

# Childhood maltreatment is associated with altered fear circuitry and increased internalizing symptoms by late adolescence

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**Maltreatment during childhood is a major risk factor for anxiety and depression, which are major public health problems. However, the underlying brain mechanism linking maltreatment and internalizing disorders remains poorly understood. Maltreatment may alter the activation of fear circuitry, but little is known about its impact on the connectivity of this circuitry in adolescence and whether such brain changes actually lead to internalizing symptoms. We examined the associations between experiences of maltreatment during childhood, resting-state functional brain connectivity (rs-FC) of the amygdala and hippocampus, and internalizing symptoms in 64 adolescents participating in a longitudinal community study. Childhood experiences of maltreatment were associated with lower hippocampus–subgenual cingulate rs-FC in both adolescent females and males and lower amygdala–subgenual cingulate rs-FC in females only. Furthermore, rs-FC mediated the association of maltreatment during childhood with adolescent internalizing symptoms. Thus, maltreatment in childhood, even at the lower severity levels found in a community sample, may alter the regulatory capacity of the brain’s fear circuit, leading to increased internalizing symptoms by late adolescence. These findings highlight the importance of fronto–hippocampal connectivity for both sexes in internalizing symptoms following maltreatment in childhood. Furthermore, the impact of maltreatment during childhood on both fronto–amygdala and –hippocampal connectivity in females may help explain their higher risk for internalizing disorders such as anxiety and depression.**

child maltreatment | sex differences | ventromedial prefrontal cortex

In both clinical and community samples, maltreatment during childhood represents one of the strongest risk factors for developing depression and anxiety (1–3). Childhood maltreatment and other adversities account for up to a third of the risk for mood and anxiety disorders (4). Further, depression and anxiety disorders are major public health problems, affecting 15 and 32% of youth, respectively, by the age of 18 y (5). The burden of these disorders is significant, representing the second and fifth leading causes, respectively, of years lived with disability in the United States (6). Some evidence suggests that maltreatment may impart greater risk for the development of internalizing symptoms in females than in males (e.g., refs. 7–9). This differential risk could account, in part, for the higher incidence of internalizing problems in females than in males (10, 11). However, the neurobiological pathways from maltreatment during childhood to the expression of internalizing problems, including potential differences for females and males, remain poorly understood. Such information is crucial for improving the treatment of depression and anxiety disorders and for mitigating the effects of maltreatment during childhood.

Both maltreatment during childhood (12) and internalizing disorders (13, 14) have been associated with altered activity in specific brain circuits involved in the processing and regulation

of threat and fear, including the amygdala, hippocampus, and prefrontal cortex (PFC). Maltreated children show increased amygdala and hippocampus activation in response to threatening faces (15–18), reduced hippocampus activation in a declarative memory task (19), and variable findings regarding PFC activation (12). These brain areas do not act in isolation but interact to regulate the fear response. The ventromedial (vm)PFC inhibits amygdala-based expression of fear responses and is required for fear extinction (14), whereas the hippocampus contextually limits fear responses via connections to both the amygdala and vmPFC (20). In rats, chronic stress impairs hippocampus–vmPFC long-term potentiation, which is required for the proper gating of conditioned fear (21, 22). Together, these findings suggest that prolonged exposure to stress may impair connectivity among the components of the fear circuitry, thereby impairing the regulation of emotion and amplifying fear responses.

The impact of maltreatment during childhood on the functional connectivity of this circuitry in humans has been explored only recently. Symptomatic adults reporting a history of maltreatment during childhood show altered local connectivity and hub-like properties of the amygdala and PFC (23), decreased functional connectivity strength of the PFC (24), and decreased functional connectivity of the amygdala with neighboring limbic areas (25). However, to our knowledge, no studies have investigated how maltreatment during childhood may affect brain

## Significance

**Childhood maltreatment is a major risk factor for internalizing disorders including depression and anxiety, which cause significant disability. Altered connectivity of the brain’s fear circuitry represents an important candidate mechanism linking maltreatment and these disorders, but this relationship has not been directly explored. Using resting-state functional brain connectivity in adolescents, we show that maltreatment predicts lower prefrontal–hippocampal connectivity in females and males but lower prefrontal–amygdala connectivity only in females. Altered connectivity, in turn, mediated the development of internalizing symptoms. These results highlight the importance of fronto–hippocampal connectivity for both sexes in internalizing symptoms following maltreatment. The additional impact on fronto–amygdala connectivity in females may help explain their higher risk for anxiety and depression.**

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functional connectivity in adolescence, and none have included connectivity of the hippocampus, a key node in the fear-regulatory network. Furthermore, no studies have investigated which, if any, of these differences in connectivity may mediate the link between maltreatment during childhood and the development of internalizing symptoms. Finally, no published work has investigated sex differences in the alterations in functional connectivity related to maltreatment during childhood that may lead to the increased rates of internalizing disorders seen in females.

We addressed these knowledge gaps by examining the associations between experiences of maltreatment in childhood, resting-state brain functional connectivity (rs-FC), and internalizing symptoms in a group of 64 late adolescents (30 female; age 18 y) who had been followed longitudinally from birth as part of the Wisconsin Study of Families and Work. Experiences of maltreatment were assessed via the Childhood Trauma Questionnaire (CTQ) (26) completed at age 18 y. As expected, adolescent reports of experiences of maltreatment in childhood were significantly and substantially associated with earlier maternal reports of family stress during late childhood and early adolescence (*SI Methods* and *Table S1*). We then examined the correlation of experiences of maltreatment in childhood with seed-based rs-FC of the hippocampus and amygdala. We predicted that maltreatment during childhood would be associated with decreased connectivity of the hippocampus and amygdala with the vmPFC. Next, we investigated whether altered rs-FC mediated the association of maltreatment during childhood and persistent adolescent internalizing symptoms (anxiety and depression symptoms averaged across four annual assessments at ages 15–18 y). Finally, we predicted that these associations would be stronger for females, given known sex differences in the development and stress sensitivity of the amygdala and hippocampus (11) and our prior study in this sample linking family stress during infancy and cortisol levels during childhood with adolescent amygdala–vmPFC rs-FC in girls but not in boys (27).

