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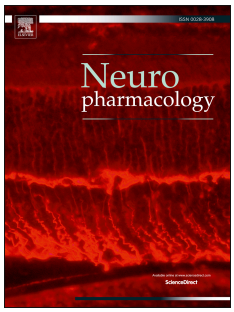

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Nicolas Senese, Max Oginsky, Richard R. Neubig, Carrie Ferrario, Emily M. Jutkiewicz, John R. Traynor

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Role of hippocampal 5-HT1A receptors in the antidepressant-like phenotype of mice expressing RGS-insensitive Gαi2 protein

[1]Nicolas Senese, [1]Max Oginsky, [2]Richard R. Neubig, [1]Carrie Ferrario, [1]Emily M. Jutkiewicz and [1]John R. Traynor

[1]Department of Pharmacology and Edward F Domino Research Center, University of Michigan, Ann Arbor, MI 48109. [2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48823.

Corresponding Author:

John Traynor

Department of Pharmacology

University of Michigan Medical School

Ann Arbor

MI 48109-5632

Phone: 734-647-7479

e-mail: jtraynor@umich.edu

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Abstract

A single base mutation in the $G\alpha_{i2}$ protein (G184S) renders this Gα subunit insensitive to the negative modulatory effects of Regulator of G-protein Signaling (RGS) proteins. Mice expressing this RGS insensitive (RGSi) variant of $G\alpha_{i2}$ (RGSi $G\alpha_{i2}$) display a spontaneous antidepressant-like phenotype that is reversible by treatment with the 5-HT1A receptor (5-HT1AR) antagonist WAY100635. Here we test the hypothesis that increased activity of 5-HT1ARs in the hippocampus of RGSi $G\alpha_{i2}$ knock-in mice is responsible for the expression of the observed antidepressant-like behavior. We administered the 5-HT1AR antagonist WAY100635 or the agonist 8-OH-DPAT *via* bilateral intra-hippocampal infusion cannulae and evaluated antidepressant-like behavior using the tail suspension test (TST). WAY100635 reversed the antidepressant-like phenotype of the RGSi $G\alpha_{i2}$ knock-in mice and 8-OH-DPAT produced an antidepressant-like response in wild type mice that was blocked by systemic WAY100635. Furthermore, intra-hippocampal infusion of the RGS4/19 inhibitor CCG-203769 produced an antidepressant-like effect in female mice. Ex-vivo slice recording confirmed the 5-HT1AR-mediated decrease in hippocampal CA1 pyramidal neuron excitability was enhanced in the RGSi $G\alpha_{i2}$ knock-in mice. There was no change in hippocampal 5-HT1AR expression as measured by ligand binding or in the ability of 8-OH-DPAT to activate G-protein as measured in the [^{35}S]GTPγS binding assay. The findings demonstrate that RGS protein control of hippocampal 5-HT1AR signaling is necessary and sufficient to account for the antidepressant-like phenotype in the RGSi $G\alpha_{i2}$ knock-in mice and that RGS proteins highly expressed in the hippocampus should be investigated as targets for novel antidepressant therapies.

1. Introduction

Although selective serotonin reuptake inhibitors (SSRIs) are widely used in psychiatric treatments this class of drugs suffers from serious drawbacks including limited clinical efficacy and a long delay between the initiation of treatment and the onset of therapeutic effects. This lag period is thought to be caused by the need to activate and subsequently desensitize serotonin 1A (5-HT1A) autoreceptors located predominantly in the raphe nucleus (Hjorth et al., 2000). However, a substantial body of research has identified 5-HT1A receptors (5-HT1ARs) on postsynaptic site, so called heteroreceptors, in the frontal cortex and hippocampus as potential mediators of the beneficial effects of serotonergic antidepressants (Celada et al., 2013). The involvement of 5-HT1ARs in both the therapeutic and negative effects of SSRIs has hindered the development of new antidepressant therapies which maintain the beneficial effects of SSRIs while avoiding their drawbacks.

