Dep. Moreover, we show that a genetic variant in the 3′UTR of *PPM1F*, rs17759843, was significantly associated with PTSD&Dep and with mRNA levels of *PPM1F* in human blood as well as brain. Additionally, we found that one of the substrates of *PPM1F, CAMK2G*, had significantly down-regulated blood mRNA levels in PTSD&Dep, which is consistent with *CAMK2* being a substrate of *PPM1F*. Consistently, Ppm1f protein levels and its colocalization with Camk2G were altered in the Amygdala and mPFC of male mice. Taken together, our findings suggest a novel and critical role of *PPM1F* in association with stress, anxiety, and depressive symptoms following trauma exposure.

PPM1F is a protein phosphatase and a member of the PP2C family of Ser/Thr protein phosphatases, which are negative regulators of stress response pathways. Calcium/calmodulin-dependent protein kinase II gamma is one of the substrates of this phosphatase (27–29). Interestingly, *Ppm1f* expression in mice was not altered at early time points after an aversive learning paradigm nor 1 day after IMO stress. A potential interpretation is that these changes in basal expression of *Ppm1f* require about a week to develop in mice. Also, it is possible that *Ppm1f* changes may not be related to fear learning, per se, or are not detected at the timing of the collection of the brain tissue in these experiments. In fact, our data suggest that it may be a marker of the chronic effects of prior trauma exposure.

Ppm1f specifically dephosphorylates Camk2 in rat neurons *in vitro* and in cells (30). This results in an inactive form of CaMK2. CAMK2 is critical in serotonergic regulation of prefrontal cortex neuronal activity, which plays a major role in depression, suicide, anxiety, and schizophrenia (31–33), and which may explain the neuropsychiatric behavioral patterns seen in CaMK2 knockout mice (34). CaMK levels in the brain have been previously associated with anxiety (35), depression (36), and suicide victims (37). Our present data suggests that PPM1F could be an upstream regulator – activated by traumatic stress – of CaMK, promoting the development of anxiety and depression-like symptoms. However, the consequences of regulation of PPM1F by stress could have broader implications than described here because of ubiquitous expression of PPM1F in the body and brain. For example, PTSD and depression have been associated with higher rates of coronary heart disease (38–40) and knowledge of the underlying mechanisms for this association is still limited. Since CaMK2 plays an important role in the development and progression of cardiovascular disease (41–43), it is possible that the PPM1F/CaMK2 pathway may play a role in coronary diseases. Further study of PPM1F and CAMK2 may shed light on the mechanisms linking PTSD and depression to elevated risk for heart disease.

Using a different traumatic model than ours, previous studies have shown that exposing rats to a predator results in Amygdala upregulation but mPFC downregulation of phosphorylated Camk2 (pCamk2) one week later (44). Consistently, we found significant changes in mRNA levels of *CaMK2G* in blood in human with PTSD&Dep symptoms and in the Amygdala of mice exposed to stress. Concordantly, we found that stress also regulated Camk2g protein in

the mouse brain. Moreover, Ppm1f protein level in the Amygdala is regulated by stress in male mice suggesting that IMO alters Ppm1f levels. Regarding directionality, the *Ppm1f* mRNA level and *Ppm1f* protein level are in the same direction in the Amygdala but in opposite direction in the mPFC. Interestingly, Ppm1f/Camk2g colocalization is regulated by stress in the mouse brain. Thus, it is possible that PPM1F levels may be regulating CaMK2G after stress, promoting the development of depression and anxiety behaviors.

We also examined whether post-stress manipulation, i.e. systemic administration of corticosterone, may decrease the stress-related behavioral and molecular phenotypes (44–46). Systemic corticosterone did not rescue the depressive-like phenotype elicited by IMO in male mice. However, corticosterone rescued the depressive-like behavior induced by IMO in female mice. EPM results showed that corticosterone rescued the anxiety-like behavior in male mice. In contrast, IMO did not induce an anxiety-like behavior in female mice. Analysis of the mRNA levels showed that corticosterone treatment shortly after stress rescued the genetic regulation of *Ppm1f* in the Amygdala and mPFC in males and in the mPFC in females. Thus, there appears to be relevant sex differences in *Ppm1f* regulation in the brain, acute exposure to traumatic stress and its treatment with corticosterone.

