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Development of a genetically encoded fluorescent indicator for facilitating deorphanization of GPR52

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eLife Assessment

GPR52 is an orphan receptor implicated in neuropsychiatric disorders, and this study addresses the lack of real-time monitoring tools by developing GPR52-1.0, a genetically encoded fluorescent sensor built on the GRAB platform. The design of the sensor is elegant and the validation is thorough. The authors also utilized the sensor to discover that striatal neuron excitation may activate the sensor, providing exciting new biological insights into GPR52 functional mechanisms. The work could be **useful** to the field if presented in the correct context, but as it stands, the work remains **incomplete** as it overlooks GPR52's well-documented high constitutive activity (*PMID: 32076264*, *PMID: 26384023*), which raises major questions about the sensor's physiological relevance.

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Abstract

GPR52 is an orphan G protein-coupled receptor implicated in psychiatric and neurodegenerative disorders, but its endogenous ligand remains unidentified, limiting the exploration of its physiological functions and therapeutic potential. We pioneered a novel methodology for orphan GPCR ligand discovery utilizing the GPCR-activation-based (GRAB) strategy by developing GPR52-1.0, a genetically encoded fluorescent sensor. GPR52-1.0 exhibits excellent membrane trafficking and high sensitivity in HEK293T cells, cultured neurons, and acute mouse brain slices. Notably, it detects neuronal activity–dependent endogenous ligand release in the striatum, with responses abolished by a specific antagonist. This sensor provides a powerful tool for identifying GPR52's endogenous ligand(s) and enables real-time monitoring of its activation. Our work lays the foundation for uncovering GPR52's physiological roles and supports future efforts to develop GPR52-targeted therapeutics.

Introduction

G protein-coupled receptors (GPCRs) represent one of the largest and most versatile families of membrane proteins, characterized by seven-transmembrane domains and activation by diverse extracellular ligands[1]. Upon ligand binding, GPCRs initiate intracellular signaling cascades that regulate a wide array of processes across organ systems. As such, GPCRs constitute the largest class of drug targets, with over one-third of FDA-approved drugs acting on this receptor family[2]. In the

nervous system, GPCRs are abundantly expressed and critically involved in modulating synaptic transmission, neural excitability, and behavior outputs[3]. These features make them particularly important for understanding brain function and treating neurological and psychiatric conditions.

Despite substantial progress in GPCR research, a significant number of GPCRs remain classified as orphan receptors, with their endogenous ligands unknown[4]. This knowledge gap limits the mechanistic understanding of these receptors and hinders their therapeutic exploitation. Among these orphan GPCRs, GPR52 has emerged as a particularly interesting candidate due to its involvement in neuropsychiatric disorders—including schizophrenia and anxiety—and its potential neuroprotective roles in Huntington's disease, as shown in both genetic and pharmacological studies[5-8]. However, the lack of identified endogenous ligands has posed a major obstacle to further functional and translational exploration.

To address this challenge, we employed the GPCR-Activation-Based (GRAB) strategy[9,10] to develop a genetically encoded fluorescent sensor based on GPR52. In this study, we report the engineering, optimization, and validation of the GPR52-1.0 sensor in live cells and intact brain tissues. We demonstrate its utility in detecting synthetic ligands and, importantly, the sensor revealed endogenous ligand release upon electrical stimulation in the striatum. These findings not only establish a robust platform for the deorphanization of GPR52 but also open new avenues for investigating its physiological functions and accelerating drug discovery efforts targeting this clinically relevant receptor.

Results

Development of a genetically encoded GPR52 sensor

To facilitate the deorphanization of GPR52, we leveraged the GRAB strategy to develop a genetically encoded fluorescent sensor capable of reporting GPR52 activation (Fig. 1A)[11]. Specifically, we first replaced the ICL3 in the human GPR52 receptor with the ICL3 in already existing sensor $GRAB_{NE1m}$ to generate a prototype green GPR52 sensor, and then systematically optimized the linker sequences flanking the circularly permutated green fluorescent protein (cpEGFP) and key residues influencing fluorescence intensity and protein folding in the cpEGFP (Fig. 1B). Through the screening of ~800 sensor variants, we identified GPR52-1.0 as the top-performing construct, which exhibits the highest response ($\Delta F/F_0$) to the application of a selective GRP52 agonist[12] (Fig. 1B).