The 5-HT1AR is a typical 7-transmembrane domain G-protein-coupled receptor (GPCR) with high expression throughout the brain (Ito et al., 1999). The 5-HT1AR couples to heterotrimeric G proteins comprised of Gαi/o and βγ subunits and as such its signaling is moderated by the regulators of G-protein signaling (RGS) proteins (Beyer et al., 2004; Ghavami et al., 2004; Wang et al., 2014). RGS proteins are a family of intracellular proteins that regulate G-protein function by directly interacting with and inactivating heterotrimeric G-proteins (Berman et al., 1996). RGS proteins have GTPase accelerating (GAP) activity which promotes the hydrolysis of active Gα-GTP to form inactive Gα-GDP. This allows for reformation of the inactive Gαβγ heterotrimer thus halting downstream signaling activity of both the Gα and βγ subunits.

The high degree of functional redundancy between individual RGS proteins has provided a significant hurdle to understanding the specific function of individual RGS proteins (Dong et al., 2000) (Doupnik et al., 2001; Chen et al., 2010). In order to overcome this issue a series of RGS insensitive (RGSi) Gα protein variants have been developed (Lan et al., 1998; Huang et al., 2006). These mutant Gα proteins have a single base mutation (Gly to Ser) at the conserved site where RGS proteins interact with their cognate Gα subunit (Tesmer et al., 1997). For $G\alpha_{i2}$ this is Gly184. The mutation prevents interaction of the Gα protein with all RGS proteins while maintaining normal enzyme kinetics and interactions with receptor and downstream effectors (Fu et al., 2004; Clark et al., 2003) and so provides the opportunity to determine the effect of removing RGS control of a specific Gα protein.

Homozygous mice expressing the RGSi $G\alpha_{i2}$ protein ($G\alpha_{i2}{}^{GS/GS}$) display a baseline antidepressant-like phenotype which is fully reversible by 5-HT1AR antagonist treatment (Talbot et al., 2010), suggesting an important role for $G\alpha_{i2}$ and RGS proteins downstream of the 5-HT1AR. However, hypothermia, an action traditionally associated with 5-HT1A autoreceptor activation in the raphe nucleus, is not affected by the mutation, indicating that postsynaptic 5-HT1A heteroreceptors are the important mediators of the antidepressant-like behavior in the $G\alpha_{i2}{}^{GS/GS}$ mice. Identifying the brain locus responsible for the antidepressant-like phenotype in these mice would be an important step forward and allow us to study individual RGS proteins expressed in regions of the brain that regulate $G\alpha_{i2}$ downstream of the 5-HT1AR.

The hippocampus and frontal cortex of the $G\alpha_{i2}{}^{GS/GS}$ mice have increased levels of the Ser-9 phosphorylated version of glycogen synthase kinase-3β (GSK3β) that is reversed by treatment

with the 5-HT1AR antagonist WAY100635 (Talbot et al., 2010). GSK3β is a neurogenic factor that is phosphorylated by antidepressant drugs and may contribute to their therapeutic effects (Malberg et al., 2000; Tsai et al., 2008). The increased levels of phospho-GSK3β in the $G\alpha_{i2}^{GS/GS}$ mice suggest the hippocampus and/or frontal cortex as potential critical sites of their 5-HT1AR dependent antidepressant-like behavior. To test this hypothesis, we targeted the hippocampus of wild type and $G\alpha_{i2}^{GS/GS}$ mice and measured antidepressant-like behavior using the tail suspension test (TST). We find that hippocampal microinjection of a 5-HT1AR antagonist fully reverses the $G\alpha_{i2}^{GS/GS}$ antidepressant-like phenotype, while hippocampal injection of a 5-HT1AR agonist to wild type animals produces effects consistent with the RGSi $G\alpha_{i2}^{GS/GS}$ behavioral phenotype. We also show that 5-HT1AR agonists have an enhanced inhibitory effect on the intrinsic excitability of CA1 hippocampal neurons from heterozygous $G\alpha_{i2}^{+/GS}$ mice. Finally, we demonstrate that inhibiting an RGS protein with high hippocampal expression can produce an antidepressant-like effect in female wild type mice. No changes in 5-HT1ARs in the hippocampus of the RGSi $G\alpha_{i2}$ mice compared to their wild type littermates were observed.

2. Materials and Methods

2.1 Drugs

(R)-(+)-8-OH-DPAT hydrobromide ((R)-(+)-2-Dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide) and WAY100635 maleate salt (N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt) were purchased from Sigma-Aldrich(St Louis, MO). CCG-203769 was synthesized as previously described (Blazer et al., 2015). [^{3}H] 8-OH-DPAT was from Perkin Elmer (Waltham, MA).

