NEUROSCIENCE

Orphan receptor GPR158 serves as a metabotropic glycine receptor: mGlyR

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Glycine is a major neurotransmitter involved in several fundamental neuronal processes. The identity of the metabotropic receptor mediating slow neuromodulatory effects of glycine is unknown. We identified an orphan G protein–coupled receptor, GPR158, as a metabotropic glycine receptor (mGlyR). Glycine and a related modulator, taurine, directly bind to a Cache domain of GPR158, and this event inhibits the activity of the intracellular signaling complex regulator of G protein signaling 7–G protein β5 (RGS7-Gβ5), which is associated with the receptor. Glycine signals through mGlyR to inhibit production of the second messenger adenosine 3′,5′-monophosphate. We further show that glycine, but not taurine, acts through mGlyR to regulate neuronal excitability in cortical neurons. These results identify a major neuromodulatory system involved in mediating metabotropic effects of glycine, with implications for understanding cognition and affective states.

Glycine is the simplest amino acid ubiquitously present in all mammalian tissues. Glycine serves as an inhibitory neurotransmitter, but it can be excitatory in developing neurons (*1*, *2*). Glycinergic neurons are distributed across the brain; however, glycine can also be released by glial cells (*3*). Known receptors for glycine belong to the family of pentameric ligand-gated ion channels (*4*). Glycine also serves as a coagonist of *N*-methyl-D-aspartate (NMDA) receptors (*5*). Metabotropic neuromodulatory effects of glycine have been observed (*6*, *7*), but no receptors mediating these actions have been found. Glycine has distinct effects on neural circuits (*3*), and glycinergic transmission has been implicated in pathological conditions, including depression (*8*–*10*).

Metabotropic neuromodulation in the nervous system is mediated mainly by heterotrimeric GTP-binding protein (G protein)–coupled receptors (GPCRs). GPCRs play essential roles in neuronal physiology and pathology and present targets for drug development (*11*). Canonically, GPCRs transduce their signals by activating heterotrimeric G proteins (*12*, *13*). However, G protein–independent modes of signal transduction triggered by the recruitment of β-arrestins and other scaffolds to activated GPCRs have also been described (*14*–*16*). G protein signaling is controlled by regulator of G protein signaling (RGS) proteins, which facilitate their deactivation (*17*). RGS proteins also interact with several GPCRs (*18*–*22*).

GPCRs mediate the effects of all major neurotransmitters except glycine and taurine. However, many GPCRs still have no identified endogenous ligands. Orphan GPCRs may have potential for obtaining insights into physiology and for drug development (*23*, *24*).

GPR158 is one of the most abundant orp GPCRs in the brain that transduces signal coupling to RGS proteins (*25*, *26*). In neurons, it regulates signaling to the second messenger adenosine 3′,5′-monophosphate (cAMP) and controls key ion channels, kinases, and neurotrophic factors involved in neuronal excitability and synaptic transmission (*25*, *27*). Accordingly, GPR158 has been heavily implicated in cognition and affective states (*25*, *28*, *29*). Genetic suppression of GPR158 in mice results in a prominent antidepressant phenotype and stress resiliency, making GPR158 an attractive target for development of new antidepressants (*25*).

The endogenous ligand for GPR158 remains unknown. Recent structures of GPR158 revealed the presence of an extracellular Cache domain, a putative ligand-binding module (*30*, *31*).

Results

Glycine signals through GPR158 to regulate cAMP

The structure of GPR158 revealed the presence of a Cache domain, which serves as a ubiquitous ligand-binding module in bacterial chemoreceptors (*30*). We found that