When expressed in HEK293T cells, GPR52-1.0 was efficiently localized to the plasma membrane and exhibited robust fluorescence increases in response to the GPR52 agonist. This fluorescence increase was almost completely abolished upon co-application of a BI-derived GPR52 inverse agonist[13] (Fig. 1C). Dose–response analysis revealed an apparent half-maximal effective concentration (EC50) of 5 μM for the agonist and a half-maximal inhibitory concentration (IC50) of 75 nM for the antagonist (Fig. 1D and 1E). High-speed line scan imaging demonstrated rapid fluorescence increases upon local puffing of agonist, with an average activation time constant of ~1.1 s (Fig. 1F). Notably, this sensor showed high specificity, showing no response to a panel of neurotransmitters (Fig. 1G). Collectively, these results indicate that GPR52-1.0 is a sensitive and selective tool for monitoring GPR52 activation in living cells.

Characterization of GPR52-1.0 in cultured neurons and acute mouse brain slices

We next expressed GPR52-1.0 in cultured rat cortical neurons and acute mouse brain slices for further characterization. In cultured neurons, GPR52-1.0 exhibited efficient membrane trafficking and retained fluorescence responses, ligand affinity, and specificity comparable to those observed in HEK293T cells (Fig. 2A–2C). To evaluate sensor performance in intact brain tissue, we delivered GPR52-1.0 to the mouse striatum—a brain region with high endogenous GPR52 expression[5]—via adeno-associated virus (AAV) (Fig. 2D). In acute brain slices containing striatum, we observed robust fluorescence signals in response to agonist perfusion, which were