Our study should be interpreted in light of its limitations. First, the data obtained with IMO should be interpreted with caution. While most rodents develop anxiety and depression-like behavior after IMO, only a small percentage of the human population develops PTSD and/or depression. Second, rs17759843 is associated with blood expression of *PPM1F* in a sample of mostly European descent. rs17759843 has an MAF of 0.11 in Caucasians and 0.01 in African Americans per dbGAP HapMap populations. Whether this SNP is an eQTL in the African Americans remain to be determined, as we were likely underpowered to detect this relationship in our GTP dataset. Third, the Blood eQTL Browser provides statistics on bivariate association between rs17759843 and blood *PPM1F* expression. Hence, rs17759843 may be associated with *PPM1F* due to its linkage disequlibrium with other stronger eQTL SNPs for *PPM1F*. Fourth, we did not have access to human brain amygdala to examine association between rs17759843 and *PPM1F* expression. Fifth, we analyzed cases with significant current symptoms of PTSD&Dep but we did not have rigorous clinical assessment to determine if these cases had the diagnosis of PTSD or Major Depressive Disorder. Additionally, our cutoff score of 14 for the PSS and BDI, though based on published psychometric studies, may be lenient and thus could increase the risk of false positive. Sixth, the CHDWB replication sample uses the GAD-7, which is a good screening tool but could provide a false positive detection if not followed by an accurate clinical evaluation. Seventh, we found blood mRNA *PPM1F* level significantly down-regulated in cases with PTSD&Dep symptoms in the GTP sample and replicated this association in cases with anxiety symptoms in an independent CHDWB cohort. Our replication is only a quasi-replication since anxiety symptoms can be PTSD symptoms but they can also be symptoms of generalized anxiety disorder or social anxiety disorder. Lastly, only a subset of GTP participants with genetic data had gene expression data. However, the two datasets are comparable as reflected by the distribution of their sex, BDI, and PSS scores (Supplemental Table S8).

In conclusion, mRNA and protein levels of phosphatase Ppm1f and Camk2g are regulated in the brain after exposure to traumatic stress in male and female mouse models and are associated with chronic anxiety-related (PTSD) and depression symptoms in traumatized humans. Because of the widespread expression of PPM1F in the body and brain, these changes in expression of PPM1F by stress may possibly have other pathophysiological implications that should be examined in future studies. Insights into the role of CAMK2 and PPM1F following trauma exposure may contribute to discovering novel approaches to treat or prevent the disabling conditions of PTSD and depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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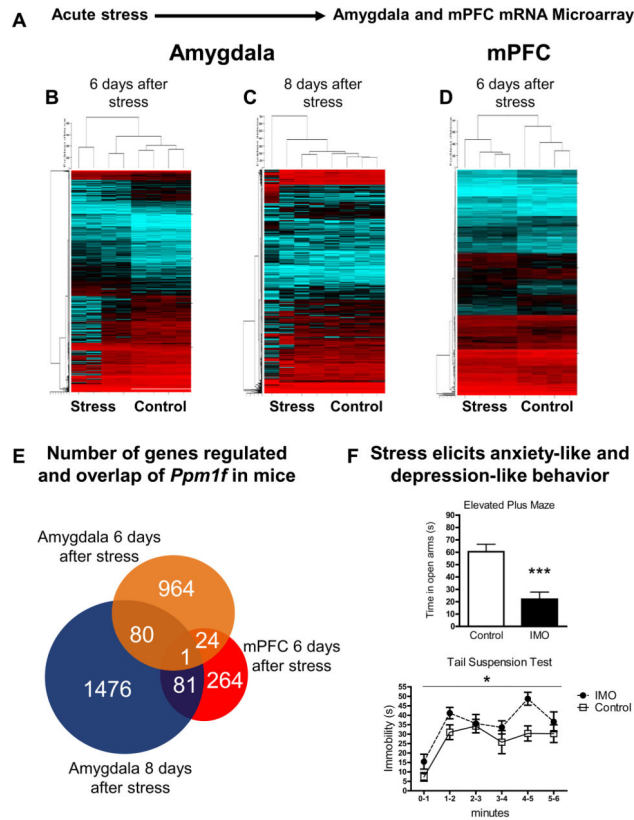**Figure 1. *Ppm1f* brain regulation after stress, anxiety-like and depression-like behaviors**
(A) Schematic of the experimental protocol (N=4 per group). **(B)** Amygdala microarray 6 days after acute immobilization stress (IMO). **(C)** Amygdala microarray 8 days after IMO stress. **(D)** mPFC microarray 6 days after IMO stress. **(E)** Gene regulation of the microarrays show overlap in the *Ppm1f* gene. **(F)** Top, 6 days after stress mice spend less time in the open arms in the elevated plus maze indicating enhanced anxiety-like behavior (p=0.0004, n=8 per group). Below, 6 days after stress mice present enhanced levels of immobility in the tail suspension test indicating enhanced depressive-like behavior (p=0.0361, n=8 per group).