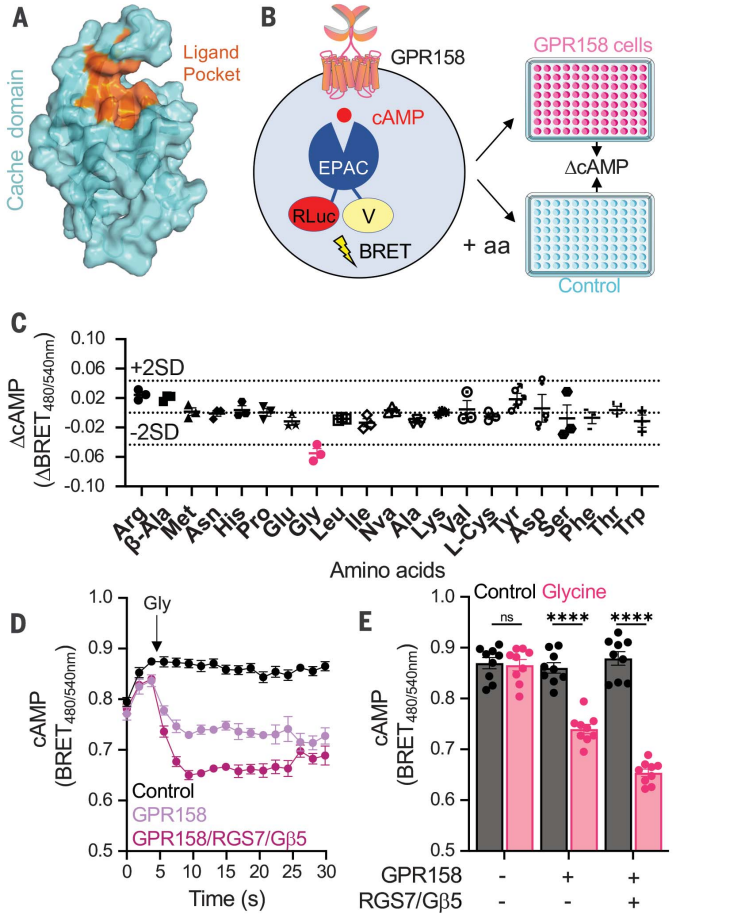

Fig. 1. Identification of glycine as GPR158 ligand. (**A**) Three-dimensional model of the GPR158 Cache domain (cyan) with putative ligand-binding pocket (orange). (**B**) Schematic of the screening assay design. (**C**) Quantification of cAMP changes mediated by GPR158. BRET signal in control cells is subtracted from the signal from cells expressing GPR158, and the difference is plotted. Dotted lines denote 2× SD confidence interval. Data represent mean ± SEM determined from three independent experiments performed in triplicate. (**D**) Time course of cAMP change induced with 10 μM forskolin in U87 MG glioblastoma cells in response to glycine (100 μM) application (arrow). (**E**) Quantification of maximal amplitudes of responses to glycine in (D). Data represent mean ± SEM determined from three independent experiments performed in triplicate. $^{**}p < 0.01$, one-way analysis of variance (ANOVA).

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GPR158 Cache domain had a small pocket with organization similar to that of the amino acid binding pocket in other Cache domains (Fig. 1A). We hypothesized that GPR158 may have an amino acid ligand. We screened a library of amino acids for their ability to alter GPR158-mediated signaling. Because GPR158 has been linked to regulation of cAMP in the brain (*25*, *27*), we used a bioluminescence resonance energy transfer (BRET)–based cAMP biosensor (*32*) (Fig. 1B). Out of all amino acids tested, only glycine showed significant decrease in cAMP when applied to human embryonic kidney (HEK) 293 cells expressing GPR158 relative to nontransfected cells (Fig. 1C).

To study this effect in more detail, we analyzed the individual responses to glycine in a kinetic mode. We found that glycine application to U87 glioblastoma cells expressing GPR158 resulted in cAMP decrease. No glycine-induced changes in cAMP were observed in cells lacking GPR158 (Fig. 1, D and E). This inhibitory effect of GPR158 was further potentiated by coexpressing RGS7-G protein β5 (RGS7-Gβ5), suggesting that GPR158 signals by means of this protein complex to affect cAMP levels (Fig. 1, D and E).

We further tested the effect of taurine, a compound closely related to glycine, which binds to several common receptors (*33*), including ionotropic glycine receptors (*34*). Taurine caused a significant decrease in cAMP levels only in HEK293 cells expressing GPR158 (fig. S1, A and B). Again, this effect was potentiated by coexpressing RGS7-Gβ5, suggesting that these proteins act in complex with GPR158 in mediating the effects of taurine. However, when compared directly, the effect of taurine on GPR158-mediated suppression of cAMP was weaker than the effect of glycine (fig. S1C).

Glycine inhibits modulation of RGS7-Gβ5 by GPR158

To understand how glycine action on GPR158 regulates intracellular cAMP, we focused on GPR158 interaction with RGS7-Gβ5, an established guanosine triphosphatase (GTPase)–activating protein (GAP) for the $G\alpha_{i/o}$ proteins (*35*) known to regulate cAMP production (*26*). We used a cell-based assay to monitor GAP activity by following kinetics of G protein deactivation (*36*) (Fig. 2A). In this assay, activation of G proteins by GPCR stimulation generates the BRET signal upon interaction of liberated Venus-Gβγ subunits with the masGRK3CT-Nluc reporter. This signal is quenched when Gα deactivation is triggered by GPCR antagonism and recombines with Venus-Gβγ to form inactive heterotrimer. As previously reported (*22*), we found that introduction of RGS7-Gβ5 accelerated deactivation of its substrate, $G\alpha_o$ (Fig. 2, B and D). Application of glycine had no effect on either baseline $G\alpha_o$ deactivation or the RGS7-Gβ5-assisted process (Fig. 2, B and D). However, when GPR158 was coexpressed together with RGS7-Gβ5, glycine significantly decelerated $G\alpha_o$ deactivation (Fig. 2, C and D), suggesting that it specifically inhibited the GAP activity of RGS7-Gβ5 by engaging GPR158.

