Stress profoundly affects physiological properties of neurons across brain circuits and thereby increases the risk for depression. However, the molecular and cellular mechanisms mediating these effects are poorly understood. In this study, we report that chronic physical restraint stress in mice decreases excitability specifically in layer 2/3 of pyramidal neurons within the prefrontal subarea of the prefrontal cortex (PFC) accompanied by the induction of depressive-like behavioral states. We found that a complex between G protein–coupled receptor (GPCR) 158 (GPR158) and regulator of G protein signaling 7 (RGS7), a regulatory GPCR signaling node recently discovered to be a key modulator of affective behaviors, plays a key role in controlling stress-induced changes in excitability in this neuronal population. Deletion of GPR158 or RGS7 enhanced excitability of layer 2/3 PFC neurons and prevented the impact of stress. Investigation of the underlying molecular mechanisms revealed that the A-type potassium channel Kv4.2 subunit is a molecular target of the GPR158–RGS7 complex. We further report that GPR158 physically associates with Kv4.2 channel and promotes its function by suppressing inhibitory modulation by cAMP–protein kinase A (PKA)–mediated phosphorylation. Taken together, our observations reveal a critical mechanism that adjusts neuronal excitability in L2/3 pyramidal neurons of the PFC and may thereby modulate the effects of stress on depression.

Maladaptive chronic stress impairs appropriate neuronal response to incoming stimuli, resulting in pathologic behaviors such as depression. At the cellular level, it involves abnormal changes in structural and functional properties of the neurons in key brain circuits that control mood. For instance, chronic stress significantly reduces the length of apical dendrites and the density of dendritic spines in the prelimbic subregion of the medial prefrontal cortex (mPFC)\(^3\) (1–3). In addition, chronic stress alters electrophysiological properties of the neurons in prefrontal cortex (4), amygdala (5), and nucleus accumbens (6).

Studies in animal models indicate that one of the key physiological parameters impacted by stress is the hypoexcitability of mPFC neurons (7–9). Consistent with these observations, clinically depressed humans also feature a prominent reduction in neuronal activity within dorsolateral PFC (10–12), a region functionally corresponding to rodent mPFC (13, 14). Conversely, optogenetic stimulation (10) or pharmacological activation (15) of mPFC neurons in mice exerted a potent antidepressant-like effect, suggesting that increasing the excitability of mPFC neurons has positive effects on mood and might be exploited for the treatment of depression. Evidence suggests that layer 2/3 (L2/3) neurons of mPFC are particularly vulnerable to stress-induced depression. For instance, induction of the transcription factor ΔFosB in L2/3 of mPFC mediates stress resilience and antidepressant responses (16–18), and deletion of Wfs1 gene from these neurons impairs the ability of mPFC to suppress stress-induced depressive behaviors (19).

There has been significant progress in understanding the molecular factors and mediators of physiological changes impacting depression-related behaviors acting in various brain regions. A significant role has been shown for the second messenger cAMP. Notably, low cAMP levels have been detected in post-mortem brains of depressed suicide victims (20, 21). In contrast, most effective antidepressants are known to enhance cAMP signaling (22–24). Furthermore, chemogenetic elevation of cAMP levels by designer receptors exclusively activated by designer drugs (DREADDs) can reverse depressive-like behaviors induced by stress (25). In addition to cAMP, inhibitory K\(^+\)-conducting channels have also been implicated in the process with significant diversity of types and brain regions involved. It has been shown that prolonged social isolation induces anxiety- and anhedonia-like symptoms, with reduced intrinsic excitability mediated by inward rectifier K\(^+\) and A-type potassium channels (6). Chronic mild stress was also shown to increase the expression of K-ATP channel subunits Kir6.1 and Kir6.2 (26) as well as voltage-gated channel Kv2.1 (27). However, molecular mechanisms and signaling reactions...
GPR158 and RGS7 regulate mPFC excitability

that underlie changes in the excitability of layer 2/3 PFC neurons in depressive states are unknown.

G protein–coupled receptors (GPCRs) mediate the effects of neuromodulators and play key roles in controlling neural activity and excitability (28, 29). In fact, their actions are critically involved in mood regulation, and several GPCRs have been directly and indirectly targeted by existing mainstream and emerging antidepressant medications (30–32). However, current GPCR interventions are limited in efficacy, requiring prolonged administration to exert effects, with nearly half of patients being refractory to the treatment. In this light, significant attention has been focused on poorly explored “orphan” GPCRs for their promise as drug targets for developing more efficacious and precise treatments for depression (33, 34).

We have recently reported the links of the orphan receptor GPR158 to depression. The levels of GPR158 are significantly elevated in human subjects with major depressive disorder and in chronically stressed mice, whereas knockout of GPR158 in mice produces an antidepressant phenotype and stress resilience (35). Interestingly, GPR158 is enriched in L2/3 neurons of mPFC where it is involved in controlling cAMP via an unusual mechanism involving an association with the negative regulator of GPCR signaling, regulator of G protein signaling 7 (RGS7) (35–37). Despite this intriguing initial discovery, the downstream effectors of GPR158 and its role in modulating neuronal activity are not well-understood.