A) Functions altered in the amygdala and mPFC after stress

Phosphoproteins

Cytoplasm

Alternative Splicing

32%

Ppm1f

27%

21.6%
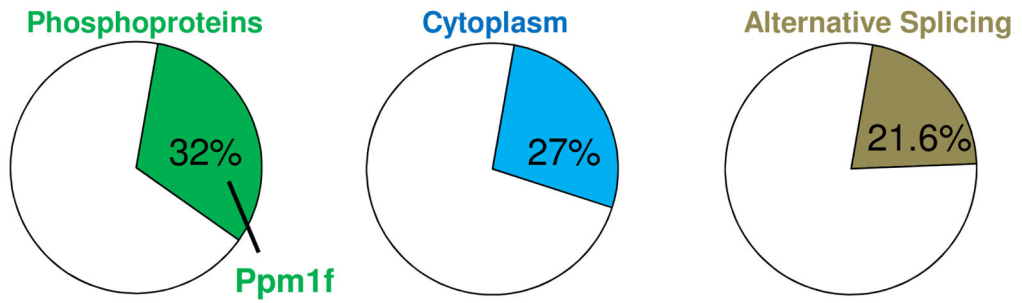
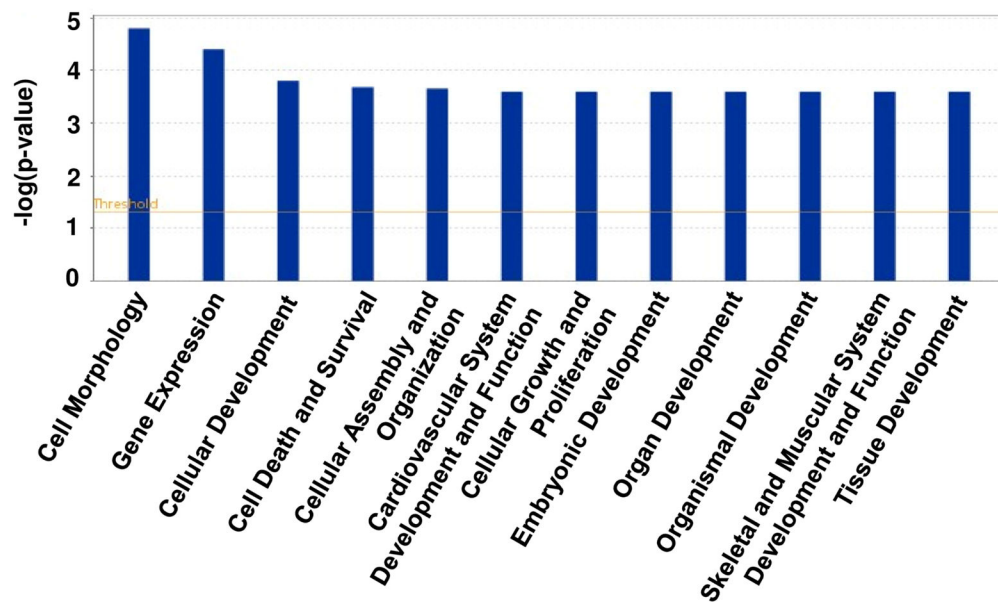## B) Detailed altered functions in the amygdala and mPFC after stress

