Brain galanin system genes interact with life stresses in depression-related phenotypes

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Galanin is a stress-inducible neuropeptide and cotransmitter in serotonin and norepinephrine neurons with a possible role in stress-related disorders. Here we report that variants in genes for galanin (*GAL*) and its receptors (*GALR1, GALR2, GALR3*), despite their disparate genomic loci, conferred increased risk of depression and anxiety in people who experienced childhood adversity or recent negative life events in a European white population cohort totaling 2,361 from Manchester, United Kingdom and Budapest, Hungary. Bayesian multivariate analysis revealed a greater relevance of galanin system genes in highly stressed subjects compared with subjects with moderate or low life stress. Using the same method, the effect of the galanin system genes was stronger than the effect of the well-studied *5-HTTLPR* polymorphism in the serotonin transporter gene (*SLC6A4*). Conventional multivariate analysis using general linear models demonstrated that interaction of galanin system genes with life stressors explained more variance (1.7%, P = 0.005) than the life stress-only model. This effect replicated in independent analysis of the Manchester and Budapest subpopulations, and in males and females. The results suggest that the galanin pathway plays an important role in the pathogenesis of depression in humans by increasing the vulnerability to early and recent psychosocial stress. Correcting abnormal galanin function in depression could prove to be a novel target for drug development. The findings further emphasize the importance of modeling environmental interaction in finding new genes for depression.

galanin receptors | mood disorders | network-based analysis | neurogenesis | transmitter coexistence

Major depressive disorder (MDD) is a common and serious disease afflicting more women than men, and a leading cause of disability worldwide, associated with much suffering and major costs for society (1, 2). Environmental psychosocial stressors are important in pathogenesis, because episodes are usually preceded by adverse life events, and early childhood experiences of physical and emotional abuse and parental neglect are important vulnerability factors (3, 4). Genetic vulnerability is significant with a heritability of about 35% (5). We remain ignorant about the brain processes that translate these genetic and environmental influences into depressive symptoms or risk. A major clue is that effective antidepressant drugs act directly or indirectly to enhance neurotransmission in serotonin (5-HT) and norepinephrine monoamine pathways, proving the monoamine hypothesis of depression (6–8). Many other candidate mechanisms have been identified in anatomical, pharmacological, and behavioral studies of stress in rodents. However, the demonstration of state- or trait-related abnormalities in human monoamine or other neural systems remains frustratingly elusive, despite modern brain-imaging methods. To determine whether the neuropeptide galanin has a role in depression, we used a unique Bayesian systems-based analysis to dissect out the influence of variation in genes for the peptide and its receptors on the interaction between different psychosocial stressors and risk of depression.

Current drug treatment of depression is far from satisfactory; the drugs target a limited range of monoamine mechanisms, they have an appreciable side-effect burden, and response is often partial (8, 9). In the search for better antidepressants, much attention has focused on neuropeptides and their receptors, the most diverse neurotransmitter system in the brain (8, 10–21), which includes galanin. As yet, however, there is no compelling evidence of efficacy of the neuropeptide approach or that particular peptides are involved in the pathogenesis of MDD.

Galanin, a 29-aa (30 in humans) peptide (22), is widely distributed in the rodent (23, 24) and human (25–27) brain. In rat it coexists with noradrenaline (NA) in the locus coeruleus (LC) and with 5-HT in the dorsal raphe complex (28). Like other peptide cotransmitters (29), it is released when neurons fire in high-frequency bursts in response to strong behavioral and pharmacological challenge (30–32). Galanin exerts its action via three cloned receptors, GALR1, GALR2, and GALR3 (33, 34) with a broad distribution in rat (35) and primate brain (26, 36). Animal behavioral studies (31, 32, 37–41) and a single study in humans (42) suggest that galanin has a role in stress, depression-like behavior, and anxiety. In addition, there is indication from

Significance

Early and recent environmental stressors, such as maltreatment in childhood, or stressful life events in adulthood, are important risk factors for depression. Nevertheless, not all people who suffer from these will be depressed. The resilience or vulnerability to these stressors, and thus depression, is likely to reside in our genes. In the present study, we used different statistical methods to demonstrate that variations in genes for galanin and its receptors increase the risk of depression only in heavily stress-exposed subjects. The work was predicated on the finding that galanin expression is strongly stimulated by stress in animal studies. In humans, variation in galanin function would appear to be important determinants of the outcome of psychosocial stress.

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previous genetic studies on humans that the galanin system is involved in psychiatric disorders including alcoholism/addiction (43–47), panic disorder (48, 49), and chronic pain-associated depression (50). Furthermore, recent functional studies provided the first evidence that polymorphisms in a highly conserved genetic region upstream from the *GAL* gene regulates *GAL* expression in brain areas, such as the amygdala and hypothalamus, implicated in the pathogenesis of depression (51, 52).