## Results

All results of rs-FC analysis associated with experiences of maltreatment in childhood are displayed in *Table 1*. Here we feature rs-FC findings that also were predictive of adolescent internalizing symptoms. Additional connectivity findings that were not predictive of internalizing symptoms are detailed in *SI Methods*.

**Experiences of Maltreatment in Childhood and Adolescent Amygdala rs-FC.** Across all participants, experiences of maltreatment in childhood predicted lower connectivity ( $R^2 = 0.27$ ) between the right amygdala and the vmPFC, specifically the subgenual anterior cingulate cortex (sgACC, Brodmann area 25) (*Fig. 1A* and *Table 1*). A similar association was found with the left amygdala but did not survive multiple comparison correction (cluster size = 62 voxels). The association of experiences of maltreatment in childhood with right amygdala–sgACC connectivity remained statistically significant when controlling for the afternoon cortisol level in childhood (age 4.5 y), which in our prior study (27) mediated the association between family stress during infancy and resting connectivity between the amygdala and a more anterior portion of the vmPFC (*Fig. 2*). Sex differences were examined by

ANCOVA on the extracted rs-FC values. Results revealed a significant sex by CTQ score interaction ( $F_{1,60} = 4.20, P = 0.045$ ). The association of CTQ score with amygdala–sgACC rs-FC was driven entirely by females ( $R^2 = 0.59$  and 0.03 for females and males, respectively), even though the CTQ scores of the two sexes were quite similar ( $t_{62} = 0.85, P = 0.40$ ). These findings remained significant when controlling for adolescent current life stress (CTQ score main effect:  $F_{1,59} = 17.44, P < 0.001$ ; sex–CTQ score interaction:  $F_{1,59} = 3.94, P = 0.05$ ). The CTQ score also significantly predicted amygdala rs-FC to postcentral gyrus and dorsolateral PFC, but these connectivity results did not predict adolescent internalizing symptoms (see below and *SI Methods*).

**Experiences of Maltreatment in Childhood and Adolescent Hippocampus rs-FC.** Childhood experiences of maltreatment predicted lower connectivity between the left hippocampus and the sgACC across all participants ( $R^2 = 0.39$ ) (*Fig. 1B* and *Table 1*). Analysis of extracted rs-FC values in an ANCOVA revealed only a main effect of CTQ score ( $F_{1,60} = 36.69, P < 0.001$ ), with similar associations in females and males ( $R^2 = 0.45$  and 0.34 respectively). This finding remained significant when controlling for adolescent current life stress (CTQ score main effect:  $F_{1,59} = 33.32, P < 0.001$ ). No significant associations were found between CTQ score and right hippocampus connectivity.

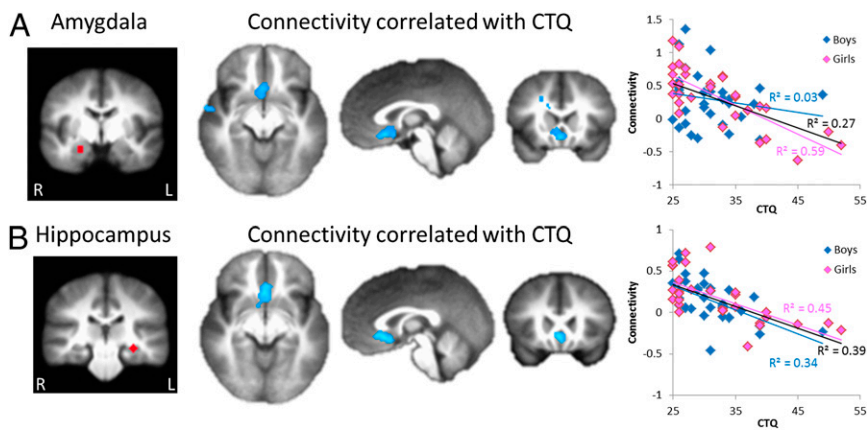
**Experiences of Maltreatment in Childhood, Adolescent rs-FC, and Internalizing Symptoms.** We next investigated the association of experiences of maltreatment during childhood with adolescent internalizing symptoms, considering potential sex differences and mediating effects of rs-FC. ANCOVA revealed that there was no statistically significant sex by CTQ score interaction ( $F_{1,60} = 1.94, P = 0.17$ ); rather, both CTQ score ( $F_{1,60} = 11.35, P = 0.001$ ) and sex ( $F_{1,60} = 4.24, P = 0.04$ ) were significant predictors of internalizing symptoms. As expected, higher CTQ scores predicted more internalizing symptoms, and females had higher levels of internalizing symptoms than males (average  $2.50 \pm 0.55$  and  $1.99 \pm 0.53$ , respectively). Of the rs-FC regions associated with CTQ score, bivariate correlations revealed that only amygdala– and hippocampus–sgACC rs-FC significantly predicted adolescent internalizing symptoms (*SI Methods* and *Table S2*). These results indicated that only amygdala– and hippocampus–sgACC rs-FC were potential mediators of the link between CTQ score and adolescent internalizing symptoms; thus these links became the focus of subsequent path-model analyses.