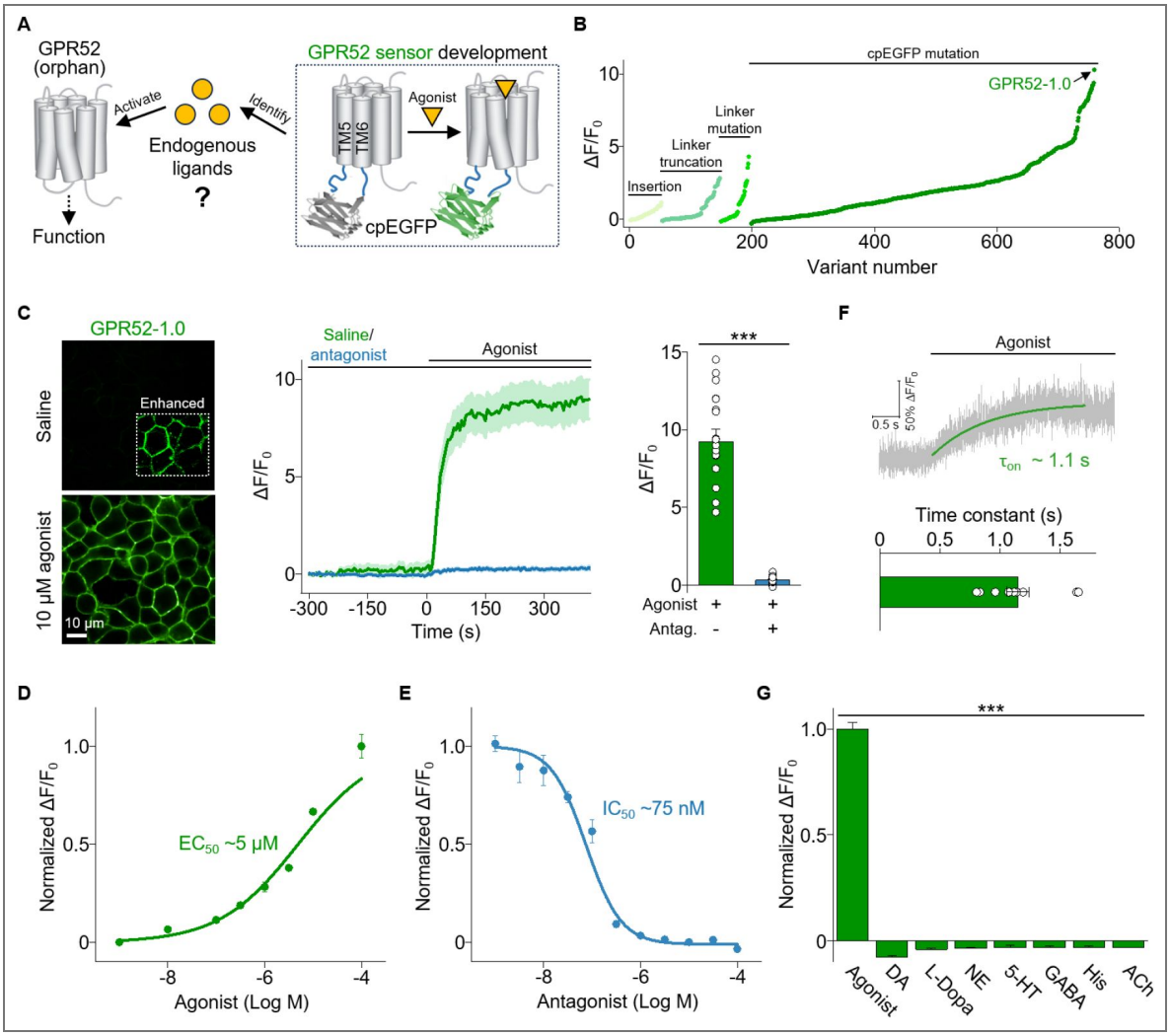

Fig. 1. Development and characterization of GPR52 sensors in HEK293T cells.

(**A**) Schematic drawing shows the strategy of developing GRAB GPR52 sensors to identify endogenous ligands of GPR52. (**B**) Screening and optimization steps of GRAB GPR52 sensors and the fluorescence response to 10 μM synthetic agonist; the black arrow indicates the best candidate GPR52-1.0. (**C**) Example images (left), traces (middle) and quantification (right) of the change in GPR52-1.0 fluorescence in response to 10 μM agonist with (blue) or without (green) antagonist pretreatment; n = 17–20 cells from 3 coverslips; ***$p < 0.001$; Two-Sample *t*-test. (**D**) Normalized dose–response curves measured in HEK293T cells expressing GPR52-1.0, with the corresponding EC_{50} value for agonist shown; n = 3 repeats. (**E**) Normalized dose–response curves measured in HEK293T cells expressing GPR52-1.0, with the corresponding IC_{50} value for antagonist in the presence of 10 μM agonist shown; n = 3 repeats. (**F**) Top, representative fluorescence changes in GPR52-1.0-expressing cells in response to the local perfusion (100 μM agonist in pipette with normal bath solution). Bottom, group data summarizes on time constants measured upon application of agonist; n = 10 cells from 3 coverslips. (**G**) Normalized fluorescence change in response to the indicated compounds (each at 10 μM) measured in cells expressing GPR52-1.0. DA, dopamine; L-Dopa, levodopa; NE, norepinephrine; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid; His, histamine; ACh, acetylcholine; n = 3 repeats; ***$p < 0.001$; One-Way ANOVA. In this and subsequent figures, unless indicated otherwise summary data are presented as the mean±SEM.

blocked by antagonist treatment (Fig. 2D). Collectively, these data suggest that GPR52-1.0 is functional in native neuronal environments and suitable for detecting ligand-induced activation in brain tissue.

GPR52-1.0 reports endogenous GPR52 ligand release