Genetic studies have the potential to identify molecular mechanisms of MDD vulnerability (53), but even mega- and meta-analyses of large genome-wide association studies (GWAS) have not identified genetic variants associated with MDD that survive genome-wide statistical correction (54, 55). Nominally significant associations will include many false-positives. Nevertheless it is noteworthy that SNPs in the gene for galanin (*GAL*) were among the top 10 genes whose variation was associated with MDD in a recent GWAS (55). One way to improve sensitivity is to take a system-based approach: if galanin is mechanistically involved in depression, genetic variation in the peptide and its receptors should exert similar influences, despite the fact the genes are located on entirely different chromosomes without linkage disequilibrium (LD) and with a low probability of randomly similar effects. Others have argued that improved sensitivity will come from deeper phenotyping (56) and characterization of environmental factors (3, 4, 57), because neither genetic nor environmental factors can be identified in isolation, if they modify each other's action to a high degree. Combining these two approaches, and in view of its preclinical properties, we predicted that variation in galanin genes would strongly interact with environmental stress in determining depression vulnerability. However, including more phenotypic and environmental variables exacerbates the problem of false-positives from multiple comparisons. Consequently, analyses of gene–environment interactions involving multiple phenotypes face a similar burden as GWAS in terms of correction for multiple testing. Furthermore, the conditional nature of such interactions frequently leads to separate analysis of multiple subpopulations (i.e., to even more statistical tests). To cope with multiple hypothesis testing, we applied a Bayesian systems-based approach both at structural and parametric levels, which allows multiple correlated outcomes. This approach supported the joint exploration of the underlying mechanism at genotype, haplotype, and diplotype levels in different depression-related phenotypes, and we validated the results by conventional multivariate analysis using independent subsamples.

Results

Genetic Association and Gene × Environment Interaction Analysis with Linear and Logistic Regression. Table 1 summarizes the demographic and phenotypic characteristic of the studied population. To show the genetic effects alone or in interaction with environmental factors, the effects of single SNPs (*SI Methods*, Figs. S1–S4, and Table S1) and their combination (haplotypes, HT) (Table S2) were studied. First, we carried out a traditional linear and logistic regression analysis using additive genotypic and diplotypic models for the selected variables. (For power calculations see *SI Methods* and Table S3.) Of the 12 SNPs studied, 7 statistically associated with one or more of the three clinical phenotypes (Fig. 1). Furthermore, all but one (*GAL* rs3136541) of the seven acted through interaction with either childhood adversity or recent life events. Two of the six *GALR1* SNPs interacted with recent life events (rs1893829, rs1162010) and two with childhood adversity (rs5375, rs11665337) to influence phenotypes. Three *GALR1* haplotypes (HT2:GAGTAG, HT6: GAGTGA, HT12:GGTCGG) interacted with childhood adversity and one with recent life events (HT10:AAGCAG). The single SNP representing *GALR2* (rs8836) interacted with life events, whereas a *GALR3* SNP (rs2285179) and the main haplotype (HT1:GA) interacted with childhood adversity. These

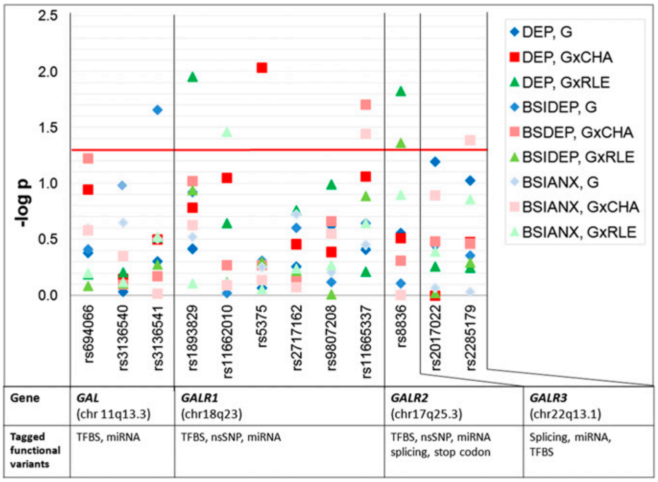

Fig. 1. Summary figure of the SNP association and SNP × environment interaction results. This figure shows the −log *P* values (vertical axis) of the genetic main effects (G), gene and childhood adversity interaction (G×CHA), and gene and recent negative life events interaction (G×RLE) analysis (additive genetic model in PLINK v1.07, http://pngu.mgh.harvard.edu/purcell/plink). The red line represents $P = 0.05$ nominal significance level; above that line significant results can be seen. Outcome variables were lifetime depression (DEP), current depression scores (BSIDEP), and current anxiety scores (BSIANX). Age and sex were covariate in all analysis. Horizontal axis lists the investigated SNPs, the genes and their chromosomal positions, and the tagged functional variants based on in silico functional analysis (see also Table S7). TFBS, transcription factor-binding site; splicing, SNPs that are located at 2 base pairs of intron–exon junction region; miRNA, miRNA binding site activity; nsSNP, SNPs in protein-coding regions that can cause amino acid change; stop codon, SNPs that may lead to premature termination of peptides (nonsense), which would disable the protein function.