In this study, we report that GPR158 specifically regulates the intrinsic excitability of layer 2/3 pyramidal neurons of mPFC and that this regulation involves its association with RGS7. We further identified Kv4.2 as a major effector regulated by the GPR158–RGS7 complex, physically associated with each other to control A-type K+ currents in layer 2/3 PFC neurons via a cAMP-dependent mechanism. This establishes a key downstream signaling mechanism by which the antidepressant target GPR158 modulates neuronal function.

**Results**

**Induction of depressive-like behaviors by stress is accompanied by selective reduction in intrinsic excitability of L2/3 prelimbic neurons**

We began our studies by examining the effects of stress on the intrinsic excitability of L2/3 neurons in the prefrontal area of the mPFC. Following physical restraint stress (PRS), the depression-like behavior of mice was evaluated in a forced swim test (FST), and whole-cell patch-clamp recordings were obtained from the L2/3 pyramidal neurons (Fig. 1, A and B). The cell identity was confirmed by action potential (AP) properties and input–output relationship (38, 39). Analysis of the behavioral data indicated that mice subjected to PRS displayed significantly increased immobility during the FST compared with control mice (t(6) = 6.97, p = 0.0004), demonstrating a depressant-like effect (Fig. 1C). Whole-cell recordings revealed a significant (t(22) = 2.17, p = 0.041) hyperpolarization of the resting membrane potential (RMP) in mice subjected to PRS relative to control subjects (Fig. 1D). Interestingly, the excitability of L2/3 pyramidal neurons was also significantly reduced in PRS-exposed mice, as evidenced by fewer action potentials in response to depolarizing current steps compared with control neurons, when the cells were held at their resting potentials (Fig. 1, E and F). A repeated-measures analysis of variance (ANOVA) revealed significant main effects of group (F(1,22) = 5.55, p = 0.028) and current intensity (F(1,4, 30.5) = 223, p < 0.001; Greenhouse–Geisser-corrected). There was also a significant interaction of group with current density (F(1,4, 30.5) = 3.88, p = 0.045; Greenhouse–Geisser-corrected). Post hoc analysis using t test revealed a significant group effect when depolarizing current steps were ≥200 pA (all values, p < 0.05).

The observed modulation of intrinsic excitability by stress was specific to L2/3 neurons (Fig. 1G), as the excitability of layer 5 (L5) neurons obtained from stressed animals was comparable with those from control unstressed mice (F(1,14) = 0.57, p = 0.46 for main effect of group and F(2, 112) = 1.6, p = 0.22 for interaction; repeated-measures ANOVA). Taken together, these data suggest that chronic stress induces specific reduction in excitability of L2/3 pyramidal neurons in prefrontal cortex in parallel with development of depressive-like behaviors.

**Stress-induced changes in excitability of L2/3 neurons of mPFC are mediated by GPR158–RGS7 complex**

In search for the molecular mechanisms underlying changes in intrinsic excitability, we focused on probing the involvement of a GPR158–RGS7 complex, which is prominently enriched in L2/3 neurons as knockout of either GPR158 or RGS7 results in a marked antidepressant phenotype and resilience to stress (35). To exclude the impact of changes in RMP, in the following experiments all cells were held at ~70 mV unless noted otherwise. Indeed, elimination of GPR158 or RGS7 significantly increased the intrinsic excitability of layer L2/3 mPFC neurons (Fig. 2). Specifically, Gpr158−/− or Rgs7−/− neurons fired more spikes as compared with littermate control Gpr158+/+ neurons (Fig. 2A). A repeated-measures ANOVA revealed significant main effects of group (F(1,23) = 8.77, p = 0.007) and current intensity (F(1,4, 31.7) = 540, p < 0.001; Greenhouse–Geisser-corrected). There was also a significant interaction of group by current intensity (F(1,4, 31.7) = 7.6, p = 0.005; Greenhouse–Geisser-corrected). Post hoc analysis revealed a significant group effect when depolarizing current steps were ≥200 pA (all values, p < 0.01; t test). In addition to enhanced excitability, knockout of GPR158 also induced a significant depolarization of RMP (t(27) = 2.42, p = 0.022), increase in input resistance (t(27) = 2.25, p = 0.033), decrease in rheobase current (t(24) = 2.43, p = 0.023), and decrease in delay to the first spike evoked by depolarizing current injection (t(23) = 2.99, p = 0.007; Table 1). The effect of RGS7 ablation was quantitatively similar to that of GPR158 loss. Layer 2/3 mPFC neurons of Rgs7−/− also fired more action potentials relative to Rgs7+/+ neurons in response to depolarizing current injections, whereas chronic stress did not significantly change the excitability of Rgs7−/− neurons (Fig. 2B). A repeated-measures ANOVA revealed significant main effects of group (F(2,40) = 4.43, p = 0.018) and current intensity (F(1.5,61.8) = 580.0, p < 0.001; Greenhouse–Geisser-corrected), but not interaction of group by current intensity (F(3.1,61.8) = 2.5, p = 0.066; Greenhouse–Geisser-corrected). Post hoc analysis revealed that the excitability of Rgs7+/+ neurons was significantly different from Rgs7−/− neurons either...
Table 1