To investigate whether GPR52-1.0 can detect endogenously released ligands, we virally expressed the sensor in the mouse striatum and prepared acute brain slices 3 weeks post-injection (Fig. 3A). Upon electrical stimulation, we observed robust fluorescence increases of GPR52-1.0 (Fig. 3B1–3B3). Critically, these responses were significantly reduced by co-application of the GPR52 antagonist, indicating that the observed signal was specific to GPR52 activation (Fig. 3B2–3B3). Taken together, these results support the existence of neuronal activity-dependent endogenous molecules capable of activating GPR52 in the striatum.

Discussion

Orphan GPCRs, including GPR52, remain underexplored despite their therapeutic relevance in brain disorders. Identifying endogenous ligands is essential not only for understanding their native signaling mechanisms but also for unlocking new drug targets. In this study, we report the development of a genetically encoded fluorescent sensor, GPR52-1.0, which enables dynamic visualization of GPR52 activation in living cells and intact brain tissue. Critically, we pioneered a novel methodology for orphan GPCR ligand discovery utilizing GRAB sensors. The sensor revealed endogenous ligand-induced GPR52 activation upon electrical stimulation in mouse striatal slices.

By enabling real-time tracking of GPR52 activation, GPR52-1.0 serves as a valuable tool to guide biochemical purification or genetic screens for endogenous ligands. Such discoveries could advance our understanding of GPR52's role in brain function and its contribution to neuropsychiatric and neurodegenerative diseases. Furthermore, the sensor offers a high-throughput platform for screening pharmacological modulators, accelerating the development of GPR52-targeted therapies. More broadly, this approach exemplifies how GRAB-based sensors can facilitate the deorphanization of GPCRs, advancing both basic neuroscience and translational drug discovery.