nominally significant findings can be seen in Tables S4 and S5, which summarize all of the regression results. The results suggested to us that *GALR1* and probably *GALR3* modulate neurodevelopmental processes relevant to the effects of childhood adversity, whereas *GALR2* might modulate neuroplastic changes connected with stress responses to recent life events. Despite their interest and the corroboration that functionally related, genomically distant genes show similar gene-by-environment (G×E) interactions, these nominally significant effects did not survive Bonferroni correction for multiple testing. To reach an optimal correction for multiple-hypotheses testing concerning the numerous potential dependencies between multiple predictors and phenotypes, we applied a systems-based approach in the second phase using the Bayesian model averaging framework (58–60). This approach allowed the principled and detailed investigation of G×E interactions as model properties. The analysis consisted of a joint multivariate analysis of *GAL*, *GALR1*, *GALR2*, and *GALR3* genes on all three phenotypes—reported lifetime depression, current depression, and anxiety—both in the Bayesian and conventional (traditional regression) statistical framework.

Bayesian Network-Based Bayesian Multilevel Analysis of Relevance. Bayesian network-based Bayesian multilevel analysis (BN-BMLA) was carried out using a method that allows a detailed investigation of the relevance of factors with respect to multiple dependent variables such as phenotype descriptors (61). The resulting scores are posterior probabilities of relevance (Pr) ranging from 0 to 1. This method involves Bayesian model averaging over possible models reflecting relationships between variables, thus handling the multiple hypothesis testing problem optimally by taking into consideration the potential interdependencies of the predictors (for detailed description of the BN-BMLA method, see *SI Methods*).