Table 2

**Figure 1. Chronic stress selectively decreases intrinsic excitability of L2/3 prelimbic neurons in mPFC.** A, experimental design. Mice were either subjected to physical restraint stress for 2 weeks or maintained in their home cages (control group) before behavioral testing. All mice were euthanized, and brain slices were prepared within 1 h following the behavioral evaluation. B, schematic diagram of a PFC brain slice and the location of L2/3 pyramidal neurons recorded in this study. Inset, a fluorescence microscopy image of representative L2/3 pyramidal cells filled with biocytin during recording and visualized with Alexa Fluor 488. Scale bar, 50 μm. C, effect of chronic stress on immobility during the FST. Compared with control, stressed mice spent significantly more time immobile during the FST (**, p < 0.01 control versus stressed mice; Student’s t test). D, effect of chronic stress on the RMP of L2/3 pyramidal neurons in prelimbic cortex (n = 13 and n = 11 for control and stress, respectively). E and F, representative traces and average data showing the effect of stress on the excitability of L2/3 pyramidal neurons within mPFC (*, p < 0.05 control (n = 13) versus stressed (n = 11) neurons; repeated-measures ANOVA followed by Student’s t test for each current step). G, effect of chronic stress on the excitability of L5 pyramidal cells in prelimbic cortex (n = 9 and n = 7 for control and stress, respectively). Error bars represent S.E.M. values.

with (p = 0.024) or without (p = 0.007) chronic stress. Furthermore, ablation of RGS7 phenocopied the effects seen upon GPR158 loss, including depolarization of RMP (t(27) = 2.41, p = 0.028), increase in input resistance (t(27) = 13.9, p = 0.002), decrease in rheobase current (t(17) = 2.33, p = 0.033), and decrease in the delay to first spike evoked by depolarizing current injection (t(17) = 2.38, p = 0.029; Table 1). To test molecular specificity of this effect, we next probed the involvement of R7BP that also prominently regulates RGS7 but does not have an impact on depression-related behaviors (35). We found that elimination of R7BP did not change the intrinsic excitability of L2/3 neurons (Fig. 2C). Furthermore, the observed changes following Gpr158 or Rgs7 deletion were specific to L2/3 neurons, as no genotype differences were observed in excitability of L5 neurons, with or without stress (Fig. 2D, E, and F, and Table 2). Together, these data suggest that the GPR158–RGS7 complex specifically modulates intrinsic excitability of layer 2/3 neurons in PFC in response to stress.

**GPR158–RGS7 complex exerts its effects on excitability via cAMP**

The GPR158–RGS7 complex has been shown to modulate signaling via the second messenger cAMP (37). Thus, we next tested whether the effects of GPR158 and RGS7 on excitability involve changes in cAMP. We found that in WT neurons elevating cAMP by adding the nonhydrolyzable analog S_cAMP into the internal recording solution significantly increased intrinsic excitability of layer 2/3 mPFC neurons, mimicking the effect of GPR158 or RGS7 ablation (Fig. 3A and B; repeated-measures ANOVA; F(1,28) = 11.18, p = 0.002). Importantly, elimination of GPR158 completely prevented the effects of S_cAMP, which was not observed in Gpr158−/− neurons (Fig. 3C and D; repeated-measures ANOVA; F(1,6) = 0.13, p = 0.73). Similarly, S_cAMP failed to alter the excitability in neurons lacking RGS7 (Fig. 3E and F; repeated-measures ANOVA; F(1,13) = 1.2, p = 0.29). These data indicate that GPR158 and RGS7 modulate intrinsic excitability of L2/3 prelimbic neurons through regulating intracellular cAMP.

**GPR158 and RGS7 modulate A-type potassium channels**

The decrease in RMP amid increase in input resistance upon deletion of GPR158 and RGS7 suggests the likely closing of inhibitory ion channels in these neurons as a mechanism for increasing excitability. To establish the identity of such channels, we investigated barium-sensitive potassium current, which includes both inward and outward currents and greatly...
affects membrane properties and excitability (40–42). We found that deletion of GPR158 or RGS7 did not significantly change inward currents when the membrane potential was stepped to a range from −90 to −120 mV (Fig. 4, A and B). Instead, the outward current was significantly inhibited in the range from −70 to −50 mV (main effects of voltage when tested with repeated-measures ANOVA, $F(1,18) = 9.27, p = 0.007$ and $F(1,15) = 7.10, p = 0.018$ for Gpr158 and Rgs7, respectively). This difference was also significant when we normalized the barium-sensitive current to a fixed potential at −80 mV (Fig. 4C; $t(18) = 3.5, p = 0.002$ and $t(15) = 3.0, p = 0.009$ for Gpr158 and Rgs7, respectively). These observations suggest the involvement of A-type potassium channels, whose genetic deletion or pharmacological inhibition has been shown to produce effects on depolarizing RMP, input resistant, and firing frequency as well as delaying the first spike in response to depolarizing current injection (43–45), which are similar to changes seen in Gpr158−/− and Rgs7−/− neurons. To test this hypothesis, we directly measured $I_A$ in brain slices using an established protocol (Fig. 5A) (46). Indeed, we observed that loss of GPR158 and RGS7 regulate mPFC excitability