Using structural equation modeling (SEM), we then examined the mediating effects of amygdala– and hippocampus–sgACC rs-FC on the association between CTQ score and internalizing symptoms, as well as possible sex differences. Amygdala–sgACC connectivity and hippocampus–sgACC connectivity were highly correlated ( $r = 0.52, P < 0.001$ ). Thus, to reduce problems of multicollinearity that occur when both connectivity measures are included in the same model, we constructed two independent (uncorrelated) measures that distinguish the total connectivity, or what the amygdala– and hippocampus–sgACC measures share in common (i.e., the sum of the two connectivity measures), from what differentiates them (i.e., amygdala–sgACC minus hippocampus–sgACC). For both females and males, maltreatment

**Table 1. Summary of adolescent rs-FC estimates with amygdala and hippocampus seeds as predicted by experiences of maltreatment during childhood (CTQ total score)**

Seed	Identified cluster	Talairach coordinates	Peak t	Volume, $\mu\text{L}$
Left hippocampus	sgACC (BA 25)	2, 22, –6	–4.67	1,304
	R. PCG (BA 4)	22, –32, 28	–4.53	2,912
Left amygdala	L. dlPFC (BA 10)	–36, 50, 20	4.80	1,176
	sgACC (BA 25)	28, –24, 38	–4.73	976
Right amygdala	R. PCG (BA 4)	2, 18, –10	–4.22	1,080

Results were significant at  $P < 0.05$  with family-wise error correction at the whole-brain level. Note that the CTQ score also predicted lower connectivity between left amygdala and sgACC, but this prediction did not survive family-wise error correction.



**Fig. 1.** Childhood experiences of maltreatment predict lower rs-FC of the amygdala and hippocampus ( $n = 64$ ; 30 female). (A) CTQ scores negatively correlated with rs-FC between the right amygdala and sgACC across all participants. This association was specific to females (scatterplots, Right). (B) CTQ scores negatively correlated with rs-FC between the left hippocampus and sgACC in all participants. This association was present in both females and males (scatterplots, Right). Seed regions are displayed in the left panels. Connectivity results are displayed at  $P < 0.05$ , family-wise error corrected at the whole brain level.

experiences predicted lower total rs-FC of the amygdala and hippocampus to sgACC, and the lower rs-FC in turn mediated the association of maltreatment experiences with internalizing symptoms (Fig. 3). However, the mediating effect of differential connectivity was moderated by sex. In males, maltreatment experiences predicted lower rs-FC primarily between the hippocampus and sgACC, predicting greater internalizing symptoms; in females, maltreatment experiences predicted lower rs-FC between both the hippocampus and the sgACC and the amygdala and the sgACC, again predicting greater internalizing symptoms (Figs. 3 and 4). Reversal of this model (CTQ score  $\rightarrow$  internalizing  $\rightarrow$  functional connectivity) yielded poor model fit and decreased fit statistics, suggesting that altered rs-FC mediates internalizing symptoms but not the reverse (Methods).

### Discussion

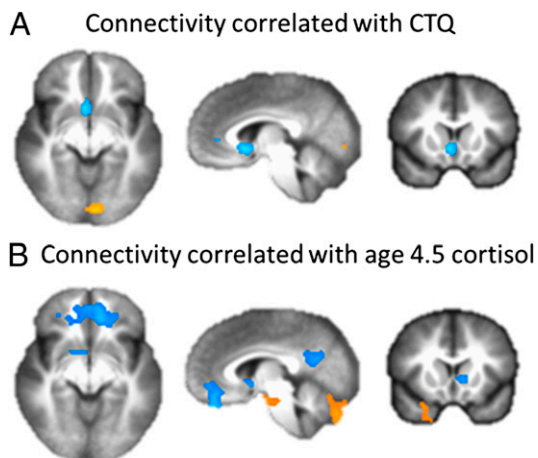
Our study details findings suggesting a direct neural mechanism mediating the link between childhood experiences of maltreatment and the development of anxiety and depressive symptoms in adolescence. This neural mechanism involves altered connectivity within the brain's fear-regulatory circuit including the hippocampus, amygdala, and sgACC. The sgACC, as part of the vmPFC, is a putative homolog of the rat infralimbic (IL) cortex, which mediates recall of fear extinction by suppressing amygdala-based fear responses through activation of inhibitory intercalated neurons in the amygdala (14). Consistent with rodent

data, fear-extinction studies in humans demonstrate increased activation of the vmPFC during fear-extinction learning and recall (14, 28). In humans, the sgACC also is involved more broadly in the automatic (nonconscious) regulation of negative affect. sgACC activation has been observed with the induction of sadness and the recall of traumatic events, and persistent hyperactivation has been observed in clinical depression (29). Furthermore, exogenous glucocorticoids reduce sgACC activation and simultaneously increase arousal to sadness-invoking stimuli in healthy individuals (30). Consistent with these findings, both adolescents and adults with anxiety disorders show reduced sgACC activation when appraising their own fear (31). Within this framework, maltreatment-associated uncoupling of the amygdala and sgACC may result in impaired modulation of negatively valenced emotional responses, including a failure to extinguish fear responses in the absence of threat.

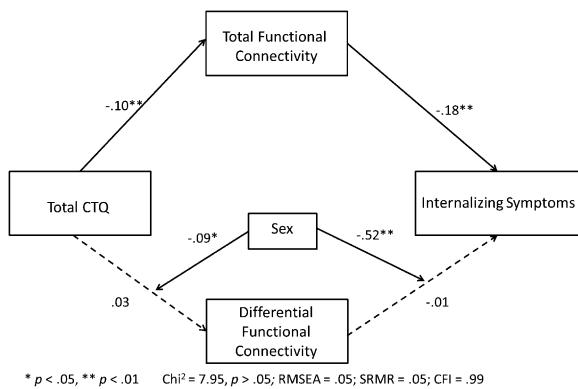
Maltreatment-associated uncoupling of the hippocampus and sgACC may lead to additional disruptions in the regulation of negative affect. In this case, impaired communication between the hippocampus and sgACC may reduce contextual gating of the expression of conditioned fear, leading to more generalized and persistent negative affect states. In rodents, both the IL cortex and hippocampus are required for the recall of fear-extinction memory (22, 32, 33), which involves enhanced synaptic plasticity between the hippocampus and medial PFC (mPFC) (21, 34). Furthermore, chronic stress in rodents blocks extinction-related enhancement of hippocampus-mPFC plasticity and the recall of fear extinction (21). Our findings are consistent with this effect and suggest that experiences of maltreatment during childhood may reduce the capacity of the hippocampus to engage PFC-mediated recall of fear extinction, thereby leading to greater internalizing symptoms.