Figure 2. Bioinformatics analysis of the altered functions in the Amygdala and mPFC after exposure to stress in mice

(A) Analysis with DAVID 6.8 revealed general altered functions 6 to 8 days after acute stress in both Amygdala and mPFC. Phosphoproteins (including *Ppm1f*), Cytoplasm and Alternative Splicing were the more affected functions in these analysis. **(B)** IPA analysis showed more detailed functions altered after a single exposure to 2 hours stress immobilization.
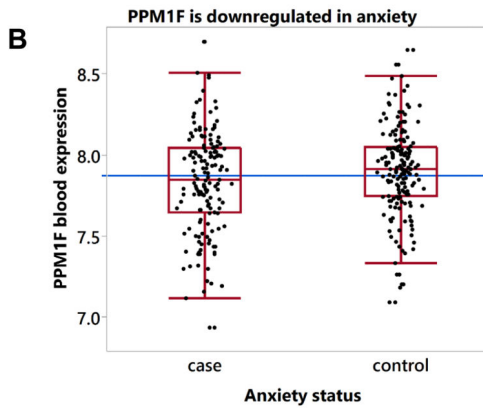
PPM1F blood mRNA levels in PTSD&Depression
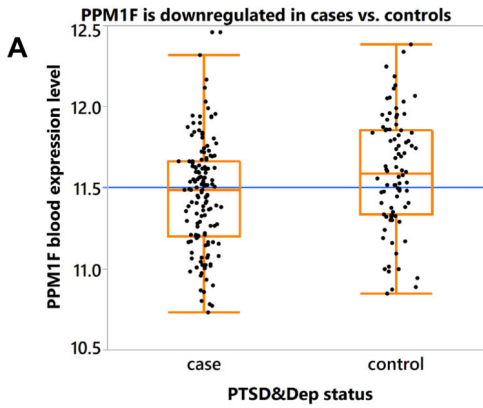
PPM1F blood mRNA levels in Anxiety

Figure 3. PPM1F blood mRNA levels in PTSD&Depression and in Anxiety
(A) Box plot showing that *PPM1F* mRNA level is lower in cases with symptoms of PTSD&Depression versus controls after adjusting for gender, age, and population substructure (p=0.005, n=230). The black dots represent the *PPM1F* expression level of each sample. The blue horizontal line represents the mean of *PPM1F* level in the overall sample; **(B)** *PPM1F* mRNA level is significantly down-regulated in anxiety vs. controls after adjusting for gender, age, and ethnicity (p=0.044, n=316).