Figure 2. Intrinsic excitability of L2/3 of mPFC neurons is specifically regulated by the GPR158 –RGS7 complex. A, representative traces and summarized graph showing the effect of genetic knockout of Gpr158 on the excitability of prelimbic L2/3 pyramidal neurons (**, $p < 0.01$ between genotypes; repeated-measures ANOVA followed by Student’s t test; $n = 12$ and $n = 13$ for Gpr158−/− and Gpr158+/+ neurons, respectively). B, representative traces and summary graph showing the effect of genetic knockout of Rgs7 on the excitability of prelimbic L2/3 pyramidal neurons (**, $p < 0.01$ control Rgs7−/− versus control Rgs7+/+; repeated-measures ANOVA followed by least significant difference post hoc tests; $n = 12, n = 15$, and $n = 16$ for control Rgs7−/− control, Rgs7−/−, and stress Rgs7−/− neurons, respectively). C, representative traces and summary graph showing the effect of genetic knockout of R7bp on the intrinsic excitability of prelimbic L2/3 neurons ($n = 13$ and $n = 12$ for R7bp−/− and R7bp+/+ neurons, respectively). D, representative traces and summary graph showing the effect of genetic knockout of Rgs7 on the intrinsic excitability of prelimbic L5 neurons ($n = 9$ and $n = 8$ for Rgs7−/− and Rgs7−/− neurons, respectively). E, representative traces and summary graph showing the effect of stress and Gpr158 knockout on the intrinsic excitability of prelimbic L5 neurons ($n = 23$ and $n = 16$ for Gpr158−/− and Gpr158+/+ neurons, respectively). F, representative traces and summary graph showing the effect of genetic knockout of Gpr158 on the intrinsic excitability of prelimbic L5 neurons ($n = 25$ and $n = 19$ for Gpr158−/− and Gpr158+/+ neurons, respectively). Error bars represent S.E.M. values.

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Table 1

Electrophysiological properties of L2/3 pyramidal neurons in prelimbic cortex

Data are means ± S.E. (number of cells). AP amp, action potential amplitude; AP thld, action potential threshold; AP width, action potential halfwidth; mAHP, median afterhyperpolarization. RMPS were measured immediately after whole-cell mode was established by switching to current mode; R0 was the slope of the linear fit of series of subthreshold currents that did not evoke any active conductance; AP threshold was defined as the voltage when dV/dt first exceeded 28 mV/ ms. AP amplitude was measured from threshold. AP width was measured as the width at half of the AP amplitude (from threshold). Delay to 1st spike was the time to the first spike after the stimulus (300-pA current injection) onset. * statistically different between genotypes, p < 0.05; †, statistically different between genotypes, p < 0.01. Statistical analysis was performed using two-tailed Student’s t test. MΩ, megohms.

| RMP (mV) | Rheobase (µA) | AP thld (mV) | AP amp (µA) | AP width (ms) | mAHp (mV) | Delay to 1st spike (ms) |
|----------|---------------|--------------|-------------|--------------|-----------|-----------------------|
| Gpr158+/− | −62 ± 0.28 (5) | 131 ± 12 (25) | 114 ± 10 (16) | −35 ± 0.4 (16) | 86 ± 1.4 (16) | 790 ± 79 (16) | 3.7 ± 0.2 (23) |
| Gpr158−/− | −63 ± 0.18 (9) | 139 ± 17 (19) | 126 ± 19 (13) | −36 ± 0.9 (12) | 84 ± 1.8 (12) | 910 ± 91 (12) | 3.7 ± 0.2 (17) |
| Rgs7+/− | −61 ± 1.6 (9) | 107 ± 18 (9) | 148 ± 18 (9) | −36 ± 36 (9) | 88 ± 1.9 (9) | 984 ± 98 (9) | 3.9 ± 0.5 (5) |
| Rgs7−/− | −61 ± 1.1 (8) | 113 ± 14 (8) | 135 ± 16 (8) | −37 ± 37 (8) | 88 ± 1.4 (8) | 966 ± 96 (8) | 3.4 ± 0.3 (7) |

or RGS7 (Fig. 5, B and C) significantly reduced I anchor (t(16) = 2.41, p = 0.029 for Gpr158 and t(25) = 2.89, p = 0.009 for RGS7). These data support the involvement of A-type potassium channels in modulating membrane properties and intrinsic excitability of L2/3 pyramidal neurons caused by inactivation of GPR158 − RGS7 complex.