The uncoupling of the hippocampus and sgACC associated with maltreatment in childhood also may have relevance for emotional disorders such as posttraumatic stress disorder (PTSD). Adult PTSD has been characterized by impaired fear extinction recall and lower activation of the hippocampus and vmPFC during extinction recall (20, 35). Interestingly, adult trauma (combat) exposure actually increases hippocampus-vmPFC connectivity, whereas failure to increase this connectivity was associated with PTSD symptoms (36). Together with our findings, this observation suggests that fronto-hippocampal connectivity may be uniquely vulnerable to trauma exposure during development. The failure of this circuit to modulate fear and anxiety responses contextually would be expected to lead to internalizing symptoms as observed in our sample. In addition, impairments in hippocampal connectivity induced by maltreatment in childhood may place the brain at further risk following subsequent adult trauma by reducing the capacity of the brain to engage this fear-gating circuit appropriately.

Of note, our study did not reveal any significant associations between maltreatment experiences in childhood and amygdala/hippocampus connectivity to the dorsal anterior cingulate cortex



**Fig. 2.** In girls, experiences of maltreatment during childhood remain negatively correlated with amygdala-sgACC connectivity when cortisol values at age 4.5 y are included in the model (A). In addition, experiences of maltreatment and cortisol values at age 4.5 y are associated with amygdala connectivity to different regions of the vmPFC (B).



**Fig. 3.** SEM examining the effect of rs-FC in mediating between childhood experiences of maltreatment (measured by CTQ scores) and internalizing symptoms at age 18 y, as moderated by sex. Connectivity variables included total connectivity of the amygdala and hippocampus to sgACC and differential connectivity (the difference between the amygdala- and hippocampus-sgACC scores) to examine overall and relative effects of each region. Models are shown by sex in Fig. 4.

(dACC), even at an uncorrected  $P = 0.001$ . The dACC is a putative homolog of the rat prelimbic (PL) cortex which, in contrast to the IL cortex, facilitates the expression of conditioned fear via excitatory connections to the basolateral amygdala (33, 37). In human studies, the dACC shows greater activation during acquisition and expression of fear (14). dACC activation to threat is associated with both childhood and adult exposure to trauma (38), and increased dACC-amygdala connectivity has been observed following combat exposure in adults (39, 40). Our findings suggest that maltreatment experiences were associated more strongly with impaired vmPFC connectivity. However, it is possible that abnormalities in dACC connectivity would emerge with additional task provocation, as in the studies cited above, or with greater levels of maltreatment than were observed in this community sample.

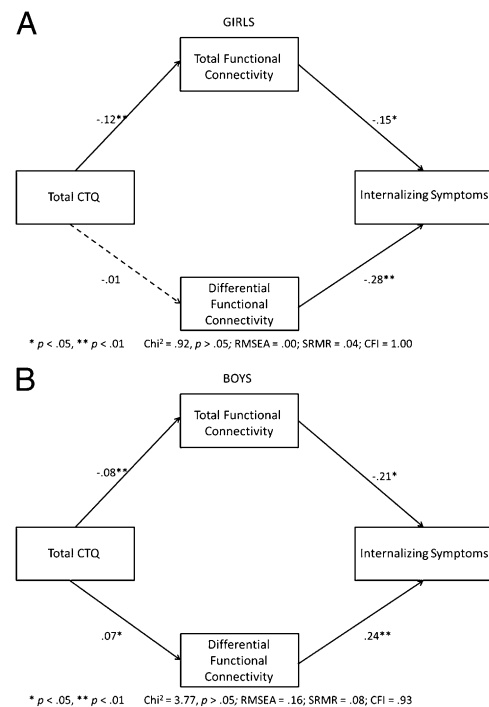
Childhood experiences of maltreatment also were associated with altered connectivity between the amygdala and other brain regions, including lower connectivity to the postcentral gyrus and greater connectivity to dPFC. However, none of these other areas mediated the development of internalizing symptoms. Thus, the functional significance of altered connectivity in these areas remains unclear at this time. Further studies would be warranted to explore their potential relevance to other psychiatric domains such as externalizing symptoms.

Our study also has revealed sex differences in the neural impact of exposure to maltreatment during childhood. Specifically, our results suggest that at the neural level females are more vulnerable to childhood experiences of maltreatment, because in females these experiences impact both the amygdala- and hippocampal-sgACC regulatory pathways. In contrast, in males maltreatment during childhood appeared to impact only the hippocampus-sgACC pathway. This “double hit” in females may explain, in part, their higher levels of internalizing symptoms in our sample and the broadly observed greater risk for anxiety and depression in females. The amygdala and hippocampus are known to exhibit sex differences in their developmental trajectories that could confer differing vulnerability to experiences of maltreatment. Initial studies using linear modeling suggested that, between the ages of 4–18 y, the amygdala has a more extended period and greater rate of growth in males, whereas the hippocampus has a more extended period and greater rate of growth in females (41, 42). More recent work using nonlinear modeling and a wider age range (1 mo to 25 y) revealed greater rates of growth for both amygdala and hippocampus in females during the first several years of life, with sex differences in the period of growth only in the amygdala (i.e., longer for males) (43). Periods of rapid brain maturation are particularly sensitive to the negative effects of early experiences (44) and may account

for the greater neural impact of maltreatment in females by affecting functional connectivity of both the amygdala and hippocampus with the sgACC. In addition, prolonged amygdala growth in males may confer greater protection in terms of neuroplasticity following repeated stress, perhaps allowing sgACC-amygdala connectivity to remain more intact in males.