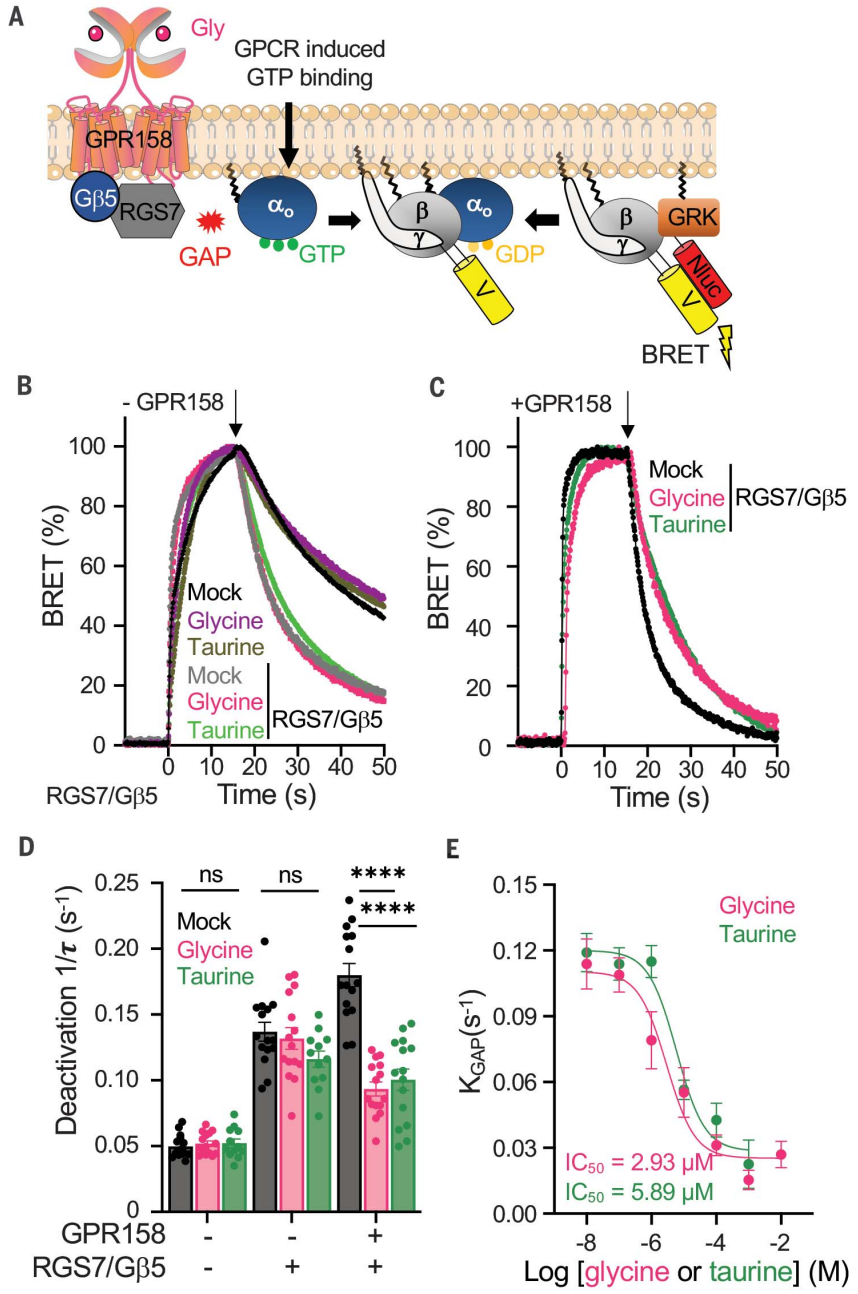

Fig. 2. Glycine and taurine slow deactivation of $G\alpha_o$ by GPR158-RGS7-Gβ5 complex. (**A**) Schematics of the BRET-based GAP assay. G proteins are activated at t = 0 s by stimulating GPCR (dopamine DR receptor, 0.1 mM). After reaching steady state, the GPCR activity is terminated by injection of haloperidol (0.1 mM) at t = 15 s (arrow). G protein deactivation is then monitored by following quenching of the BRET signal. (**B** and **C**) Traces of BRET signal showing $G\alpha_o$ activation and deactivation time course with or without glycine or taurine (100 μM) treatment in cells without GPR158 (B) or cells transfected with GPR158 (C). (**D**) Quantification of deactivation time constant of the reactions presented in (B) and (C). $1/\tau$ is calculated from deactivation curves of n = 5 independent experiments conducted in triplicate from each cell transfection group. Data represent mean ± SEM. ****$p < 0.0001$, ns (not significant) = $p > 0.05$, two-way ANOVA. (**E**) Dose–response profile of changes in GAP activity (K_{GAP}) calculated by subtracting the baseline deactivation rate ($1/\tau$) from the rate of the reaction in the presence of GPR158-RGS7-Gβ5. Data represent mean ± SEM of n = 4 independent experiments conducted in triplicate.

Dose–response studies showed that the median inhibitory concentration (IC_{50}) of glycine on GPR158 is ~3 μM (Fig. 2E). Taurine displayed a similar inhibitory effect on $G\alpha_o$ deactivation only in cells coexpressing GPR158