While our current study stops short of fully deorphanizing GPR52, the sensor we developed lays the groundwork for achieving this in future experiments. The observed activity-dependent activation in striatal slices strongly suggests the presence of endogenous ligands, and GPR52-1.0 provides a robust platform to identify them. A logical next step would involve biochemical fractionation of brain tissue extracts, particularly from the striatum, followed by activity-guided screening using the sensor to detect fractions that elicit fluorescence responses. These active fractions could then be analyzed using mass spectrometry to identify candidate molecules. Together, these strategies would enable systematic identification and validation of endogenous GPR52 ligands, ultimately advancing the deorphanization process and opening new avenues for therapeutic exploration.

Methods

Animals

C57BL/6N mice (6 to 8 weeks of age) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd., and group-housed under a 12-hours/12-hours light/dark cycle with a 25°C ambient temperature. All animal experiments were approved by the Animal Care and Use Committee of Peking University School of Life Sciences.

Compounds

The GPR52 agonist used here was synthesized based on the structure described in WO 012020738[12]. The GPR52 antagonist was previously published[13]. Both compounds were synthesized and provided by Boehringer Ingelheim. Compounds were dissolved in water or DMSO

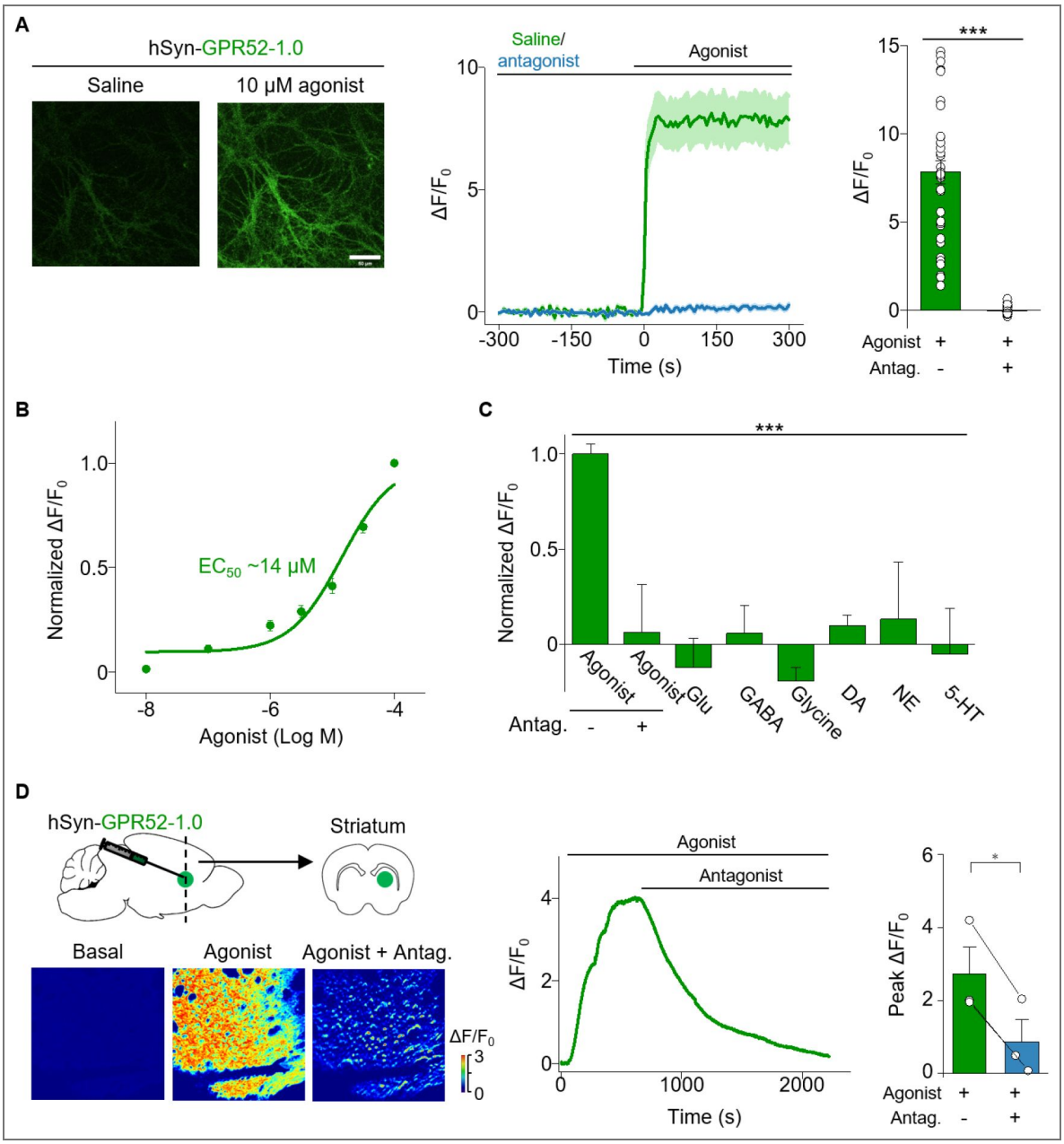

Fig. 2. Characterization of GPR52-1.0 in cultured neurons and acute mouse brain slices.

(**A**) In GPR52-1.0-expressing neurons, example images (left), traces (middle) and quantification (right) of the change in GPR52-1.0 fluorescence in response to 10 μM agonist with (blue) or without (green) antagonist pretreatment; n = 35–36 neurons from 3 coverslips; ***$p < 0.001$; Two-Sample *t*-test. (**B**) Normalized dose–response curves measured in neurons expressing GPR52-1.0, with the corresponding EC_{50} value for agonist shown; n = 3 repeats. (**C**) Normalized fluorescence change in response to the indicated compounds (each at 10 μM) measured in neurons expressing GPR52-1.0. When indicated, the antagonist was also added. Glu, glutamate; n = 3 repeats; ***$p < 0.001$; One-Way ANOVA. (**D**) Left: top, schematic illustration depicting the experimental design of virally expressing GPR52-1.0 in the striatum; bottom, representative pseudo-color images of the fluorescence change in GPR52-1.0-expressing acute mouse brain slices; where indicated, 10 μM agonist and 10 μM antagonist were applied. Middle, a representative trace; where indicated, agonist and antagonist were added. Right, summary data; n = 3 slices; s; *$p < 0.05$; Paired *t*-test.