Sex differences in white matter development also may have particular relevance for our functional connectivity findings. Males have more rapid increases in white matter volume during development (45) and have greater structural integrity in white matter tracts connecting the vmPFC and amygdala/hippocampus (46, 47). These differences may confer greater protection in males following maltreatment experiences, although this hypothesis would require further study.

Aside from the period and rate of growth, the amygdala, hippocampus, and sgACC also may exhibit inherent sex differences in sensitivity to stress. Unfortunately there is very little work in humans (especially developmentally) directly examining this question. A meta-analysis of volumetric brain studies of PTSD revealed that exposure to trauma had a relatively greater effect on hippocampal volume in males than in females (48). Consistent with this finding, animal studies have shown that chronic stress impairs neurogenesis and causes dendritic retraction in the hippocampus, along with spatial memory deficits, to a much greater degree in adult male than in female rats (49). These sex differences in sensitivity to the effects of chronic stress may reflect, in part, the effects of estrogen, which exhibits



**Fig. 4.** SEM examining the effect of functional connectivity in mediating between childhood experiences of maltreatment (measured by CTQ scores) and adolescent internalizing symptoms in girls (A) and boys (B). Connectivity variables included both total connectivity of the amygdala and hippocampus to sgACC and differential connectivity (the difference between the amygdala- and hippocampus-sgACC scores) to examine overall and relative effects of each region. (A) In girls, lower total functional connectivity of the amygdala and hippocampus to sgACC mediated the association between CTQ scores and internalizing symptoms. Lower amygdala-sgACC connectivity (i.e., a lower differential score) contributed more substantially to the association between CTQ scores and internalizing symptoms. (B) In boys, findings were similar except that lower hippocampus-sgACC connectivity (i.e., a higher differential score) contributed more substantially to this effect. The full model with moderation by sex is shown in Fig. 3.

a number of neuroprotective effects in the hippocampus (49, 50). However, in our sample, maltreatment during childhood showed similar associations with hippocampal rs-FC in both males and females. One possibility is that the greater vulnerability of the male hippocampus to stress may be counterbalanced by greater structural connectivity between the vmPFC and hippocampus in males, yielding similar effects for both sexes in terms of the functional connectivity in this pathway. Even less is known about sex differences in the impact of stress on the amygdala and PFC. Chronic stress increases dendritic arborization of the amygdala and decreases arborization of the PFC in male rats, but there are few such studies in female rats (49, 51). In humans, structural connectivity between the vmPFC and amygdala/hippocampus is reduced in children who have suffered early neglect/maltreatment (52, 53), but whether there are sex differences in the effects of stress on these white matter tracts remains unclear. Ultimately, more studies exploring developmental sex differences in the sensitivity of these brain regions and their connecting fibers to stress will be needed in humans as well as in animal models.

The results of this study suggest important brain pathways linking maltreatment in childhood to the development of anxiety and depression. Strengths of the study include the relatively large sample size, longitudinal design, and the inclusion of measures of adolescent brain connectivity. However, there are potential caveats regarding these findings. First, it is possible that altered brain connectivity represents a preexisting factor (trait) for vulnerability to experiences of maltreatment or for the development of internalizing symptoms. Although our path modeling suggests otherwise, it will be important in future studies to supplement this type of data with earlier brain measures, before maltreatment has occurred, to address this question. Second, connectivity measures based on the resting state may not be equivalent to those obtained during a task. On the other hand, resting-state analysis avoids task performance as a potential confound. Finally, although our data suggest strong developmental sensitivities to trauma exposure in the fear circuit, it will be important in future work to tease out more precisely which developmental periods are the most vulnerable in different brain areas and how these periods may differ by sex.

In conclusion, the current data suggest a direct neural mechanism, via altered connectivity of the brain's fear circuitry, by which experiences of maltreatment during childhood lead to anxiety and depressive symptoms by late adolescence. These findings highlight the importance of sgACC–hippocampal connectivity for both sexes in internalizing symptoms following maltreatment in childhood. Furthermore, the impact of maltreatment in childhood on both sgACC–amygdala and –hippocampal connectivity in females may help explain their higher risk for internalizing disorders such as anxiety and depression. We observed these associations even with the relatively low levels of maltreatment experiences found in a community sample. These results suggest that maltreatment, even below the threshold of reportable childhood maltreatment, leads to significant changes in the brain's emotion-regulating circuitry. These findings will help point the way to new and developmentally sensitive interventions following maltreatment with the goal of averting the development of internalizing disorders, which are major public health problems. Our findings also suggest that additional or more extensive interventions may be needed to help female victims recover from the many deleterious effects of maltreatment during childhood.

## Methods

**Participants.** Participants were 64 adolescents [30 female; age  $18.79 \pm 0.19$  y (mean  $\pm$  SD)] from the larger Wisconsin Study of Families and Work (originally the Wisconsin Maternity Leave and Health Project) (54). For further details see *SI Methods*.