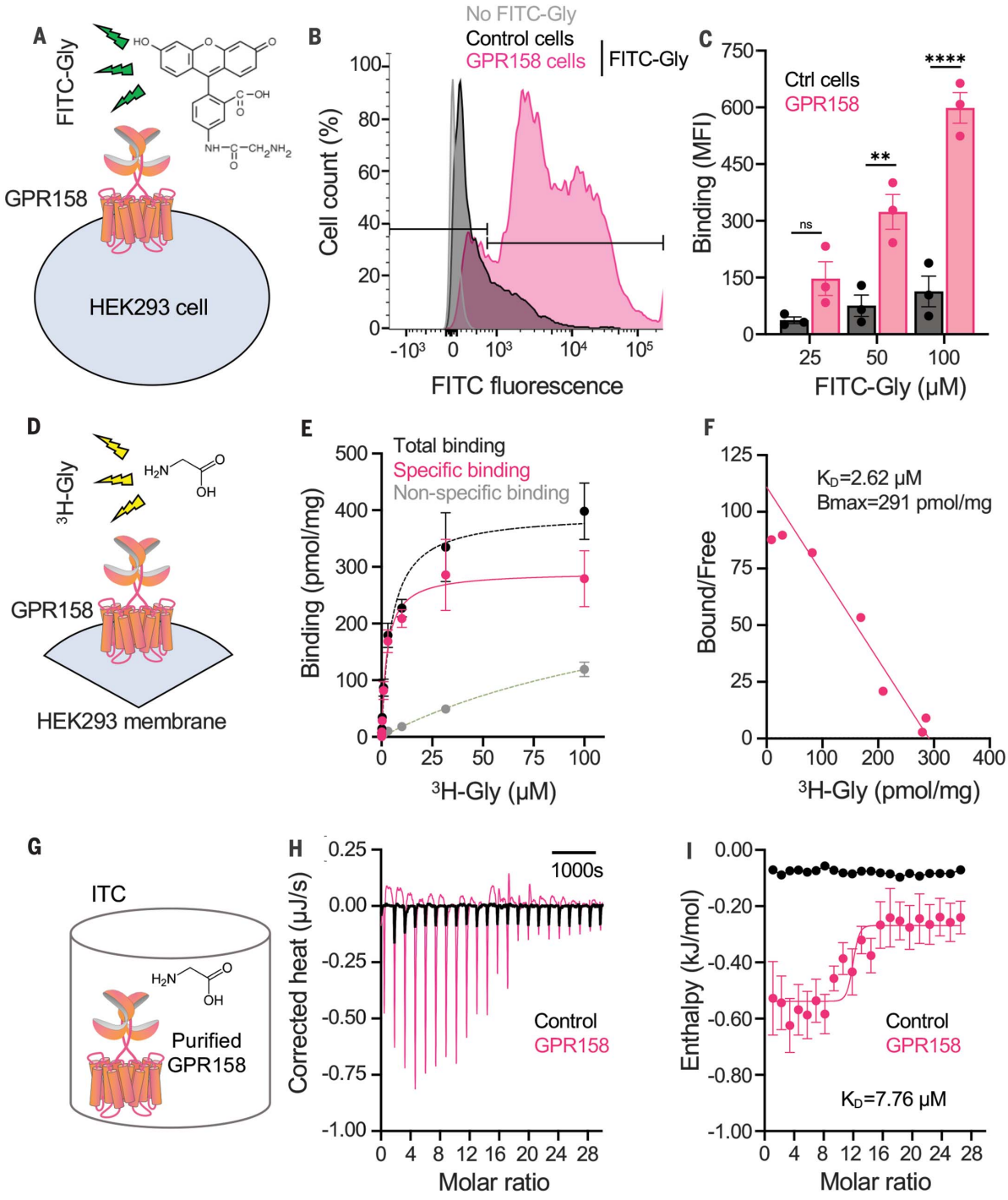

Fig. 3. Direct interaction of glycine with GPR158. (**A**) Schematics of assay design for detecting glycine binding to GPR158 by flow cytometry. (**B**) Flow cytometry histogram showing distribution of cellular populations after sorting. (**C**) Quantification of FITC-glycine binding detected in flow cytometry experiments. The median of fluorescence (MFI) is quantified and plotted. Error bars indicate SEM, $n = 3$, $^{**}p < 0.01$, $^{****}p < 0.0001$, two-way ANOVA. (**D**) Schematics of the radioligand binding assay. (**E**) Quantification of [^{3}H]glycine binding to membrane expressing GPR158. Data show mean of four independent experiments; error bars indicate SEM, $n = 4$. (**F**) Scatchard plot of the [^{3}H]glycine radioligand binding assay. Data show mean of four independent experiments; error bars indicate SEM, $n = 4$. (**G**) Schematics of the assay design detecting glycine binding to GPR158 by isothermal titration calorimetry (ITC) with purified protein. (**H**) ITC binding profile showing glycine binding to GPR158 in the initial run with fresh sample. (**I**) Quantification of binding determined by fitting the integrated isotherm to an independent binding model. Data show mean of four experimental runs; error bars indicate SEM.

with RGS7-Gβ5, but with a lower IC_{50} of ~6 μM (Fig. 2, B to E).

We further tested whether glycine or taurine could induce GPR158 to activate G proteins as canonical GPCRs do (fig. S2A). We observed no significant activation of any G proteins tested with either glycine or taurine (fig. S2, B to I). We also tested whether glycine could induce β-arrestin recruitment to GPR158 using a BRET assay and obtained no significant response (fig. S3).