A SNP within *PPM1F* is associated with PTSD&Depression

rs17759843 (number of its minor allele in additive genetic model) vs PTSD&Depression
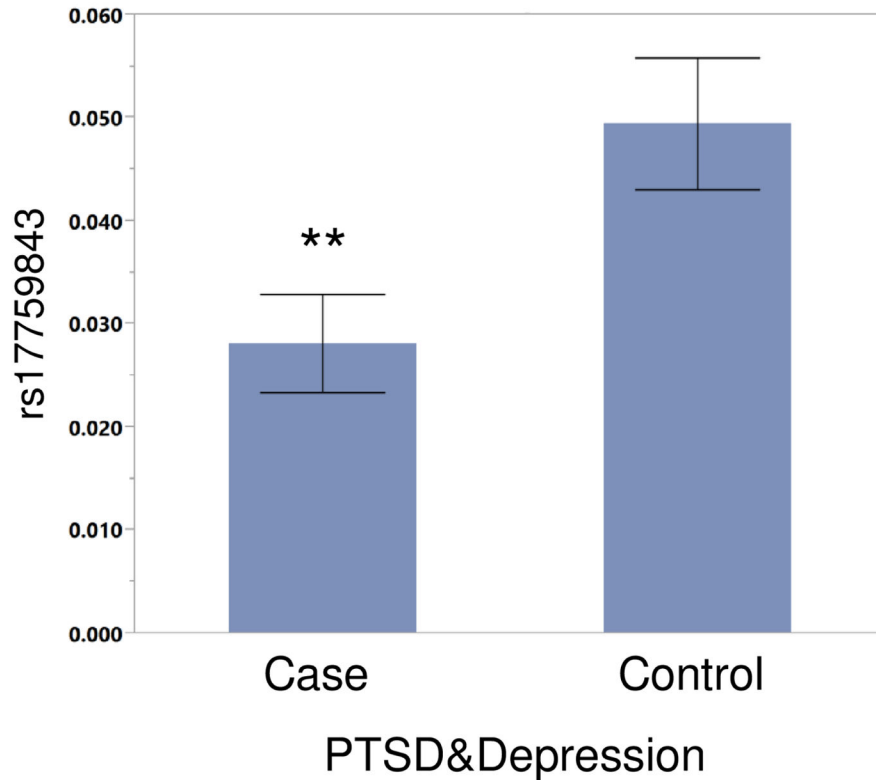

Figure 4. A SNP within the PPM1F gene is associated with PTSD&Depression
The minor allele of rs17759843, a PPM1F 3′UTR SNP, is significantly associated with lower odds for PTSD & Depression after adjusting for gender and population substructure (OR=0.53; p=0.005; permuted p=0.046; n=2361). Each error bar is constructed using 1 standard error from the mean.
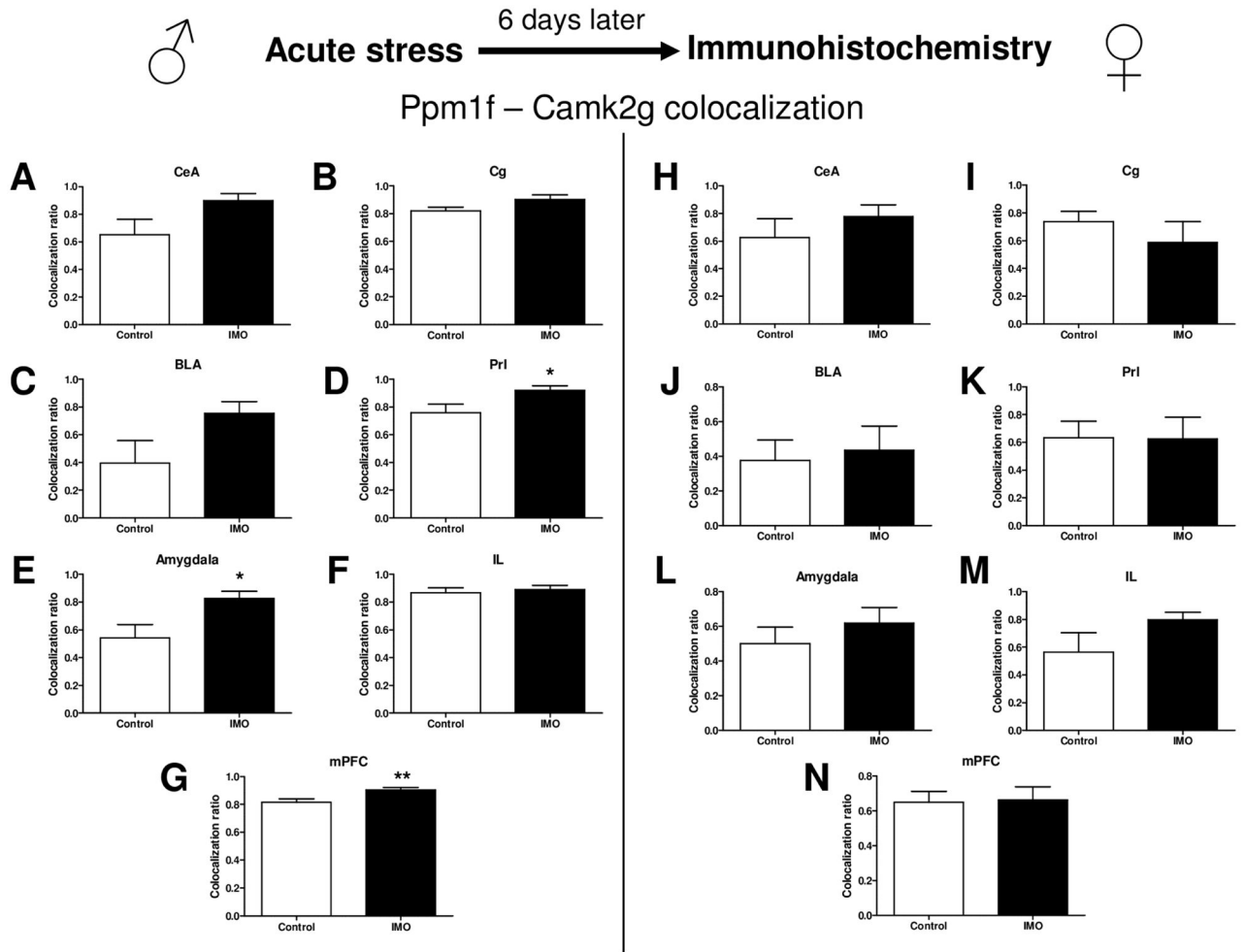
Figure 5. Stress immobilization and Ppm1f/Camk2g levels in the Amygdala and mPFC in both male and female mice: an immunohistochemistry study