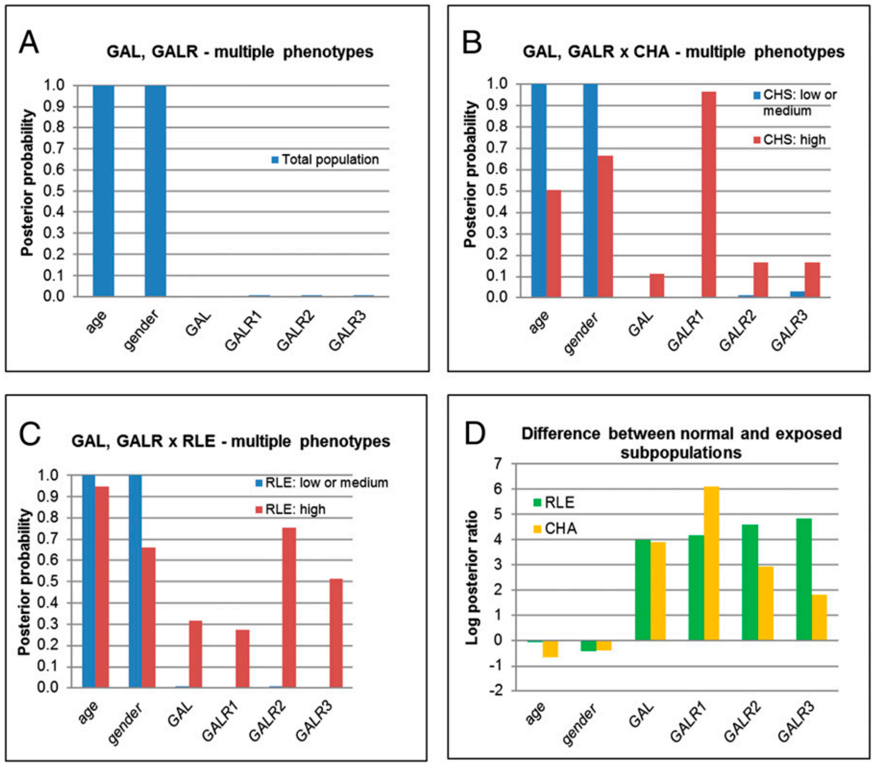

Fig. 2. Comparison of posterior probabilities of relevance for the total population and for subpopulations under the influence of different environmental factors. Subpopulations were created by dividing the original sample into two groups based on childhood adversity (CHA) and recent negative life events (RLE). The posteriors range from 0 to 1 and are estimated with respect to all three phenotypes (reported lifetime depression, current depression, and anxiety). A high posterior probability indicates that the corresponding factor is highly relevant. *GAL*, *GALR1*, and *GALR3* represent corresponding diplotypes, whereas *GALR2* denotes the single related SNP. Age and sex were included as cofactors. (*A*) The posterior probability of relevance of factors for the total population, not taking into account life stressors. Age and sex are highly relevant, but none of the genetic factors are relevant. (*B*) The posterior probability of relevance of factors for patients with low-medium CHA versus patients with high CHA. None of the genetic factors are relevant in the low-medium CHA group. In contrast, in case of patients with high CHA there is at least one highly relevant genetic factor, the *GALR1* with a high posterior probability (Pr = 0.96). Furthermore, the corresponding log posterior ratio is high (6.09), which means that there is a strong difference between the relevance of *GALR1* in the two subpopulations. (*C*) A comparison of the posterior probability of relevance of factors in case of patients with low or medium RLE versus patients with high RLE. In the case of the former subpopulation, none of the genetic factors are relevant, contrary to the high RLE group, where several factors are found to be relevant. The results indicate that *GALR2* is the most relevant factor in case of high RLE having a relatively high posterior probability for relevance (Pr = 0.75). *GALR3* has the second largest probability score, although it is only moderately relevant (Pr = 0.51). Furthermore, *GAL* and *GALR1* are even less relevant, and can be considered as weak results. (*D*) To compare the Bayesian posteriors across exposures we calculated log posterior ratios. The high (>3) log posterior ratios indicate in case of every genetic factor that there is a substantial difference in terms of posterior probability of relevance between those who experienced high life stresses and who did not. In contrast, the effects of age and sex factors do not differ substantially between those who experienced high life stresses and who did not.

In the total population, excluding life stressors, the galanin pathway genes showed minimal relevance (Fig. 2*A*). This finding is supported by the moderate/weak genetic main effects in the initial regression analysis. Next, we performed separate analyses in subpopulations defined by childhood adversity categories: low or medium (0–6) versus high (≥7) on the short version of the Childhood Trauma Questionnaire or by the number of recent negative life events: low or medium (0–2) versus high (≥3). In people with exposure to high childhood adversity, the *GALR1* diplotypes were highly relevant (Pr = 0.96) with respect to multiple phenotypes, but it was nonrelevant (Pr = 0.002) in the low/medium childhood adversity group (Fig. 2*B*). To compare the Bayesian posteriors across exposures, we calculated log posterior ratios. The striking magnitude of the difference is confirmed by the sixfold log posterior ratio. In contrast, *GALR1* showed little relevance to the effect of exposure to recent negative life events; the *GALR1* diplotypes had a relatively low posterior probability (Pr = 0.27) in the high negative life-events group, and a negligible posterior probability (Pr = 0.004) in those with low/medium exposure. (Fig. 2*C*). The single SNP rs8836 related to *GALR2* had high relevance (Pr = 0.75) to multiple phenotypes in the high negative life-event group but had no relevance in the low/medium life-events group. This substantial difference was also indicated by the high log posterior ratio of 4 (Fig. 2*D*). Although the other galanin pathway genes have only moderate or low probability of relevance in the high life-stressor groups, the log posterior ratios (>3) indicate that for each genetic factor there is a substantial difference in terms of posterior probability of relevance between those who were highly exposed to environmental life stressors and those who were not (Fig. 2*D* and Table S6).

As an interesting comparison, the same Bayesian analysis of relevance as here used for the galanin system was carried out in the present cohorts for the well-known *5-HTTLPR* polymorphism. Note that from the statistical point of view this comparison can be seen as a benchmark and from the systems biological point of view as a comparison with an experimentally validated reference. Our results show that the *5-HTTLPR* polymorphism is moderately relevant (Pr = 0.55, log posterior ratio 4.56) in those who experienced a high level of recent negative life events, and minimally relevant in those who experienced a high level of childhood adversities (Pr = 0.04; log posterior ratio 1.67) (Fig. S5).

In addition, further testing the relevance of the *5-HTTLPR* and the galanin system genes in one model in those who experienced high level of recent negative life events, the relevance of the galanin system genes remained stable, whereas the relevance of the *5-HTTLPR* modestly decreased (from Pr = 0.55 to Pr = 0.34). This result suggests that the effect of the *5-HTTLPR* may be partially mediated by the galanin system but not vice versa. These results corroborate previous findings and suggest that the galanin system probably has similar or stronger effect on stress-induced depressive symptoms compared with the *5-HTTLPR* functional polymorphism.