GPR158 directly modulates Kv.4.2 A-type potassium channel

Kv.4.2 potassium subunit is the major contributor of A-type currents (47). Interestingly, proteomic profiling has identified GPR158 to be present among a large number of proteins associated with Kv.4.2 potassium channel (48, 49). Thus, we next turned to a transfected HEK293 cell system to specifically test the interaction of GPR158 − RGS7 with Kv.4.2 and study its functional consequences. Indeed, we found that GPR158 effectively coimmunoprecipitated with Kv.4.2 when coexpressed in HEK293 cells (Fig. 6A), validating their physical binding. In support of our model, the Kv.4.2 channel is prominently modulated by cAMP via PKA-dependent phosphorylation of Thr-38 and Ser-552, which inhibits its plasma membrane traf-
To investigate the impact of GPR158 on Kv4.2 gating and the relevance of cAMP in this process, we coexpressed GPR158 with WT Kv4.2 or a Kv4.2AA double mutant (Kv4.2 T38A/S552A) that abolishes PKA regulation at the two sites. Cells were further cotransfected with the construct encoding mCherry to identify the cells that express the desired constructs (Fig. 6B). Whole-cell patch-clamp recordings revealed that transfecting Kv4.2 resulted in a rapidly activating and inactivating $I_{K}$ current typical of $I_{A}$, which was absent in untransfected cells (Fig. 6C). Mutation of Kv4.2 channels or coexpression of GPR158 might not affect RMP because the current used to hold the cells at $-50 \text{mV}$ was comparable between groups (Fig. 6D, left; $F(3,36) = 1.46, p = 0.24$). Furthermore, coexpression of GPR158 did not affect the peak amplitude of the current carried by either WT or mutant Kv4.2 channel (Fig. 6D, right; $F(3,36) = 0.84, p = 0.48$; one-way ANOVA), suggesting the lack of the channel modulation at the baseline when cAMP levels are low (47).

We next studied the effect of GPR158 on Kv4.2 upon elevating cAMP concentration by forskolin treatment, which activates the cAMP-producing enzyme adenylyl cyclase (Fig. 6E). Consistent with previously noted inhibitory effects of cAMP, forskolin quickly inhibited the peak amplitude of Kv4.2 current in all groups of cells (main effect of time, $F(3.1,102.7) = 36.4, p < 0.001$; repeated-measures ANOVA, Greenhouse–Geisser-corrected). There was also a significant effect of group ($F(3,33) = 7.59, p = 0.001$) and group by time interaction ($F(9.3,102.7) = 3.60, p = 0.001$). This effect was further underscored by the analysis of the maximal effect size at the effect plateau (Fig. 6F); one-way ANOVA ($F(3,33) = 5.57, p = 0.003$). The follow-up post hoc tests confirmed the role of cAMP in this effect: the Kv4.2AA mutant was insensitive to cAMP modula-
tion, and forskolin was significantly less effective in inhibiting its currents \((p < 0.05)\). Similarly, introduction of GPR158 also made Kv4.2 less sensitive to inhibition by forskolin \((p < 0.05)\).

Finally, addition of GPR158 to Kv4.2AA did not further alter its forskolin inhibition, indicating an occlusion of the effect \((p < 0.05)\). It is notable that forskolin also induced a slight reduction of the currents in cells expressing Kv4.2AA constructs. This is likely due to the phosphorylation of the pore-forming subunit mediated by the extracellular signal-regulated kinase/mitogen-activated protein kinase signaling cascade \((52, 53)\). These observations suggest that the GPR158–RGS7 complexes counteract the inhibitory action of cAMP on A-type conductances via phosphorylation of the PKA-dependent sites on Kv4.2 channels (Fig. 6G).

**Discussion**

The present study demonstrates for the first time that GPR158 and RGS7 regulate intrinsic excitability of pyramidal cells in superficial layers (L2/3) of mPFC. This effect was specific to this cellular population and was not observed in L5 neurons. Neither was it observed in mice lacking R7BP. Our data also indicate that GPR158 and RGS7 regulate the A-type potassium channels of L2/3 neurons in mPFC via cAMP-dependent phosphorylation. Taken together, this study suggests that modulating intrinsic excitability via A-type potassium channels may contribute to the antidepressant-like phenotype observed in GPR158- and RGS7-knockout animals \((35, 37)\).

The current observations are consistent with the critical role of mPFC in regulating behavioral response to stress and the susceptibility to detrimental effects induced by stress \((54, 55)\). In particular, chronic stress has been noted to cause dendritic branch shrinking, spine loss \((56, 57)\), and synaptic modifications \((58, 59)\) in mPFC neurons. Furthermore, deletion of Wfs1 (gene responsible for Wolfram syndrome) in L2/3 pyramidal cells resulted in neuronal hyperactivation and engagement of the hypothalamic–pituitary–adrenal axis, impairing the suppression of stress-induced depressive behaviors \((19)\). Thus, our findings suggest that the GPR158–RGS7 complex may be a key modulator that gates the excitability of L2/3 mPFC neurons in response to stress and regulates behavioral outcomes.