**Behavioral Measures.** Experiences of maltreatment during childhood were assessed by self-report at the age of 18 y using the CTQ (26). The total CTQ score (sum of physical abuse and neglect, emotional abuse and neglect, and sexual abuse) was used in the analyses. Variance in the CTQ score was driven most strongly by emotional abuse and neglect (Subscore means: emotional

abuse  $6.83 \pm 2.45$ , emotional neglect  $8.06 \pm 2.53$ , physical abuse  $5.33 \pm 0.82$ , physical neglect  $5.73 \pm 1.23$ , sexual abuse  $5.69 \pm 2.58$ ). Maternal reports of earlier childhood stress were based on averages for three developmental periods: infancy/preschool, late childhood (age 9–11 y), and early/mid-adolescence (age 13–15 y). Maternal stress measures included maternal depression, negative parenting, marital conflict/family anger, maternal role overload, and financial stress (54). Adolescent anxiety and depression symptoms were assessed four times annually from ages 15–18 y via self-report with the adolescent version of the MacArthur Health and Behavior Questionnaire (HBQ) (55), a well-validated measure of mental health, physical health, and social and academic functioning. Of interest for the current analysis were the HBQ subscales measuring symptoms of anxiety and depression, which were averaged across the 4 y to provide a measure of persistent internalizing symptoms. Finally, current adolescent life stress was indexed using a 61-item life-events inventory modeled on the Adolescent Perceived Events Scale (56) and the Life Experiences Survey (57). Events covered age-appropriate life domains (e.g., relationships, change in parental marital status or finances, serious illnesses and deaths). The current analyses include the summed impact of negative events in the past 6 mo.

**Imaging Data Acquisition and Processing.** Structural and resting-state functional images were collected on a 3T MRI scanner (GE Discovery MR750) with an eight-channel RF head coil array. Data preprocessing was conducted with Analysis of Functional and Neural Images (AFNI) and FMRIB Software Library software. For further details, see *SI Methods*.

**Functional Connectivity Analyses.** rs-FC estimates were computed using a seed region-based approach. Binary masks of the left and right amygdala and hippocampus were defined in AFNI by placing spheres with a 4-mm radius at the locations of the amygdala and hippocampus. Participant connectivity maps were entered into two-tailed regressions (AFNI's 3dttest++) while covarying childhood experience of maltreatment (CTQ scores), childhood basal cortisol levels, and/or other behavioral variables of interest. Note that CTQ scores were not correlated with subject motion (Table S3). At an individual voxel  $P < 0.001$ , a minimum cluster size of 111 voxels is required to have a corrected  $P \leq 0.05$ . For further details, see *SI Methods*.

**Path Modeling.** Mplus software (version 5.2, Muthén & Muthén, Los Angeles) was used to construct a structural equation model testing the mediating effects of brain connectivity in the association between childhood experiences of maltreatment and persistent internalizing symptoms. To test for mediation, both direct and indirect effects were examined. To determine if pathways differed in males and females, main and appropriate interactive effects of sex were included on all indirect pathways (58).

Because the measures of hippocampus–sgACC and amygdala–sgACC connectivity were highly correlated, including both in the model introduced potential problems of multicollinearity. To address this problem, we chose to conduct a principal components analysis specifying two independent (uncorrelated) components. This approach allowed us to isolate what the two measures had in common (i.e., the first component reflected total, or shared, connectivity) from what was different about them (i.e., the second component reflected differential connectivity, with a positive score reflecting increased amygdala connectivity and a negative score reflecting increased hippocampal connectivity). We chose this approach because we are equally interested in both connectivity measures; other approaches, such as residualization, would require us to attribute the shared variance arbitrarily to only one of the measures (e.g., including in the model hippocampus–sgACC connectivity and amygdala–sgACC connectivity residualized for hippocampus–sgACC connectivity, or vice versa). Given that previous analyses did not identify significant associations with any control variables, no additional predictors were included in the model because of unnecessary reductions in power. This model demonstrated good fit [ $\chi^2 = 7.95$ ,  $P > 0.05$ , root mean square error of approximation (RMSEA) = 0.05, standardized root mean square residual (SRMR) = 0.05, comparative fit index (CFI) = 0.99] and accounted for 49.7% of the variance in persistent internalizing symptoms. Furthermore, this model revealed that experiences of maltreatment during childhood led to lower total connectivity, which in turn led to internalizing symptoms (Fig. 3). The differential pathway revealed significant sex interactions, suggesting that for boys the total connectivity findings were driven by less connectivity in the hippocampus–sgACC pathway (Figs. 3 and 4). Formal testing of both mediating pathways suggests significant mediating effects of connectivity (total connectivity estimate = 0.02,  $P < 0.001$ ; differential connectivity estimate = 0.04,  $P = 0.05$ ). Finally, because the timing in the measurement of connectivity and internalizing symptoms overlapped, a second SEM was constructed with the positions of internalizing symptoms and connectivity reversed. The fit statistics of this model were all within the unacceptable range,

suggesting that the observed data did not fit the proposed model ( $\chi^2 = 47.71$ ,  $P < 0.05$ , RMSEA = 0.33, SRMR = 0.13, CFI = 0.37). For further details see *SI Methods*.

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# Supporting Information

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## SI Methods

**Participants.** Recruitment for the Wisconsin Study of Families and Work began in 1990 and was designed to gather information on parental leave and health outcomes from a subsample of the general population in and around two cities in southern Wisconsin where the woman was working either outside the home or as a full-time homemaker. A total of 570 women and their partners initially were recruited from clinics and hospitals while attending routine prenatal visits. Mothers had to be over 18 y old, in their second trimester of pregnancy, and living with the baby's biological father. Selection for the present study was based on proximity to the laboratory and MRI exclusionary criteria. Participants' ( $n = 64$ ) racial background was 61 white, 1 Native American/Alaskan, and 2 African American. Resting-state data were collected during a 4-h laboratory visit. Informed consent (and parental permission in childhood) was obtained for all assessments, and participants received monetary compensation for participating. University of Wisconsin-Madison Institutional Review Boards approved all procedures.