Galanin Pathway Level Analysis. To assess the overall contribution of galanin genes to variation in risk of our depression-related phenotypes, two general linear models were constructed: a "Reduced" model containing only environmental factors (childhood adversity and recent negative life events), and a "Full" model containing environmental factors, genetic factors (*GAL, GALR1, GALR2, GALR3*), and their interactions. Table 2 shows residual variances for the phenotypes, namely reported lifetime depression, current depression, and anxiety separately, and also for the multivariate case (i.e., combining the variance across all phenotypes). The results indicate that the Full model explains more variance, resulting in less residual or unexplained variance in every case than the life stress-only model. In the overall multivariate case the difference is 0.017, which means that the investigated genetic variants and their interactions with life stressors contribute 1.7% to the total variance. In our study, the difference between the Full and Reduced models in the multivariate comparison was significant ($F = 1.838$, $F_{critical} = 1.759$, $\alpha < 0.005$). This effect was significant separately for the population recruited in Budapest ($F = 1.632$, $F_{critical} = 1.452$, $\alpha < 0.05$) and in Manchester ($F = 1.531$, $F_{critical} = 1.448$, $\alpha < 0.05$), and in the combined sample separately both in males ($F = 1.645$, $F_{critical} = 1.459$, $\alpha < 0.05$) and in females ($F = 2.108$, $F_{critical} = 2.039$, $\alpha < 0.0005$) with similar magnitude of effect size (3.9% vs. 3%, respectively). Conducting the comparison of the models for the phenotypes individually in the combined sample showed that the difference between the models was most significant in case of the current depression phenotype ($F = 2.174$, $F_{critical} = 2.031$, $\alpha < 0.0005$).

In Silico Functional Analysis and Comparison with Psychiatric Genetic Consortium GWAS Results. Finally, in silico functional prediction was carried out using the SNP Function Prediction (FuncPred) tool (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm). This process revealed that two of our investigated SNPs have functional effects. Namely, rs11662010 (near to the 5′ end of the *GALR1* gene) modifies a transcription factor binding site, and rs8836 (downstream to the *GALR2* gene in strong LD with it) has miRNA binding activity. In addition, our 12 haplotype tag SNPs captured an additional 23 potentially functional variants within the galanin system (Fig. 1), suggesting that the genetic regions covered by our haplotype-tagging SNPs have functional consequences on the gene transcription and translation, thus may reflect real functional differences (Table S7). In addition, the Psychiatric Genetic Consortium's latest mega-analysis showed several nominally significant associations and trends between MDD and the *GAL*, and *GALR1* genes (Table S7), further supporting our results.

Discussion

Galanin is, as revealed in animal experiments, a highly "dynamic" neuropeptide, frequently showing a robust up-regulation of expression in response to stress, both under physiological and extreme conditions. We tested the hypothesis that the genetic effects of the galanin system in the development of depression and anxiety would be greatest in those exposed to the most life stress. In the present study, genetic variants of *GALR1* significantly interacted with childhood adversity, suggesting it also has a role in neuronal damage and wiring during neuronal development. The interaction of *GALR1* SNPs and childhood adversity in the regression analysis was confirmed by the Bayesian multivariate analysis of relevance. Moreover, *GALR2* rs8836 significantly moderated the effect of recent negative life events, also confirmed by the Bayesian analysis. In addition, *GALR3* showed a moderate relevance in interaction with recent negative life events in our study. Finally, high log posterior ratios indicated that *GAL* gene effect was more relevant in the highly stressed population compared with the low or moderately stressed subjects. These results indicate that the galanin pathway has a role in the development of depression in humans but only in persons exposed to high levels of childhood adversity or recent

Table 1. Demographic and phenotypic characteristic of the sample

Demographics	Combined	Budapest	Manchester
Sex			
Female	1,641 (70%)	702 (69%)	939 (70%)
Male	720 (30%)	313 (31%)	407 (30%)
Age (mean ± SEM)	32.8 ± 0.2	31.1 ± 0.3	34.0 ± 0.3
Personal psychiatric history			
Reported depression	974 (41%)	217 (21%)	757 (56%)
Recurrent episodes	690 (71%)	118 (54%)	572 (76%)
Ever treated with antidepressant	637 (65%)	70 (32%)	567 (75%)
Reported suicide attempt	285 (12%)	48 (5%)	237 (18%)
Reported anxiety disorder	641 (27%)	202 (20%)	439 (33%)
Reported substance use disorder	130 (6%)	24 (2%)	106 (8%)
Family psychiatric history			
Reported depression in immediate blood relatives	632 (27%)	135 (13%)	497(37%)
Symptom scores (range 0–4)			
BSI depression (mean ± SEM)	0.85 ± 0.02	0.56 ± 0.02	1.08 ± 0.03
BSI anxiety (mean ± SEM)	0.88 ± 0.02	0.69 ± 0.02	1.02 ± 0.03
Adversities			
Recent negative life events (mean ± SEM)	1.22 ± 0.03	1.08 ± 0.04	1.3 ± 0.04
Childhood adversity (mean ± SEM)	3.3 ± 0.07	2.8 ± 0.09	3.7 ± 0.1

BSI, Brief Symptom Inventory.

negative life events, and that the different receptors have different roles in mediating the effects of different stressors.