GPR158 directly binds glycine

To confirm that GPR158 is a direct target of glycine, we used several strategies. First, we devised a flow cytometry–based assay to monitor binding of fluorescein isothiocyanate (FITC)–

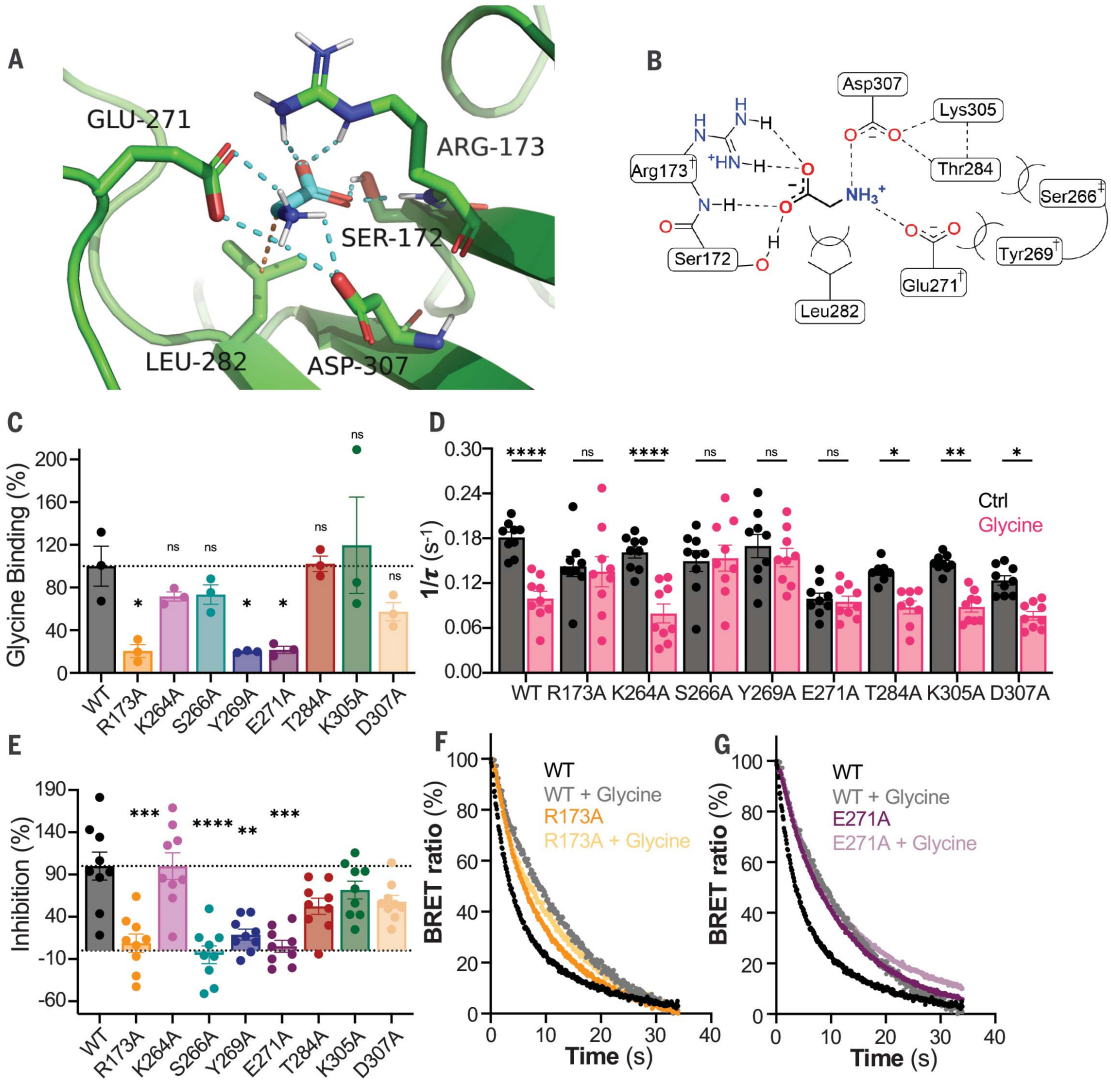

Fig. 4. Probing Cache domain of GPR158 as a ligand binding site. (**A**) Computational docking of glycine (teal) into putative ligand-binding pocket on GPR158 Cache domain (green). Glycine and directly interacting residues are shown as sticks. Hydrogen bonds (teal) and van der Waals interactions (orange) are shown as dotted lines. (**B**) Diagram showing interactions in the docked model of glycine against GPR158 Cache domain. Hydrogen bonds are shown as dashed lines, van der Waals interactions are shown as intersecting semicircles, and the secondary structural context of Ser266 and Tyr269 is shown as an arc. Dagger indicates a residue implicated in glycine binding in (A). (**C**) Radioligand binding assay of [^{3}H]glycine in cells expressing GPR158 mutants. Data show mean ± SEM of three independent experiments, *p < 0.05, ns = p > 0.05, one-way ANOVA. (**D**) Functional evaluation of GPR158 mutants in GAP BRET assay. Error bars indicate mean ± SEM of three independent experiments conducted in triplicate. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = p > 0.05, two-way ANOVA. (**E**) Quantification of glycine inhibitory effect on K_{GAP} normalized to the effect seen with wild-type (WT) receptor. Error bars indicate mean ± SEM of three independent experiments conducted in triplicate. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = p > 0.05, one-way ANOVA. (**F** and **G**) Traces of Gα_o deactivation time course upon glycine addition. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; E, Glu; K, Lys; R, Arg; S, Ser; T, Thr; and Y, Tyr.

conjugated glycine to cells expressing GPR158 (Fig. 3A). When HEK293 cells expressing GPR158 were incubated with FITC-glycine, we observed labeling of a significant population of cells (Fig. 3B). No such labeling was evident when FITC-glycine was incubated with cells not transfected with GPR158. Dose–response studies further confirmed this binding and its selectivity across the ranges of glycine used (Fig. 3C).