**Imaging Data Acquisition and Processing.** Structural and functional images were collected on a 3T MRI scanner (Discovery MR750, General Electric Medical Systems) with an eight-channel RF head coil array. T1-weighted structural images (1-mm<sup>3</sup> voxels) were acquired axially with an isotropic MPRAGE sequence (TE = 3.18 ms, TR = 8.13 ms, TI = 450 ms, flip angle = 12°). Subjects were instructed to rest silently with their eyes closed while remaining "clear, calm, and awake" during the collection of a T2\*-weighted gradient-echo echo-planar pulse sequence lasting 420 s (210 volumes) with a TE, TR, and flip angle of 25 ms, 2,000 ms, and 60°, respectively. Image volumes had a resolution of 3.5 × 3.5 × 5 mm<sup>3</sup> (matrix size = 64 × 64; 30 sagittal slices).

Most data-reduction steps were performed using the Analysis of Functional and Neural Images (AFNI) software package (1). Images were corrected for slice-dependent time shifts and motion (2) and were field-map corrected using FMRIB Software Library (FSL) PRELUDE (3, 4) and in-house software. Anatomical images then were aligned to the fifth volume of echo-planar image (EPI) time series using a Local Pearson Correlation cost function (5). The first four volumes of the time series were removed because of T1-equilibrium effects. Both anatomical and functional images were transformed to Talairach Atlas space using a nine-parameter affine transformation and were resampled to 2 × 2 × 2 mm voxels.

Resting-state fMRI time courses were temporally filtered (bandpass: 0.001 Hz <  $f$  < 0.01 Hz). In a step to reduce the influence of motion further, time points were censored if the motion of a point 87 mm from the center of rotation was greater than 2 mm per degree. Note that the Childhood Trauma Questionnaire (CTQ) was not associated with subject motion or with the number of time points censored (see below and Table S3). Variance from sources of noninterest was removed using multiple linear regression (AFNI's 3dDeconvolve function). Six rigid body-motion parameters were included as nuisance regressors, along with the average signal (calculated from all voxels) and derivatives from both eroded cerebral spinal fluid (CSF) and 2× eroded white matter (WM) masks. Masks were generated with an automated segmentation of the T1-weighted structural scan using FSL's FAST routine (3, 4, 6) and were transformed to Talairach Atlas space. EPI time series then were spatially smoothed with a 6-mm FWHM Gaussian kernel (postnuisance regression to avoid partial volume averaging within CSF and WM masks).

**Functional Connectivity Analyses.** Resting-state functional connectivity (rs-FC) estimates were computed using a seed region-based approach (7). Binary masks of the left and right amygdala and hippocampus were defined in AFNI by placing spheres with a 4-mm radius at the locations of the amygdala and hippocampus according to the Talairach Daemon (8). The average preprocessed functional MRI (fMRI) signal-intensity time course over each amygdala region of interest then was regressed against the signal-intensity time courses of all other voxels in the brain. Time points were motion censored as outlined above. The correlation coefficient of each voxel in the resultant statistical parametric maps was converted into a z-score using the Fisher z-transformation. Participant connectivity maps then were entered into two-tailed regressions (AFNI's 3dtest++) while covarying CTQ scores, childhood basal cortisol levels, and/or other behavioral variables of interest. Cluster sizes were selected based on  $\alpha$  significance values  $\leq 0.05$  and were estimated with AFNI's 3dClustSim and 3dFWHMx. In our data, at an individual voxel  $P < 0.001$ , a minimum cluster size of 111 voxels is required to have a corrected  $P \leq 0.05$ . Before any behavioral covariates were included, both the left and right amygdala showed significant positive rs-FC with the contralateral amygdala, dorso-medial prefrontal cortex and ventrolateral prefrontal cortex (PFC), ventral striatum, superior temporal gyrus, and ventromedial prefrontal cortex (vmPFC). Both left and right hippocampus showed significant positive rs-FC with the contralateral hippocampus, ventromedial and dorsomedial PFC, ventral striatum, posterior cingulate, bilateral angular gyrus, middle and superior temporal gyrus, and postcentral gyrus (PCG).

**Path Modeling.** Mplus software (version 5.2) (9) was used to construct a structural equation model (SEM) testing the mediating effects of brain connectivity in the association between childhood experiences of maltreatment and persistent internalizing symptoms. SEM provides a variety of measures indicating the associations between variables and the overall fit of the proposed model. Models must consider the number of participants relative to the number of paths being estimated; a 5:1 ratio is considered acceptable (10). A nonsignificant  $\chi^2$  test is desirable, because it indicates that the proposed model is not statistically significantly different from the observed data (11). Fit indices such as the root mean square error of approximation and the standardized root mean square residual generally are considered adequate below 0.08–0.10 (12). The comparative fit index is sensitive to the number of estimated paths in the model and is considered good at 0.93 or above.

**Association of Adolescent Report of Childhood Experiences of Maltreatment with Earlier Maternal Reports of Family Stress.** The retrospective nature of the CTQ can make it subject to memory bias. Thus, we took advantage of the longitudinal study design to investigate associations of the adolescent-reported CTQ score with earlier maternal reports of family stress obtained during the childhood and early adolescent years. A stepwise regression analysis performed within SPSS (v. 21) revealed that the CTQ score was substantially predicted by maternal reports of financial stress during infancy/preschool, negative parenting in late childhood, and financial stress during adolescence (model fit  $R^2 = 0.32$ ,  $P < 0.001$ ; Table S1). In addition, the effect of financial stress during infancy/preschool dropped to nonsignificance after the inclusion of financial stress during adolescence, suggesting mediation. These results demonstrate that adolescent reports of

experiences of maltreatment during childhood substantially reflect earlier maternal reports of family stress.