The paradigmatic example of a candidate gene interacting with recent negative life events and childhood adversity is the serotonin transporter gene (*SLC6A4*). This gene has a functional polymorphism in the promoter region (*5-HTTLPR*) (62), whose risk variant is associated with a 50% reduction in serotonin transporter protein and predisposition to depression after negative life events (63–65), although there are negative studies. In our study we used this gene as a benchmark and reference for the Bayesian analysis to allow the comparison of posterior probabilities of relevance. The results of Bayesian analysis supported the relevance of *5-HTTLPR* in stress-related depression, but the galanin system had a stronger effect. Indeed, the investigated genetic variants in the galanin pathway and their interactions with life stressors explained 1.7% of the total variance in the depression-related phenotypes. This is a large proportion in comparison with the 0.6% explained variance by the whole-genome polygenic risk score seen in a recent GWAS mega-analysis for MDD (54). According to the Psychiatric Genetic Consortium suggestion, at least 100,000 MDD cases (plus controls) would be required to achieve GWAS-significant findings for MDD (54). However, our results further emphasize that using subjects with high life stresses, because MDD is a stress-related disorder, could potentially decrease the required number of cases to 5,500–35,000 (Table S3). According to the differential sensitivity hypothesis (57), some risk genotype-by-stress interactions also involve increased sensitivity to beneficial environments, such as social supports.

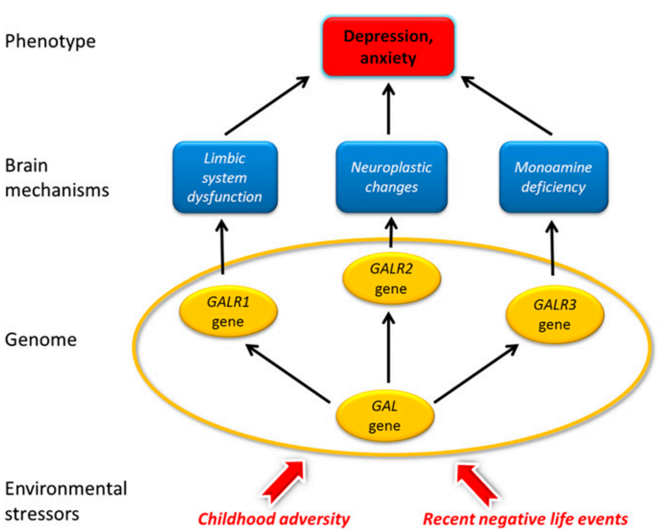

Fig. 3. Galanin mechanisms hypothetically involved in MDD in humans. Galanin, a neuropeptide, and its receptors are colocalized in some monoaminergic neurons in the brain. The galanin system is highly sensitive to experimental and naturalistic stressors. Stress-induced activation of the galanin system represents the first phase in the development of depression. Recent analysis of human brain has shown that the Gi protein-coupled GALR3 (and not GALR1 as in rodents) is the main galanin receptor in NA-LC and probably 5-HT dorsal raphe nucleus cells, and that the Gi protein-coupled GALR1 is the main receptor in the forebrain. Antidepressive effects may be achieved by (*i*) GALR3 antagonists (71), by reinstating normal monoamine turnover in the brainstem, and by (*ii*) GALR1 antagonists in the forebrain by normalization of limbic system activity, or by (*iii*) agonists at GALR2, a Gq protein-coupled receptor, promoting neuroprotection. The present genetic analysis suggests that GALR1 risk variants may compromise galanin signaling during childhood, whereas GALR2 signaling may be influenced by recent negative life events. In addition, all four galanin system genes have relevant roles in the development of depression-related phenotypes in those persons who were highly exposed to life stressors.

However, no protective effects of galanin-related genotypes were seen in the low-stress groups in our study.

Potential mechanisms that may explain the galanin system effect in the development of depression are summarized in Fig. 3. The *GAL* gene is widely expressed in the human brain [e.g., LC, forebrain, amygdala, and hypothalamus (26, 51)], but its involvement in the development of depression is not well understood. Although previous studies indicated that it might have a sex- or estrogen-dependent effect (49), in our study the galanin system genetic variants significantly influenced the depression-related phenotypes both in males and in females, with similar magnitude of effect size, providing evidence that the excessive stress effect is not mediated by sex.