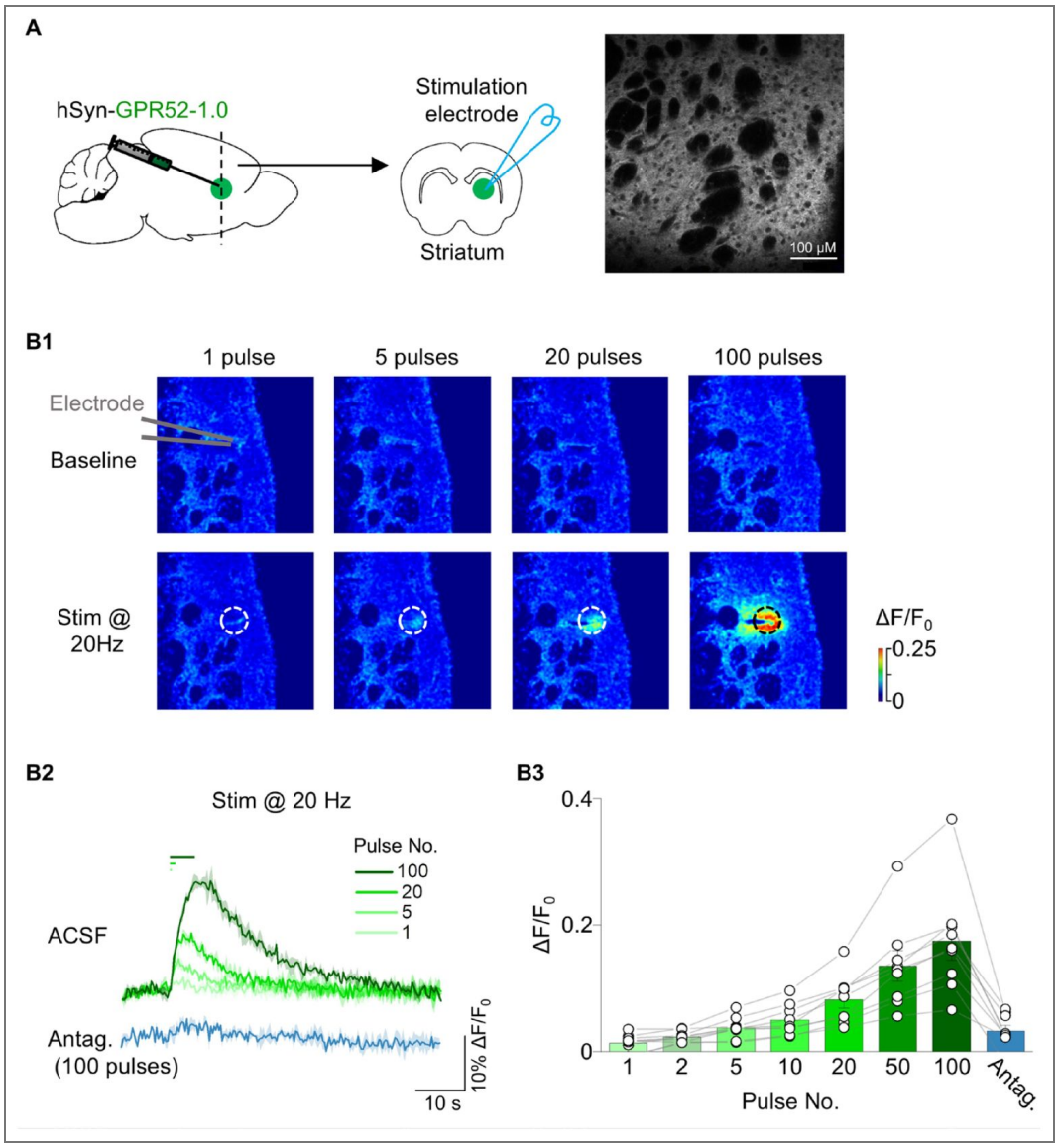

Fig. 3. GPR52-1.0 can report the release of GPR52 endogenous ligands *ex vivo*.

(**A**) Left, schematic illustration depicting the experimental design in the striatum for (B1–B3); right, a representative image of GPR52-1.0-expressing brain slices. (**B1**) Representative pseudo-color images of GPR52-1.0-expressing brain slices at baseline (top) and in response to the indicated stimuli (bottom) in the presence of artificial cerebrospinal fluid (ACSF). The dashed circles indicate the ROI used to calculate the response, and the approximate location of the stimulating electrode is indicated. (**B2–B3**) Representative traces (B2) and summary data (B3) for the change in GPR52-1.0 fluorescence in response to the indicated stimuli in ACSF or antagonist; n = 9 slices.

at stock concentrations of 10 mM or 100 mM and stored at −20 °C.

Cell culture

HEK293T cells (CRL-3216, ATCC) were used to express and test sensors. All cell lines were cultured in DMEM (Gibco) supplemented with 10% (vol/vol) FBS (Gibco) and 1% penicillin-streptomycin (Gibco) at 37 °C in 5% CO_2.

Primary neuronal cultures

Rat cortical neurons were cultured from postnatal day (P) 0 Sprague–Dawley rat pups of both sexes (Beijing Vital River). Specifically, the brain was removed and the cortex was dissected; neurons were then dissociated in 0.25% trypsin-EDTA (Gibco), plated on 12-mm glass coverslips coated with poly(d-lysine) (Sigma-Aldrich) and cultured in neurobasal medium (Gibco) containing 2% B-27 supplement, 1% GlutaMax (Gibco) and 1% penicillin-streptomycin (Gibco) at 37 °C in 5% CO2.

Fluorescence imaging of cultured cells

An inverted confocal microscope (Nikon) equipped with NIS-Elements 4.51.00 software (Nikon), a ×40/1.35-NA oil-immersion objective, a 488-nm laser and a 561-nm laser was used for imaging; the GFP and RFP signals were collected using 525/50-nm and 595/50-nm emission filters, respectively. Cultured cells expressing GRAB GPR52 sensors were either bathed or perfused with Tyrode's solution containing (in mM) 150 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 10 glucose (pH 7.4); where indicated, drugs and other compounds were delivered via a custom-made perfusion system or via bath application. An Opera Phenix high-content screening system (PerkinElmer) equipped with a ×40/1.1-NA water-immersion objective, a 488-nm laser and a 561-nm laser was also used for imaging; the GFP and RFP signals were collected using 525/50-nm and 600/30-nm emission filters, respectively. The Harmony 4.9 software of the Opera Phenix high-content screening system (PerkinElmer) was used for data collection. For imaging, the fluorescence signals of the candidate GRAB GPR52 sensors were calibrated using the GFP/RFP fluorescence ratio. To measure the response kinetics of the GPR52-1.0 sensor, the line-scanning mode of the confocal microscope was used to record rapid changes in fluorescence; a glass pipette containing 100 μM agonist was placed near the surface of HEK293T cells expressing GPR52-1.0, and agonist was puffed onto cells to measure τ_{on}.