Next, we performed radioligand binding assays examining binding of [^{3}H]-labeled glycine to HEK293 cells expressing GPR158 (fig. S4A). We detected significant binding of [^{3}H]glycine to GPR158-expressing cells across concentrations (Fig. S4B). We isolated cellular membranes and conducted classical radioligand titration experiments (Fig. 3D). We detected saturable [^{3}H]glycine binding to membranes containing GPR158 in substantial excess over linear nonspecific binding to membranes devoid of GPR158 (Fig. 3E). Scatchard analysis (Fig. 3F) estimated the dissociation constant, K_D, of GPR158 for glycine to be ~3 μM. Binding competition experiments directly comparing the ability of glycine and taurine to displace [^{3}H]glycine bound to GPR158 (fig. S5) confirmed the specificity of glycine and taurine binding to GPR158 and also revealed a twofold lower affinity of taurine relative to glycine (IC_{50}: ~3 μM versus ~6 μM).

In addition, we examined binding of non-labeled glycine directly to purified GPR158 using isothermal titration calorimetry (ITC) (Fig. 3G). Titration experiments showed saturation of the heat released upon glycine addition to GPR158 yielding K_D ranging from 2 to 16 μM across experiments conducted first with a fresh sample (Fig. 3, H and I) and subsequently rerun after removal of glycine and detergent (fig. S6). The affinity of glycine obtained in direct binding experiments is in good agreement with the affinity measured in the functional GAP assays, indicating that binding to glycine is responsible for changes in GPR158 activity.

Glycine binds to Cache domain of GPR158 and modulates GAP activity of RGS7-Gβ5 complex

We performed molecular docking experiments fitting glycine into a model of the putative ligand-binding pocket in the Cache domain of GPR158, built by supplementing experimental structure (*30*) with a missing loop taken from the AlphaFold2 prediction (Fig. 4A and table S1). Although another structure of GPR158 is available (*31*), it did not resolve side-chain conformations and thus was not considered