In this study, we identified a molecular target of GPR158–RGS7 action involved in the modulation of excitability. We show that the GPR158–RGS7 complex modulates the activity of A-type potassium channel Kv4.2 via a cAMP-dependent mechanism. Although GPR158 and RGS7 likely regulate several effectors and affect several processes, our observations suggest that their effects on excitability may be mediated, at least in part, by Kv4.2 channels, which are well-known for their role in regulating neuronal excitability \((50, 60)\). Mechanistically, pre-
Figure 6. GPR158–RGS7 regulates Kv4.2 channels in a cAMP/PKA-dependent manner. A, in vitro coimmunoprecipitation of GPR158 with Kv4.2 in HEK293 cells transfected with the indicated constructs. Immunoprecipitated (IP) proteins were detected by Western blotting using a specific antibody against GPR158 or an anti-myc antibody to recognize Kv4.2. 1.5% of the cell lysate (input) and 25% of the eluted immunoprecipitation was loaded per well. B, representative fluorescence (top) and IR differential video interference microscopy (bottom) images showing a HEK293 cell that expressed Gpr158, Kv4.2 channels, and mCherry. Whole-cell patch-clamp recordings were obtained from fluorescently labeled cells. C, recording protocol and representative current responses of HEK293 cells that expressed Kv4.2 channels (red) and cells that did not express the channels (black). D, point mutations in Kv4.2 channels (Kv4.2AA) or coexpression of GPR158 did not significantly affect the current used to hold the cells at −50 mV (left) and the peak amplitude of Kv4.2 current in HEK293 cells (right). E, effect of forskolin on the peak amplitude of Kv4.2 current (***, \( p < 0.001 \) between Kv4.2 and all other three groups; repeated-measures ANOVA followed by least significant difference post hoc test). F, summarized data with individual cells showing the maximal effect of forskolin-induced inhibition on the peak amplitude of Kv4.2 current (trial 11; *, \( p < 0.05 \) between Kv4.2 and all other three groups; one-way ANOVA followed by Bonferroni’s post hoc test). G, proposed model for GPR158–RGS7 involvement in modulation of intrinsic excitability. GPR158–RGS7 complex inhibits the production of cAMP, which inhibits potassium current through PKA-dependent phosphorylation of Kv4.2 channels to affect the extent of A-type current generation that reduces excitability. Elimination of GPR158–RGS7 complex would enhance cAMP–PKA activation, which phosphorylates A-type potassium channels, inhibiting them to increase excitability to produce antidepressant effects. Error bars represent S.E.M. values.
Prior studies have established that the phosphorylation of Kv4.2 channels by cAMP–PKA causes their internalization and thus increases neuronal excitability (44, 50, 51, 61). Because the genetic knockout of Gpr158 or Rgs7 also increases intracellular cAMP levels (35, 37), the enhanced excitability in neurons lacking GPR158 or RGS7 is likely due to smaller A-type potassium current mediated by PKA phosphorylation. The identification of A-type potassium channels as the targets for GPR158–RGS7 effects in mPFC neurons was unexpected as our working hypothesis based on prior studies in hippocampal neurons favored a role for G protein–gated inwardly rectifying potassium channels in this process (62). Another channel that could have been involved in this process is hyperpolarization-activated cyclic nucleotide–gated (HCN) that is active at resting membrane potentials and greatly facilitated by cAMP (63, 64). The net effect of activating HCN channels is a decrease in excitability primarily due to reduction in membrane resistance (65, 66). Because cAMP is increased upon the loss of RGS7–GPR158 and the excitability is also increased, we think it is unlikely that HCN channels contribute to RGS7–GPR158-mediated regulation of excitability in L2/3 neurons of mPFC. The expression pattern of HCN channels with prominent presence in hippocampal CA1 (67–69) and deep but not superficial layers (L2/3) of mPFC (39, 70) may provide an explanation for the lack of their involvement. Thus, differential expression of ion channels across brain regions may set up bidirectional effects of GPR158–RGS7 on neuronal excitability in a neuron-selective fashion.

The contribution of Kv4.2 channels to the effects of GPR158–RGS7 complex on depression remains to be fully elucidated. Based on increased inhibition of Kv4.2 observed in the absence of GPR158 or RGS7, one can expect that elimination of Kv4.2 would also result in antidepressant behavior. However, Kv4.2-knockout mouse do not exhibit a consistent phenotype when evaluated by depression/anxiety-related tests. One study reported a prodepressant increase in the immobility time in FST but lack of the effect in the tail suspension test (71). A subsequent study failed to confirm the prodepressant phenotype and found that Kv4.2-KO mice behaved similarly to controls in the FST test (72). However, both of the studies reported a mild anxiolytic phenotype (71, 72). Furthermore, Kv4.2-KO mice exhibited an abnormal response to stress diminishing its effects on depression-related behavioral changes (71), whereas increased immobility during FST may also be viewed as a learned habituation and desensitization to stress (73, 74). There are several possible explanations for why the marked antidepressant phenotype associated with GPR158–RGS7 ablation is not recapitulated by Kv4.2 KO. First, loss of the Kv4.2 channels is well-known to be compensated by the up-regulation of other K+ channels that contribute to A-type conductance (75); thus, constitutive knockout of Kv4.2 may be insufficient for altering excitability to the threshold that triggers behavioral changes. Second, GPR158–RGS7 complex may not be involved in regulating Kv4.2 in all relevant brain circuits. Studies indicate that GPR158–RGS7 action in PFC is sufficient for producing an impact on affective behaviors (35, 37) and so might be Kv4.2. However, in global Kv4.2 KO, this action may be compensated by counteracting effects from other circuits where Kv4.2 may not be regulated in similar fashion by GPR158–RGS7. Testing these possibilities would require examining the effects of manipulations with Kv4.2 in adult mice with region selectivity by either genetic or pharmacological strategies.