The monoamine neurotransmitters, NA, 5-HT, and dopamine, have been implicated in the mechanism of action of antidepressants and thus the pathogenesis of MDD for more than half a century, and also shown to interact in intricate ways in the development and treatment of this serious disease (6, 7, 66). Some of the effects of galanin may fit into this framework. Involvement of the galanin system in regulation of mood-related behavior in animals has focused on several brain sites, via different mechanisms. For example, in rat galanin may have a prodepressive role via modulating 5-HT_{1A} receptors in the forebrain (37, 67) or, when released from soma and dendrites in the LC, via inhibitory GALR1 autoreceptors (68, 69). The same receptor mediates inhibition of pyramidal neurons in the ventral hippocampus (70). Thus, galanin may cooperate with its cotransmitter norepinephrine, both at the LC cell body autoreceptor level and postsynaptically in the hippocampus. However, it is important to note that recent studies demonstrated that in humans GALR3 receptors are more prevalent in the brainstem compared with GALR1, whereas GALR1 is widely expressed in the human forebrain (26). In the 5-HT neuron-rich rat dorsal raphe nucleus/periaqueductal gray, Lu et al. (39) have suggested that mood is controlled through a balance between signaling via prodepressive GALR1/3 (71, 72) and antidepressive GALR2 receptors (38, 39). In the ventral tegmental area galanin inhibits dopamine neurons, inducing depression-like behavior (40).

Accumulating evidence suggests that hippocampal atrophy and loss of dendritic spine synapses are associated with depressive symptoms (73–75), whereas recovery of MDD patients involves normalization of the hippocampal volume (76), possibly related to enhancement of functional synapses (77–79). Interestingly, galanin has been reported to act as a neuroprotective factor for hippocampal neurons (80–82) via GalR2 (83). Moreover, it is now established that adult neural stem/progenitor cells generate new neurons in, for example, the hippocampal granule cell layer (84, 85). Subsequently, the proliferation and integration of neuronal stem cells in this brain region have emerged as a focus in attempts to understand mechanisms underlying stress, depression, and the effects of antidepressants (86–88). In the hippocampus, galanin's trophic and proliferative effects via GALR2/3 receptors, on neuronal stem cells in the subgranular zone in the dentate gyrus (89–91), may be involved and could mediate some of the effects of genetic variation that we have observed. The latter idea has gained more weight in view of the recent report that, in humans, a large subpopulation of hippocampal neurons, constituting one-third of the neurons, is subject to exchange (92), substantiating the first report of adult neurogenesis in humans (93). Thus, in adults 700 neurons are added in the hippocampus each day, and around one-third of the hippocampal neurons constantly renew, involving most neurons in the dentate gyrus (92). Interestingly, galanin was more abundant in mouse embryonic stem cells compared with any other examined tissues (94), and in human stem cells galanin was in the top 50 overexpressed genes (95). Furthermore, galanin receptors can also act through cAMP formation (96), and thus the cyclic AMP-responsive element binding (CREB) signaling pathway (97–99), which is an important modulator of the brain-derived neurotrophic factor (BDNF) production. BDNF mediates activity-dependent neuroplasticity in the hippocampus and cortex, which is critical to the adaptation of environmental stress and also contribute to antidepressant effects (77, 100, 101). It is interesting to note that our previous study demonstrated that genetic variation in the *CREB1-BDNF-NTRK2* pathway also interacts with childhood adversity to increase risk of depression (102).

There are some limitations of our study. For example, we used self-reported questionnaires to measure lifetime depression, depressive and anxiety symptoms, and negative life events that, although proven and widely used, might be influenced by recall bias. Therefore, we validated them in a subpopulation of 142 during face-to-face interviews showing good reliability (102, 103). In addition, we did not control for the timing of depression and life events. It has been demonstrated that childhood adversity has a long-term effect on the pathogenesis of depression (104), and the questionnaire we used to measure recent (last year) negative life events builds on items with long-term contextual threat (105). Finally, our nominally significant G×E interaction results did not survive traditional correction for multiple testing, which was expected in case of weak genetic effects. However, our Bayesian network-based approach accommodates multiple interdependent outcome variables and predictors (i.e., system genes and life stresses), minimizes the loss of power, and quantitatively characterizes the dependency structure of galanin G×E interactions. Results were also confirmed by conventional multivariate analysis using general linear models and comparatively evaluated against the *5-HTTLPR* as reference. Thus, development of probabilistic graphical model-based methods using Bayesian statistical framework may be essential for detecting G×E interactions in modestly heritable disorders.

In conclusion, the present results indicate that the galanin system plays a significant role in the pathogenesis of depression, almost entirely by modulating the vulnerability to early and recent psychosocial stress. The results validate the galanin system as an illness-related target for novel antidepressant drug development. In addition, our results support suggestions that G×E interactions may significantly contribute to the "missing heritability" in genome-wide case-control studies that lack environmental measures because of their large scale.