Preparation and fluorescence imaging of mouse acute brain slices

Wild-type C57BL/6N mice were deeply anesthetized by an intraperitoneal injection of avertin (500 mg kg^{-1}; Sigma-Aldrich) and then placed in a stereotaxic frame for injection of AAVs using a microsyringe pump (Nanoliter 2000 Injector, WPI). hSyn-GPR52-1.0 AAVs were injected (300 nl) into the striatum of mice using the following coordinates: anteroposterior (AP), +1.0 mm relative to bregma; mediolateral (ML), −1.0 mm; dorsoventral (DV), −3.1 mm from the dura.

Three weeks after viral injection, mice were again deeply anesthetized with an intraperitoneal injection of avertin and transcardial perfusion was performed using cold oxygenated slicing buffer containing (in mM) 110 choline chloride, 2.5 KCl, 1 NaH_2PO_4, 25 $NaHCO_3$, 7 $MgCl_2$, 25 glucose, 0.5 $CaCl_2$, 1.3 sodium ascorbate and 0.6 sodium pyruvate. Brains were then rapidly removed and immersed in the oxygenated slicing buffer, after which the cerebellum was trimmed using a razor blade. The brains were then glued to the cutting stage of a VT1200 vibratome (Leica) and sectioned into 300-μm-thick coronal slices. Brain slices containing the striatum were incubated at 34 °C for at least 40 min in oxygen-saturated artificial cerebrospinal fluid (ACSF) buffer containing (in mM) 125 NaCl, 2.5 KCl, 1 NaH_2PO_4, 25 $NaHCO_3$, 1.3 $MgCl_2$, 25 glucose, 2 $CaCl_2$, 1.3 sodium ascorbate and 0.6 sodium pyruvate. For two-photon imaging, the slices were transferred into an imaging chamber in an FV1000MPE (Olympus) microscope equipped with a ×25/1.05-NA water-immersion objective and a mode-locked Mai Tai Ti:Sapphire laser (Spectra-Physics) tuned to 920 nm with a 495-to 540-nm filter to measure fluorescence. FV10-ASW Ver.3.1a software for the FV1000MPE two-photon microscope (Olympus) was used for data collection. For

electrical stimulation, a homemade bipolar electrode (WE30031.0A3, MicroProbes) was placed onto the surface of the brain slice near the striatum under fluorescence guidance. Imaging and stimulation were synchronized using an Arduino board with a custom-written program. All other stimulation experiments were recorded at video frame rates of 0.3583 s per frame, with 256 × 192 pixels per frame. The stimulation voltage was set at 5–8 V, and the duration of each stimulation was 1 ms. Where applicable, compounds were applied to the imaging chamber by perfusion in ACSF at a flow rate of 4 ml min^{-1}.

Statistical analysis

Statistical analyses were performed using OriginPro 2020. Group data were analyzed using Two-Sample *t*-Test, Pair-Sample *t*-Test, One-Way ANOVA, or Mann-Whitney *U* test, and differences were considered significant at $p < 0.05$. Unless indicated otherwise, all summary data are presented as the mean±SEM.

Data availability

All data supporting the findings of this study are available within the main text or the Supplementary Information.

Additional information

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Peer reviews

Reviewer #1 (Public review):

Summary:

GPR52 is an orphan receptor implicated in neuropsychiatric disorders; however, the absence of tools capable of monitoring GPR52 activity in real time has stalled both mechanistic research and ligand discovery. This study addresses this gap by reporting the development of GPR52-1.0, a genetically encoded fluorescent sensor designed to detect activation of GPR52. The sensor was systematically engineered using the established GRAB platform, yielding a construct with micromolar sensitivity and high selectivity in cell culture. The authors largely achieve their stated aims, however the biological relevance of their aims is unclear, as GPR52 is reported to be a constitutively active receptor (PMID: 32076264, PMID: 26384023). GPR52-1.0 is a validated, specific, and sensitive sensor that functions in vitro and ex vivo. The claim that electrically stimulated endogenous GPR52 ligand release occurs in the striatum is supported by the specificity of the GPR52 antagonist block using ex vivo brain slices, however, once again this aim is clouded by evidence that GPR52 is constitutively active. The sensor is presented as a tool for future deorphanization; however, this assumes that the physiological ligand is an agonist, which is unclear based on the evidence that GPR52 is constitutively active. If the authors can explain or adapt their experiments and manuscript in the context of GPR52 constitutive activity, this will be useful work to the community. The impact of this work is likely to be moderate to high within the specialized communities studying orphan GPCRs, neuronal signaling, and neuropsychiatric disease. The GRAB sensor strategy has already generated widely adopted tools for other receptors, and a validated GPR52 sensor would fill a genuine gap. The GRAB technology makes GPR52-1.0 directly applicable to in vivo studies. It is likely that GPR52-1.0 could be replicated for other orphan receptors to facilitate their deorphanization.