Taken together, the current study suggests that changes in the excitability of neurons in superficial layers of mPFC may be a cellular mechanism underlying the antidepressant effect of GPR158 or RGS7 knockout. The GPR158–RGS7 complex may regulate Kv4.2 channels through controlling the cAMP–PKA signaling cascade. This may provide a new therapeutic strategy for treatment of stress-induced depression, by regulation of the excitability of L2/3 neurons in PFC via suppressing the activity of GPR158–RGS7 complex.

**Experimental procedures**

**Animal models**

All studies were carried out in accordance with the National Institute of Health guidelines and were granted formal approval by the Institutional Animal Care and Use Committee of The Scripps Research Institute. The generation of Gpr158+/− (36, 37), Rgs7+/− (76), and R7bp+/− (77) mice was described previously. All mice were maintained on C57/Bl6 background and were 2–5 months old during the experiments. All animals used for comparing genotypes were littermates derived from heterozygous breeding pairs. Mice were housed in groups on a 12-h light–dark cycle with food and water available *ad libitum*. Both male and female mice were used in this study.

**Genetic constructs**

Cloning of full-length mouse GPR158 into the pcDNA3.1/V5-His-TOPO was described previously (78). The plasmid encoding full-length mouse Kv4.2 (GenBank™ accession number NM_019697; myc-DDK-tagged in C terminus) for mammalian expression was purchased (Origene). Kv4.2 bearing mutations to express Ala at the two PKA target sites Thr-38 and Ser-552 (Kv4.2AA) was generated using the In-Fusion HD cloning system (Clontech) in pcDNA3.1. Each construct was verified by DNA sequencing.

**Behavior and physical restraint stress**

PRS was performed in plastic tubes (30-mm diameter × 115-mm length) with holes for ventilation. Stressed mice were restrained horizontally in tubes for 2 h for 14 days; nonstressed mice were kept undisturbed in their home cages. On day 15, all mice underwent an FST that was conducted using a vertical glass cylinder filled with water (25 °C). The mice spent 6 min in the water, and immobility was scored from 2 to 6 min by an independent researcher. A mouse was regarded as immobile when floating motionless or making only those movements necessary to keep its head above the water. Brain slices were prepared within 1 h following the FST, and whole-cell patch-clamp recordings were obtained from pyramidal cells in L2/3 of prelimbic area.

**Slice preparation and whole-cell recordings from brain slices**

Coronal slices (300 μm) containing prefrontal cortex (anteroorposterior +1.5–2.2) were cut in ice-cold aCSF (124 mM
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NaCl, 2.8 mM KCl, 1.25 mM NaH2PO4, 2 mM CaCl2, 1.25 mM MgSO4, 26 mM NaHCO3, 10 mM glucose, pH 7.5, bubbled with 95% O2, 5% CO2 using a vibrating tissue slicer (VT1200, Leica). Slices were transferred to a holding chamber (79) where they remained in oxygenated aCSF at 32–35 °C until use. During recording, slices were transferred to a submerged recording chamber where they were continuously perfused at 2 ml/min with oxygenated aCSF and maintained at 32–36 °C using an inline temperature controller. Neurons were visualized with IR differential video interference microscopy. Whole-cell recordings were obtained with standard-wall borosilicate glass pipettes (2–5 megaohms) filled with the following solution: 110 mM K-glucuronate, 20 mM KCl, 10 mM di-Tris-P-creatinine, 10 mM HEPES, 2 mM MgCl2, 2 mM Na2ATP, 0.3 mM Na2GTP with a pH of 7.3 and osmolarity of 290 mosmol. Prelimbic L2/3 neurons were recognized by their large somata, prominent apical dendrite, and firing properties. To verify identity, some cells were injected with 0.1% biocytin through the recording electrode and visualized using a streptavidin Alexa Flour 488 reaction as described previously (39). One neuron was studied in each slice. Only neurons with an RMP more negative than −60 mV, an input resistance (Rin) >50 megaohms, an AP amplitude >45 mV relative to threshold, and an access resistance less than 20 megaohms were included.

RMP was obtained immediately by switching to current clamp after whole-cell mode was established. The membrane capacitance was estimated by using the auto C-slow function in Patchmaster. Intrinsic properties of mPFC neurons were recorded 3 min later (to allow the diffusion of internal solution) according to the following protocols: 1) V−I relationships were obtained from a series of 500-ms current injections (range −300 to 50 pA) and plotting the plateau voltage deflection against current amplitude. Neuronal Rin was determined from the slope of the linear fit portion of the V−I plot where the voltage sweeps did not exhibit sags or active conductance (80).

2) AP properties, including the minimum current necessary to elicit an AP (Ithreshold), were studied with an ascending series of 500-ms depolarizing pulses to elicit one single spike. 3) Neuronal excitability was assessed by counting the number of spikes evoked in response to a series of 1-s depolarizing steps (range, 100–600 pA at 100-pA increments or 50–450 pA at 50-pA increases with a 20-s intertrial interval). To record barium-sensitive current, cells were held at −50 mV and V−I relationships were obtained from a series of voltage steps (from −50 to −120 mV) before and 10 min after bath application of 100 μM barium chloride. Barium-sensitive currents were calculated by subtraction of current responses to voltage steps in the presence and absence of barium. A-type current was isolated using a voltage protocol described previously (46). Briefly, the cells were first held at −40 mV to inactivate most of the outward current. After stabilization, a step was made to +40 mV for 500 ms and then returned to −40 mV for 5 s followed by a prepulse to −100 mV (150-ms duration) to allow preferential recovery of the A-type current. This prepulse duration is long enough to recover most of the A current but is too short to recover the majority of the delayed rectifier currents (81, 82). A-type current was defined as the difference between the peak current during the test potential (+40 mV) after the prepulse and current during the test pulse without prepulse (relevant to the current used to hold the cells at −40 mV).