Quantification of Ppm1f and Camk2g levels reveals that both are more densely colocalized after traumatic stress in male mice in the Amygdala and mPFC. **(A,H)** CeA = Central amygdala, **(B,I)** Cg = Cingulate cortex, **(C,J)** BLA = Basolateral amygdala, **(D,K)** Prl= Prelimbic cortex, **(E,L)** (Total) Amygdala = CeA + BLA, **(F,M)** IL= Infralimbic cortex, (G,N) mPFC = Medial prefrontal cortex = Cg+Prl+IL. *p 0.05, **p 0.01. N=2 per group.
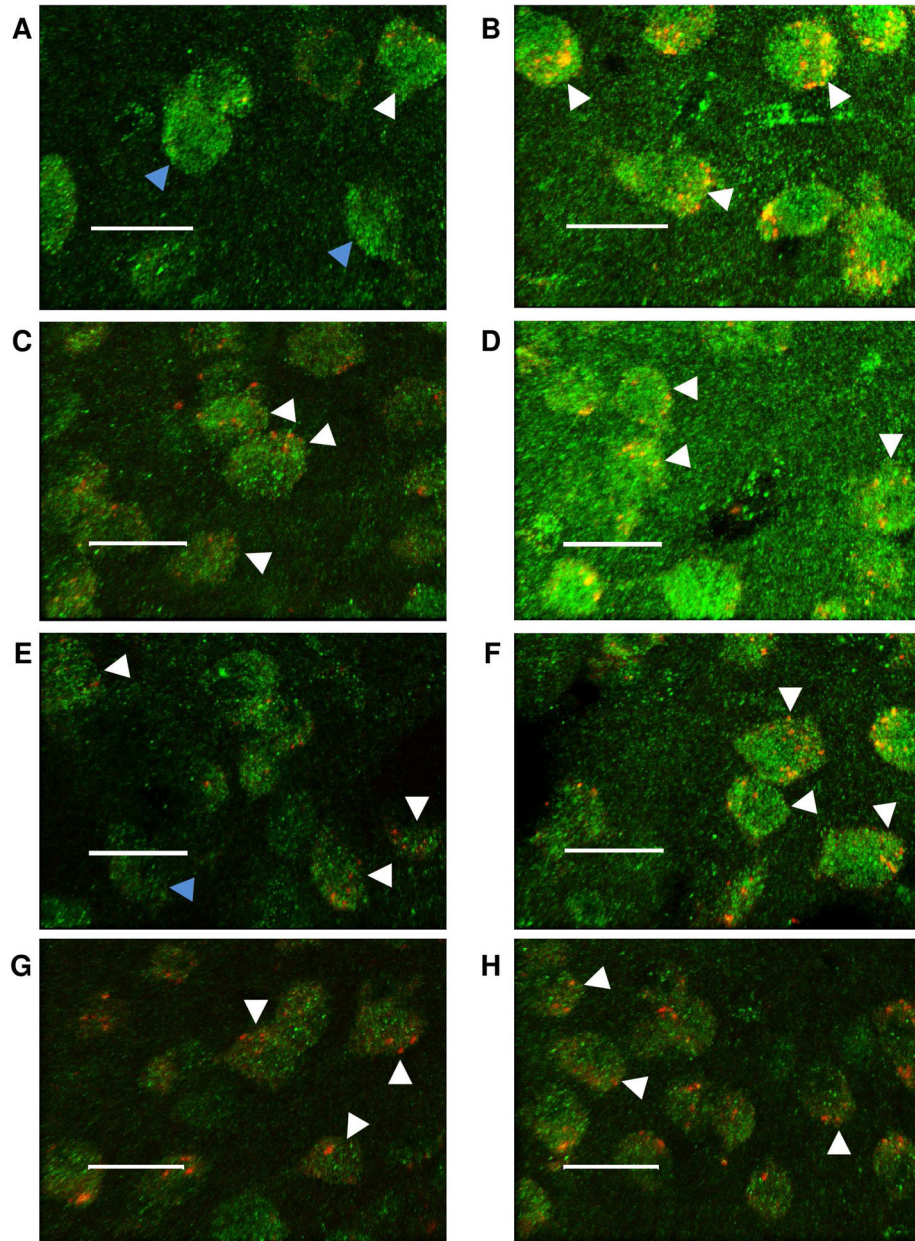
Figure 6. Representative images of the immunohistochemistry study evaluating the colocalization of Ppm1f (green) and Camk2g (red) in the Amygdala and mPFC