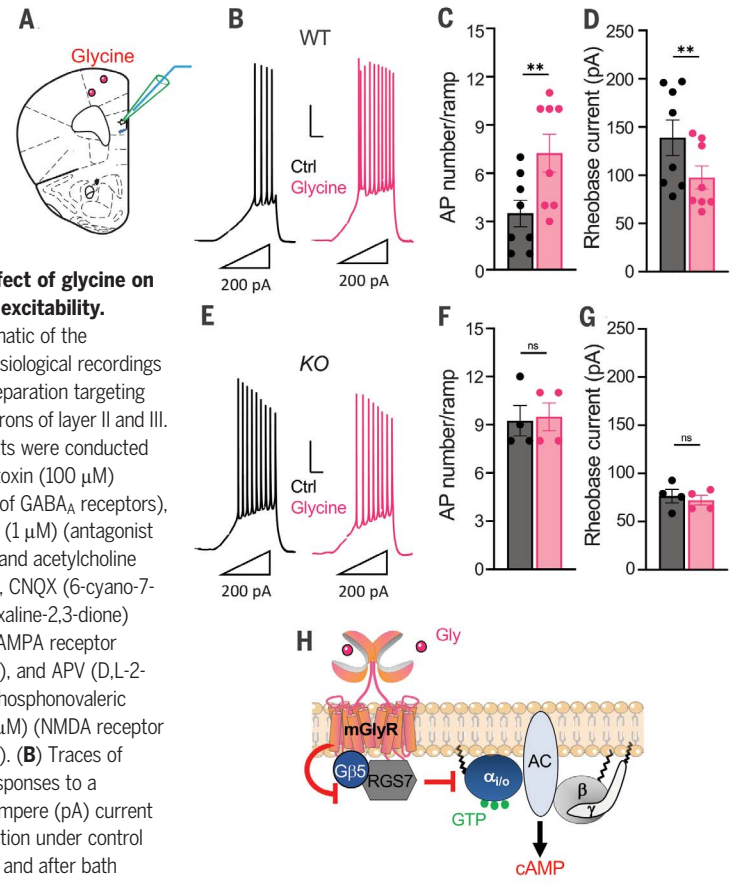

Fig. 5. Effect of glycine on neuronal excitability. (**A**) Schematic of the electrophysiological recordings in slice preparation targeting mPFC neurons of layer II and III. Experiments were conducted with picrotoxin (100 μM) (blockade of $GABA_A$ receptors), strychnine (1 μM) (antagonist of glycine and acetylcholine receptors), CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) (20 μM) (AMPA receptor antagonist), and APV (D,L-2-amino-5-phosphonovaleric acid) (50 μM) (NMDA receptor antagonist). (**B**) Traces of voltage responses to a 200-picoampere (pA) current ramp injection under control conditions and after bath application of glycine (1 mM). (**C**) Quantification of changes in excitability by number of action potentials fired in response to 200-pA current ramp (*n* = eight neurons from five mice). (**D**) Quantification of changes in excitability by rheobase current under control condition and glycine application (*n* = eight neurons from five mice). (**E**) Traces of voltage responses to a 200-pA current ramp injection obtained from layer II and III pyramidal neurons in *Gpr158* KO mice under control conditions and during bath application of glycine (1 mM). (**F**) Quantification of changes in excitability by number of action potentials (AP) fired in response to 200-pA current ramp (*n* = four neurons from three mice). (**G**) Quantification of changes in excitability by rheobase current under control condition and glycine application (*n* = four neurons from three mice). (**H**) Schematic representation of the proposed mechanism of glycine effects on mGlyR. In all graphs, nonparametric *t* test; Wilcoxon test was used for statistical analysis, ns = $p > 0.05$, $^{**}p < 0.01$.

as a source of alternate receptor conformations for docking. For the best-scored glycine pose, glycine could be well accommodated in a pocket where it is stabilized by a network of hydrogen-bonding interactions with S172, R173, E271, and D307 side chains, with the charged side chains ideally positioned to stabilize the carboxylate and amine moieties of the zwitterion. These residues are embedded in a web of other hydrophilic residues located in a close vicinity (e.g., K264, S266, Y269, T284, and K305) lining the pocket (Fig. 4B). Docking studies performed with taurine found a cluster of poses that overall matched the putative binding mode of glycine, retaining the features described for glycine (fig. S7). The size of the pocket is spatially constrained, particularly by L282, in such a way that other amino acids cannot be easily accommodated without steric clashes with side chains of residues lining the pocket, which provides a possible explanation for the selectivity of the recognition (fig. S8).

To test the role of the residues forming the putative glycine pocket in the GPR158 Cache domain, we performed site-directed mutagenesis. In radioligand binding assays, the R173A, E271A, and Y269A mutants showed near complete loss of [^{3}H]glycine binding, confirming the essential role of these residues in ligand coordination (Fig. 4B). We then tested each of the mutants in functional assays (Fig. 4, C and D). Each of the mutants defective in glycine binding also lost an ability to inhibit the GAP activity of RGS7-Gβ5 (Fig. 4E). The activity of the S266A mutant, which normally binds glycine, was not regulated by it, suggesting that some of the residues in the binding pocket are involved in conformational transitions triggered by ligand interaction (*37*). The mechanism by which mutating E271 residue resulted in loss of glycine responsiveness also deviated for that of other mutations. This mutant exhibited a much slower deactivation kinetics in the absence of glycine, generating a constitutively inhibited receptor.

Glycine modulates neuronal excitability through GPR158

Lastly, we assessed the impact of glycine modulation of GPR158 on neuronal activity. We examined the intrinsic properties of layer II and III neurons in the prelimbic cortex, where GPR158 is prominently expressed (*25*) and regulates neuronal excitability (*27*). The metabotropic effects of glycine are not well characterized across the nervous system. Therefore, we started by defining the effects of glycine on layer II and III neurons. To isolate metabotropic actions, we antagonized excitatory and inhibitory synaptic drive with pharmacological blockade and measured the current-voltage relation in response to a depolarizing current ramp. Application of glycine significantly increased the number of action potentials while decreasing the amount of current necessary to elicit the first action potential (Fig. 5, A to C) without changes in the resting membrane potential (fig. S9). This excitatory effect of glycine is distinct from its canonical inhibitory action mediated by glycine receptor (GlyR) ion channels. Interestingly, glycine application did not produce any changes in the intrinsic excitability of layer V neurons (fig. S9), which do not express GPR158 (*27*).

To confirm the involvement of GPR158 in the effects of glycine, we studied *Gpr158* knockout (*Gpr158* KO) mice. Glycine application failed to alter excitability of layer II and III neurons in prefrontal cortex of *Gpr158* KO mice (Fig. 5, D to F). We also tested the effect of taurine on the excitability of layer II and III neurons (fig. S11). These experiments revealed small effects on neuronal firing in the same direction as glycine. However, these effects did not reach the criteria for statistical significance, possibly because of the lower efficacy of taurine.