**Childhood Experiences of Maltreatment and Adolescent Amygdala rs-FC with Brain Regions Not Predictive of Adolescent Internalizing Symptoms.** As shown in Table 1, the CTQ score also significantly predicted connectivity between amygdala and additional brain regions that did not predict adolescent internalizing symptoms (see below). The CTQ score predicted lower connectivity between left and right amygdala and right PCG ( $P < 0.01$ ,  $R^2 = 0.32$  and  $0.28$ , respectively) and greater connectivity between left amygdala and left dorsolateral prefrontal cortex (dlPFC) ( $P < 0.02$ ,  $R^2 = 0.35$ ). ANCOVA of the extracted rs-FC revealed main effects of the CTQ score only for left amygdala–PCG ( $F_{1,60} = 21.84$ ,  $P < 0.001$ ) and left amygdala–dlPFC rs-FC ( $F_{1,60} = 23.79$ ,  $P < 0.001$ ). Right amygdala–PCG rs-FC showed a significant sex by CTQ score interaction ( $F_{1,60} = 4.55$ ,  $P = 0.037$ ). This effect was driven by a stronger association with CTQ score in females ( $R^2 = 0.61$  in females and  $R^2 = 0.03$  for males). These findings remained significant when controlling for adolescent current life stress (left amygdala–PCG CTQ score main effect:  $F_{1,59} = 20.07$ ,  $P < 0.001$ ; left amygdala–dlPFC CTQ score main effect:  $F_{1,59} = 20.41$ ,  $P < 0.001$ ; right amygdala–PCG sex–CTQ score interaction:  $F_{1,59} = 4.33$ ,  $P = 0.04$ ).

**Correlations Between Childhood Experiences of Maltreatment, Adolescent Internalizing Symptoms, and Functional Connectivity.** Table S2 shows the bivariate correlations between childhood experiences of maltreatment, adolescent internalizing symptoms, and extracted functional connectivity values derived from regions associated with CTQ score. Analyses were controlled for sex. Amygdala–subgenual anterior cingulate cortex (sgACC) and hippocampus–sgACC connectivity were significantly correlated with adolescent internalizing symptoms. Amygdala–PCG and amygdala–dlPFC functional connectivity were not significantly correlated with adolescent internalizing symptoms. There was a trend for an

association between left amygdala–PCG connectivity and internalizing symptoms ( $P = 0.06$ ). To examine whether this pathway contributes to internalizing symptoms independently of amygdala/hippocampus–sgACC connectivity, we performed an additional partial correlation analysis with sex and total amygdala/hippocampus–sgACC connectivity included as variables. When controlled for left amygdala–PCG connectivity, the association between total amygdala/hippocampus–sgACC connectivity and internalizing dropped only modestly (from  $-0.31$  to  $-0.23$ ;  $P = 0.07$ ). However, when controlled for total amygdala/hippocampus–sgACC connectivity, the association between left amygdala–PCG connectivity and internalizing was no longer present (from  $-0.24$  to  $-0.11$ ,  $P = 0.38$ ). These analyses indicate that only amygdala–sgACC and hippocampus–sgACC connectivity remained as potential mediators between experiences of maltreatment during childhood and adolescent internalizing symptoms and therefore became the focus of the path modeling.

**Subject Motion During Resting-State fMRI and Association with CTQ Total Score.** Summary measures of motion for each subject were obtained by (i) computing the sum of the squared differences (SSD) (13) between successive time points of the six estimated motion parameters (three rotation, three translation) and (ii) computing the total number of time points censored. In this sample, there was very little subject motion (SSD =  $0.097 \pm 0.073$  mm per time point; number of time points censored =  $1 \pm 5$  of 210 total time points). Of the 64 subjects, 59 had no time points censored. The remaining 5 subjects had the following number of time points censored: 3, 3, 6, 11, and 41. Across subjects, there was no significant association between CTQ score and subject motion as assessed with either of these motion summary measures (Pearson's correlation between CTQ score and SSD = 0.021 and between CTQ score and number of time points censored = 0.031). These results are summarized in Table S3.

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**Table S1. Association between adolescent reports of experiences of maltreatment during childhood (CTQ scores) with earlier maternal reports of family stress during childhood**

Step	$R^2$	Variable	$\beta$	$t$	$P$
1	0.12	Infancy/preschool financial stress	0.35	2.90	0.005
2	0.23	Infancy/preschool financial stress	0.24	2.02	0.047
		Late childhood negative parenting	0.34	2.87	0.006
3	0.33	Infancy/preschool financial stress	0.00	0.00	0.997
		Late childhood negative parenting	0.26	2.26	0.027
		Early/midadolescence financial stress	0.42	2.92	0.005

A stepwise linear regression examined predictors of CTQ score by average maternal stress reports from three developmental periods: infancy/preschool, late childhood (age 9–11 y), and early/midadolescence (age 13–15 y). Maternal stress measures included maternal depression, negative parenting, marital conflict, maternal role overload, financial stress, and family anger.

**Table S2. Bivariate correlations, controlled for sex, for extracted rs-FC estimates from regions in Table 1 with experiences of maltreatment during childhood (CTQ total score), and adolescent internalizing symptoms**

Seed	Identified cluster	Experiences of maltreatment during childhood		Adolescent internalizing symptoms	
		$r$	$P$	$r$	$P$
Left hippocampus	sgACC (BA 25)	−0.63	<0.001	−0.29	0.02
Left amygdala	R. PCG (BA 4)	−0.56	<0.001	−0.24	0.06
	L. dlPFC (BA 10)	0.59	<0.001	0.13	0.32
Right amygdala	sgACC (BA 25)	−0.53	<0.001	−0.26	0.04
	R. PCG (BA 4)	−0.52	<0.001	−0.08	0.51

**Table S3. Summary of subject motion parameters during resting state fMRI and association with CTQ total score**

Metric	Mean value	Correlation with CTQ score, Pearson's $r$
SSD of motion parameters	$0.097 \pm 0.073$ mm per time point	0.021
Number of time points censored (out of 210 time points)	$1 \pm 5$	0.031

Summary measures of motion for each subject were obtained by computing the sum of the SSD between successive time points of the six estimated motion parameters and computing the total number of time points censored. There was very little subject motion, and neither measure was associated with CTQ total score.