Scale bars = 20μm. **(A)** Male Basolateral amygdala, group control. **(B)** Male Basolateral amygdala, group IMO. **(C)** Male Prelimbic cortex **(PrL)**, group control. **(D)** Male PrL, group IMO. **(E)** Female Basolateral amygdala, group control. **(F)** Female Basolateral amygdala, group IMO. **(G)** Female Prelimbic cortex (PrL), group control. **(H)** Female PrL, group IMO. Blue arrow indicates no colocalization. White arrow indicates colocalization.
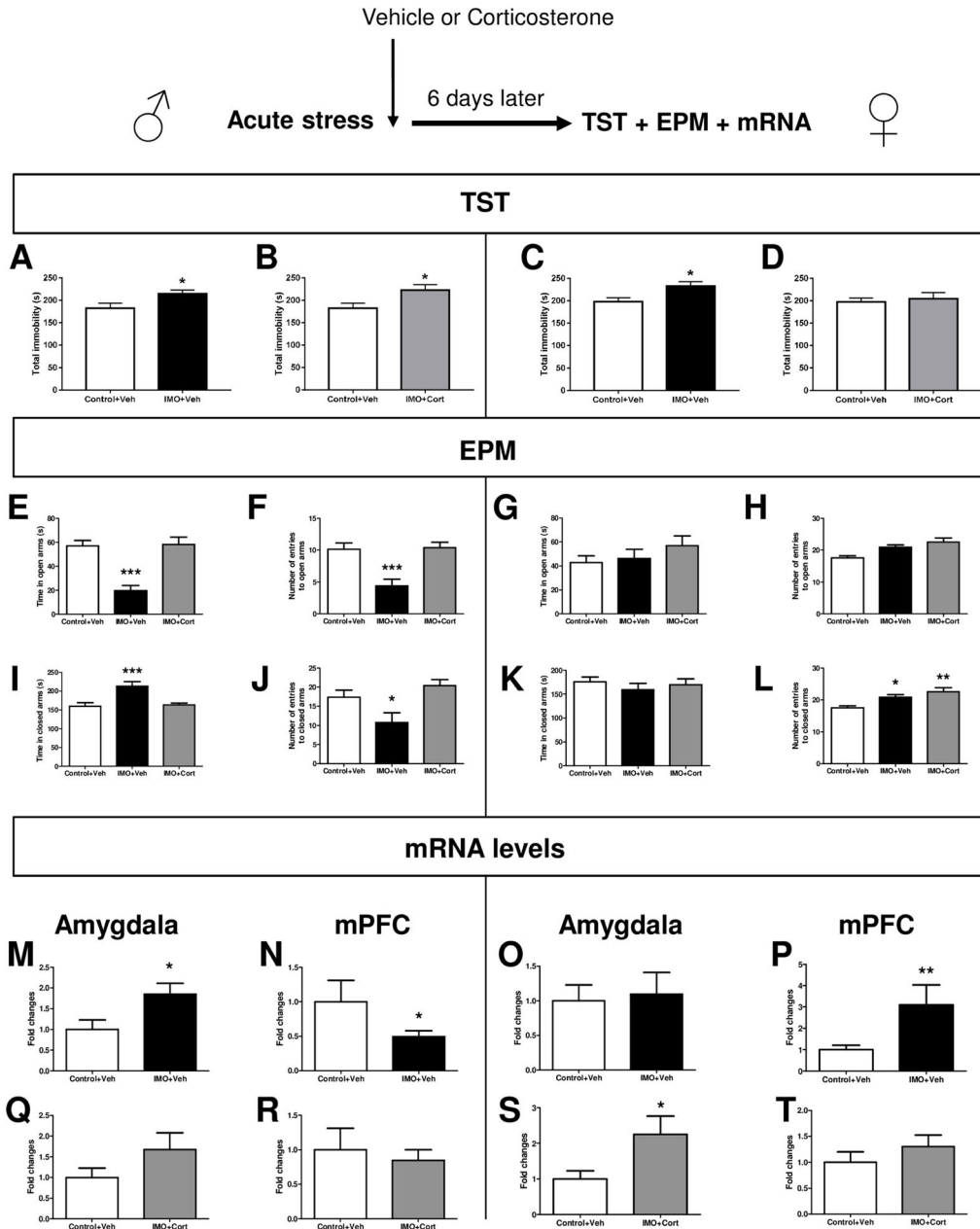
Figure 7. *Ppm1f* and corticosterone in anxiety-like behavior and depressive-like behavior in both male and female mice