Methods

Population. Population cohorts were recruited in Budapest, Hungary and Manchester, United Kingdom in the European Union-funded NewMood study (New Molecules in Mood Disorders, Sixth Framework Program of the European Union, LSHM-CT-2004-503474) using harmonized phenotyping and genotyping methods that enabled us to carry out a mega-analysis. From the recruited n = 2,588 subjects, n = 2,361 (n = 1,015 from Budapest and n = 1,346 from Manchester) were eligible for this study who filled out the questionnaires, provided DNA, which was successfully genotyped for the galanin pathway, and have European White ethnic origin. Data of all eligible participants were included in the analysis, regardless of reported psychiatric disorders (Table 1). Details of the recruitment strategy and the population cohorts can be read in previous publications (64, 102, 103). In short, we recruited participants aged between 18–60 y from Greater Manchester, United Kingdom through general practices, advertisements, and a Web site, and from Budapest, Hungary, through general practices and advertisements. Participants returning the signed consent form and the questionnaire were then sent a genetic sampling kit, which they returned. Both studies were approved by the local ethics committees and were carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Phenotypic Assessment. Three stress-related phenotypic outcome variables were analyzed. Reported lifetime depression was derived from targeted questions of a self-reported questionnaire and was validated in a subpopulation during face-to-face diagnostic interviews (102). To measure current depression and anxiety we used the Brief Symptom Inventory (106) anxiety and depression subscales with additional items for depression. A short version of the Childhood Trauma Questionnaire (107) assessed the experience of emotional and physical abuse and neglect in childhood, as validated in a previous study (102). Recent stressors were assessed using a validated measure of negative life events covering intimate relationships, financial difficulties, illnesses/injuries, and social network problems (105). Further details of the phenotypic measures can be seen in *SI Methods*.

Table 2. Residual variances for the full models and the reduced models

	Variance			
Models	Reported lifetime depression	Current depression	Current anxiety	Multivariate
Total sample				
Reduced	0.215	0.727	0.710	0.551
Full	0.210	0.701	0.692	0.534
Explained variance	0.6%	2.7%*	1.8%†	1.7%‡
Budapest				
Reduced	0.154	0.418	0.464	0.345
Full	0.147	0.387	0.440	0.325
Explained variance	0.7%	3.1%‡	2.4%	2.1%†
Manchester				
Reduced	0.225	0.888	0.882	0.665
Full	0.216	0.844	0.843	0.634
Explained variance	0.9%	4.4%†	4.0%†	3.1%†
Total males				
Reduced	0.196	0.554	0.501	0.417
Full	0.184	0.501	0.450	0.378
Explained variance	1.2%	5.2%†	5.1%‡	3.9%†
Total females				
Reduced	0.227	0.803	0.798	0.609
Full	0.220	0.759	0.759	0.579
Explained variance	0.7%	4.4%*	3.9%*	3.0%*

*Significant difference in explained variance $P < 0.001$.
†Significant difference in explained variance $0.01 < P \leq 0.05$.
‡Significant difference in explained variance $0.001 \leq P \leq 0.01$.

Genetic Data. Genetic samples (buccal mucosa cells) were collected according to a validated method (108). Because there are no known functional polymorphisms within this pathway we used haplotype tagging method (www.broad.mit.edu/personal/jcbarret/haploview) to represent the selected genes and scientific literature to identify previously investigated SNPs. Our haplotype-tagged SNPs capture genetic regions that tend to inherit together (LD $r^2 > 0.8$) in populations with European ancestry [based on the Centre d'Etude du Polymorphisme Humain population data of the International HapMap Project (www.hapmap.org) Phase I. June 2005]. The selected 12 SNPs (Figs. S1–S4 and Table S1) were genotyped with the Sequenom's MassARRAY technology (Sequenom, www.sequenom.com). Genotyping was blinded with regard to phenotype and was performed under the ISO 9001:2000 requirements.

Statistical Analysis. PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink) was used to test additive genetic association using linear and logistic regression models, G×E interactions, to impute haplotypes (Table S2), and to calculate Hardy–Weinberg equilibrium *P* values. Bayesian and non-Bayesian multivariate analyses were performed to assess the joint effect of *GAL*, *GALR1*, *GALR2*, and *GALR3* on all three phenotypes (reported lifetime depression, current depression, and anxiety). Non-Bayesian statistical analyses were performed with SPSS 21.0 for Windows (IBM). Age and sex were covariates in all analyses. All statistical testing used two-tailed $P = 0.05$ threshold. For detailed description of the statistical methods and for power calculations, see *SI Methods*. First, we carried out statistical analysis in the total sample because of the moderate sample size, and then replicated our main findings in subpopulations according to study sites (Budapest and Manchester) and sex (female and male).

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