Strengths:

(1) Systematic and rigorous sensor optimization and characterization by screening ~800 variants with iterative linker and cpEGFP mutation step. The resulting EC50 values are characterized in HEK293T and cultured neurons.

(2) Testing GPR52-1.0 against a broad panel of neurotransmitters with no detectable off-target activation strengthens confidence in sensor specificity.

(3) The use of a selective antagonist to confirm specificity, both in cell lines and in brain slices, strengthens the conclusions significantly.

(4) Electrically stimulated GPR52-1.0 fluorescence changes in ex vivo striatal slices are blocked by a GPR52 antagonist. This is the most biologically significant result in the manuscript, as GPR52-related diseases can involve the striatum.

Weaknesses:

(1) The work, both experimentally and in its presentation, is not put into the context of what is known about GPR52 pharmacology and signaling. It is reported by multiple groups that GPR52 has high constitutive activity and does not require a ligand for high levels of signaling (PMID: 32076264, PMID: 26384023). The authors should clarify whether GPR52-1.0 senses constitutive activation and whether baseline fluorescence is stable over the timescale of their experiments. The cell and mouse work needs to be reframed and conducted in the context of the high basal activity of the receptor, or the authors need to explain the differences between their study and other studies.

(2) The electrical stimulation used in brain slice experiments is non-specific. This could be activating many cell types and neurotransmitter systems simultaneously. The pharmacological block by the GPR52 antagonist is reassuring, but the identity of the molecules driving the signal remains unknown. It could be that GPR52 is constitutively active, and that the electrical stimulation drives higher expression of GPR52 and thus constitutive signaling. This constitutive signaling can then be inhibited by the GPR52 antagonist. In this scenario, there would be no endogenous GPR52 agonist invoked by electrical stimulation.

(3) The ex vivo brain slice data rely on n=9 slices without reporting the number of animals that the slices come from. Given the importance of this result, more biological replicates and clear reporting of animal numbers would strengthen confidence.

(4) The manuscript does not benchmark GPR52-1.0 against existing approaches (e.g., HTRF, BRET, or calcium mobilization assays) to contextualize its advantages in a drug-discovery or screening workflow.

(5) The paper's title references deorphanization, but the authors have made no attempts toward this deorphanization. No candidate ligand molecules are identified or tested.

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Reviewer #2 (Public review):

Summary:

This study describes the development of GPR52-1.0, a novel genetically encoded fluorescent sensor for the orphan GPCR, GPR52. The authors also utilized this sensor in vivo in brain slices and discovered that striatal neuron excititation may activate GPR52.

Strengths:

(1) The design and validation of the sensor are elegant, thorough, and rigorous. The authors conducted a systematic and impressive optimization screen of numerous variants to arrive at the top-performing GPR52-1.0 sensor. The subsequent characterization is thorough, showing excellent membrane trafficking, appropriate pharmacological profiles (EC50, IC50) by the GPR52 chemical agonist/antagonist, rapid kinetics, and high specificity against a panel of common neurotransmitters. The functional characterization was also performed in multiple experimental systems.

(2) The most exciting result is the observation that electrical stimulation may activate GPR52 in the striatum, an area where GPR52 is natively expressed. The blockade by a specific GPR52 antagonist confirms its specificity and provides the first direct evidence for activity-dependent, native GPR52 ligand in striata. This finding alone is a significant step forward and strongly justifies the sensor's development.

(3) The manuscript is well-written and logically structured. The figures are clear and effectively illustrate the key data, from the initial screening process to the final ex vivo validation. The authors did not overstate their discoveries.

Weaknesses:

(1) The sensor specificity is largely based on a single agonist/antagonist, and it might be desired for future studies to confirm this by additional agonists/antagonists or by point mutagenesis that is known to influence GPR52 activation (for example, the ones reported in (PMID: 40087539).

(2) The discovery of the existence of activity-dependent, native GPR52 ligand(s) in striata is extremely exciting. This might be further strengthened by inhibiting synaptic transmitter release with TTX, calcium channel blockers, or SNARE complex disruptors, etc.

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