N=5–8 per group. Systemic corticosterone given 1 hour after stress did not rescue the depressive-like phenotype elicited by IMO in male mice (**A, B**). However, Corticosterone rescued the depressive-like behavior induced by IMO in female mice (**C, D**) *p 0.05). EPM results showed that corticosterone rescued the anxiety-like behavior in male mice (**E,F, I, J**, *p 0.05, **p 0.01, ***p 0.001 vs the other groups). In contrast, IMO did not induce an anxiety-like behavior in female mice (**G, H, K**). The number of entries in closed arms was increased in the IMO+Veh vs Control+Veh group (**L**, *p 0.05, ***p 0.001 vs Control +Veh). Analysis of the mRNA levels in males showed that the IMO+Veh group presented

enhanced levels of ppm1f compared to Control group (p=0.0331, **M**) in the Amygdala and decreased levels in the mPFC (p=0.0371, **N**). Corticosterone rescued the ppm1f gene changes induced by IMO in the Amygdala **(Q)** and mPFC **(R)**. The mRNA levels in females were upregulated in mPFC in the group IMO+Veh vs Control+Veh **(P)** and also in the group IMO+Cort vs Control+Veh in the Amygdala **(S)**. Corticosterone rescued the ppm1f gene changes in the mPFC **(T)**.