Cell culture and whole-cell recording from transfected HEK293 cells

Cell culture was carried out using standard procedures. HEK293T/17 cells were cultured at 37 °C in 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, minimum essential medium nonessential amino acids, 1 mM sodium pyruvate, and 2 mM GlutaMAX. 80,000 cells were plated on 12-mm poly-d-lysine−coated glass coverslips and transfected using Lipofectamine LTX (Invitrogen) and Plus reagent (Invitrogen) with a 1:1 ratio of GPR158 and Kv4.2 expression constructs and used 16 h later. Transfected cells were identified by expression of the fluorescein protein mCherry. Coverslips were bathed in recording solution for electrophysiological recording containing the following: 141 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl2, 1.8 mM CaCl2, 10 mM glucose, 10 mM HEPES, pH 7.4, with NaOH. Whole-cell recordings were performed on fluorescently labeled cells at room temperature with glass pipettes filled with internal solution containing 125 mM KCl, 4 mM MgCl2, 10 mM HEPES, 10 mM EGTA, 5 mM Mg-ATP, pH 7.2, with KOH. Pipette resistance was between 2 and 5 megaohms, and series resistance ranged between 2 and 10. The cells were held at −50 mV when the whole-cell configuration was obtained. The capacitance and series resistance were compensated using the auto function of Patchmaster software before recording the currents. Under voltage clamp, Kv4.2 channel currents were evoked by a brief prepulse to −80 mV followed by pulses of depolarization (Fig. 6C, left). Kv4.2 currents exhibited rapid activation and inactivation typical of A-type potassium currents, whereas endogenous Kv4.2 currents in HEK cells were relatively negligible; therefore, they were not quantified (Fig. 6C, right). To study the effect of forskolin on the peak amplitude of Kv4.2 channel current, a test pulse (+40 mV, 500 ms) was given every 10 s, and the peak current of each pulse was measured. After a baseline recording, forskolin (100 μM) was directly applied to the cell with an SF-77B rapid perfusion system (Warner Instruments, Inc., Hamden, CT). Percent inhibition of the peak Kv4.2 current by forskolin was expressed as (1 − Itest/Ibaseline) × 100% where Itest is the peak current of each test pulse and Ibaseline is the baseline amplitude (average of four pulses before forskolin application). All chemicals were purchased from Sigma. All data were collected with a HEKA EPC10 amplifier system (HEKA Instruments, Holliston, MA) and transferred to a personal computer using an ITC-16 digital-to-analog converter (HEKA Instruments). The signals were filtered at 2.9 kHz and digitized at 10 kHz using Patchmaster software (HEKA Instruments). Data were analyzed offline using Patchmaster. Voltages were not corrected for the liquid–liquid junction potential.

Immunoprecipitation

Two million HEK293T/17 cells/well were plated in a 6-well culture plate and transfected using Lipofectamine LTX and Plus reagent with a 1:1 ratio of GPR158 and Kv4.2myc plasmids. Cells were harvested 24 h later and lysed in 500 μl of ice-cold
immunoprecipitation buffer (300 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1% Triton X-100, complete protease inhibitor mixture) by sonication. Lysates were cleared by centrifugation at 14,000 × g for 15 min. 50 μl of supernatant were saved as input fraction, and the remaining 450 μl were incubated with 20 μl of Protein G beads (GE Healthcare) and 2 μg of antibodies on a rocker at 4 °C for 1 h. After three washes with immunoprecipitation buffer, proteins were eluted with 40 μl of 2× SDS sample buffer. 10 μl of each sample were analyzed by SDS-PAGE followed by Western blotting using horseradish peroxidase–conjugated secondary antibodies and an ECL West Pico (Thermo Scientific) detection system. Both lysates and immunoprecipitated fractions were run on the same gel followed by (Thermo Scientific) detection system. Both lysates and immunoprecipitated fractions were run on the same gel followed by

**Data analysis and statistics**

A minimum of three animals were used in each group throughout the study. For statistical analyses, data were analyzed from individual cells. Statistical analysis was performed using GraphPad Prism (Prism 6.0, GraphPad, San Diego, CA) and SPSS Statistics 25 software (IBM). Student’s t test was used to compare means between two groups, and one-way or two-way ANOVA followed by Bonferroni post hoc test was used to determine significant differences among multiple groups. Repeated-measures ANOVA was used when a dependent variable was measured multiple times such as intrinsic excitability, barium-sensitive current, and forskolin-mediated Kv4.2 current inhibition. Statistical tests were performed two-sided. Differences were considered significant if p was <0.05. All data are expressed as means ± S.E.

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