Discussion

In this study, we demonstrated that GPR158 serves as a metabotropic receptor for glycine. We also report that GPR158 can be modulated by taurine, which acts as a partial agonist for this receptor. This finding was enabled by recently obtained high-resolution structure of the receptor, which revealed the presence of a ligand-binding module: the Cache domain. Cache domains are well-known receptors for amino acids and other related small molecules ubiquitously used by bacterial chemoreceptors. Only two GPCRs contain them, including GPR158 and night-blindness associated receptor GPR179 (*38*), whose ligand remains to be established. We present evidence that glycine acts as a bona fide ligand on GPR158,

including direct binding and resultant change in receptor activity eliciting cellular response. This puts GPR158 in line with other class C GPCRs, many of which are amino acid sensors, such as the metabotropic glutamate receptors (mGluRs) and the receptor for γ-aminobutyric acid (GABA), $GABA_B$. Thus, we propose a generic name for GPR158 to be metabotropic glycine receptor, or mGlyR. We did not observe significant GPR158-mediated neuronal responses to taurine in cortical neurons, consistent with weaker effects of taurine on GPR158 relative to glycine. However, it remains possible that GPR158 may still mediate the effects of taurine in other neuronal populations or under certain conditions, possibly making GPR158 a receptor for both glycine and taurine.

The mechanism by which mGlyR (GPR158) signals upon glycine or taurine binding deviates from canonical actions of GPCRs. Instead of activating G proteins, mGlyR recruits a RGS7-Gβ5 complex, docking it into the intracellular pocket that canonical GPCRs use for interacting with G proteins and relaying changes in seven-transmembrane architecture upon ligand binding into conformational changes in Gα, triggering nucleotide exchange. Thus, in the model we propose (Fig. 5H), glycine binding to the Cache domain of mGlyR changes the conformation of the intracellular surface, which in turn affects conformation of RGS7-Gβ5. This change reduces the ability of RGS7-Gβ5 to stimulate Gα GTPase, likely by disfavoring its orientation toward the membrane. In this sense, glycine serves as an antagonist of the GPR158-RGS7-Gβ5 complex by reducing its activity. Because RGS7-Gβ5 is a selective GAP for the inhibitory $G_{i/o}$ proteins, which regulate cAMP production (*39*, *40*), inhibition of RGS7-Gβ5 activity via GPR158 influences cAMP levels. The direction of the effect on the cAMP production is likely determined by the identity of the adenylyl cyclases present in a particular cell, as they are known to be differentially regulated by $G\alpha_i$ and $G\alpha_o$ (via Gβγ) (*41*). Thus, glycine signals via mGlyR by inhibiting inhibitory G protein regulation, thereby generating an excitatory influence. This regulation endows the metabotropic glycinergic system with a distinct feature that makes the degree of its influence scale with the extent of $G_{i/o}$ activation by other GPCR cascades, with its influence increasing upon the increase in $G_{i/o}$ inputs.

The discovery of mGlyR also opens many interesting avenues for exploring the metabotropic influence of glycine and its role in nervous system physiology. Indeed, metabotropic effects of glycine have been anecdotally noted (*6*, *7*, *42*), but molecular and circuit dissection of this influence have been limited. The relatively high affinity of mGlyR for glycine (~3 μM) should allow it to signal without concomitant engagement of GlyRs, which have an order-of-magnitude-lower affinity for glycine, creating an independent neuromodulatory channel (*6*). The mGlyR effects on neurons that we observe are also excitatory, contrasting with the largely inhibitory influence of ionotropic GlyR receptors (*9*, *43*). The two systems likely overlap and are involved in autotuning and homeostatic feedback, as has been noted for other pairs of ionotropic and metabotropic systems. Thus, in the context of intact neural circuitry, glycine likely triggers more complex responses that may involve interplay between ionotropic and metabotropic systems.

Furthermore, we think that glycinergic signaling by means of mGlyR has implications for understanding mood disorders and for the development of new pharmacological strategies. mGlyR is prominently expressed in the medial prefrontal cortex (mPFC) (*25*), a region critically involved in depression (*44*). Glycine and its transporters are also colocalized in the mPFC (*45*–*47*). Both taurine and glycine have been heavily implicated in the pathophysiology of depression (*8*–*10*, *48*) and are dysregulated in plasma of humans diagnosed with major depressive disorder (*10*). Furthermore, taurine has an antidepressant effect on stress-induced depressive rats (*49*). Because these amino acids inhibit mGlyR, and because knockout of mGlyR in mice also results in an antidepressive phenotype and stress resilience (*25*), it seems possible that antidepressant properties of glycine and taurine may be mediated by mGlyR. The ubiquitous nature and multitude of the effects limit the potential of glycine and taurine to be used as medications. However, identification of mGlyR presents a new target for the development of antidepressants that we postulate to be small molecules that selectively inhibit this receptor to avoid possibly related receptors, such as GPR179 in the eye.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S10
Table S1
References (*51*–*65*)
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

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Orphan receptor GPR158 serves as a metabotropic glycine receptor: mGlyR

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A metabotropic glycine receptor

Until now, ion channels were the only receptors known to mediate the inhibitory effects of glycine. However, glycine can also exert modulatory metabotropic effects through as-yet unclear mechanisms. Laboute *et al*. discovered that the orphan receptor GPR158 acts as a metabotropic glycine receptor. In analogy to the well-known metabotropic glutamate receptors (mGluRs), they named it mGlyR. This new receptor is a member of the G protein–coupled receptor family, which signals by altering the concentration of the central second messenger cyclic adenosine monophosphate. Glycine is directly recognized by a ligand-binding Cache domain present in mGlyR and regulates the activity of cortical neurons. This work introduces an additional neuromodulatory component that will give further insights into synaptic transmission. —PRS