2.2. Animals

Wild type ($G\alpha_{i2}^{+/+}$), heterozygous ($G\alpha_{i2}^{+/GS}$) and homozygous ($G\alpha_{i2}^{GS/GS}$) RGSi $G\alpha_{i2}$ knock-in mice were derived from heterozygous breeding as described previously (Huang et al., 2006). Animals were backcrossed onto the C57BL/6J background strain for four generations. Wild type (+/+) and heterozygous (+/GS) RGSi $G\alpha_o$ knock-in mice were generated as described previously on a 129S1/SvIMJ background (Fu et al., 2004; Fu et al., 2006; Huang et al., 2006; Goldenstein et al., 2009). As mice on the 129S1/SvIMJ background typically produce small, inconsistent litters heterozygous female RGSi $G\alpha_o$ knock-in mice on a 129S1/SvIMJ were bred with male wild type C57BL/6J mice. The resulting F1 mice were used for experiments involving RGSi $G\alpha_o$. All animals were between 8 and 16 weeks of age at time of testing, and animals were age and sex matched in each experiment. In experiments where sex is not specified a pilot experiment was performed to identify potential sex differences. If no difference was observed results from male and female animals were pooled. Mice were group housed with up to five same-sex littermates per cage. The vivarium was maintained on a 12-hour light/dark cycle with lights on at 7:00 AM. All testing occurred during the light phase. Drugs were typically administered i.p. 30 min before testing unless otherwise indicated. All experimental procedures were approved by the University of Michigan Institutional Animal Care and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Council 2011).

2.3 Intra-hippocampal infusions

Mice were anesthetized with a combination of Ketamine (100mg/kg i.p.) and Xylazine (10mg/kg i.p.). Carprofen (5mg/kg s.c.) was administered before and every 24 h following surgery for 48 h as an analgesic. Mice were placed into the stereotax (Kopf Instruments Model 902 Dual Small

Animal Stereotaxic Instrument) and a midline incision made over the top of the skull. Bregma and lambda were located and marked to determine implant position. Bilateral implant coordinates were 1.5 mm posterior, 1.0 mm ventral and 1.0 mm distal from the midline on both sides. Bilateral guide cannulae were custom ordered from Plastics One Inc. with a center-to-center distance of 2.0 mm between each cannula arm and with 2.0 mm cannula arm length. In order to prevent blockage within the guide cannula a bilateral dummy cannula was kept in the guide cannula at all times following surgery except during intra-hippocampal infusions. Animals were allowed to recover for at least seven days following surgery before any experimental testing took place. Any animals that showed signs of distress during this period were removed from the experiment and euthanized. Following experimental testing, a solution containing Fast Green FCF dye was infused through the cannula. Brains were then dissected and rapidly frozen before sectioning. When staining indicated a misplaced cannula, data from this animal was excluded from the experiment.

2.4. Intra-hippocampal infusions

Immediately before infusion animals were placed in a drop jar containing isoflurane and breathing was monitored until rate reached approximately one breath per second. A bilateral injection cannula attached by flexible plastic tubing to two Hamilton syringes (Hamilton #86274 syringe) was then inserted through the guide cannula. A 500 nl infusion was then delivered to each side at a rate of 250 nl per second using a syringe pump (NE-300 pump, New Era Pump Systems Inc.). Following infusion, the injection cannula remained in place for a further two minutes to prevent backflow away from the infusion site. Drugs were administered 30 min before testing unless otherwise indicated. For experiments involving repeated intra-hippocampal

infusions this process was repeated once every 24 h for three days, and experimentation occurred 30 min after the final infusion.

2.5 Tail suspension test

A piece of adhesive tape was affixed to the distal end of the mouse' tail and attached to a metal bar elevated 30cm above the table surface (Steru et al., 1985). Behavior was video-recorded for 6 min and later scored for immobility time. Immobility was defined as any period without continuous movement. Isolated head movements, and swinging without other movement were also defined as immobile.

2.5 Hypothermia

Mice were moved from group housing to individual cages 2 h before testing with free access to food and water. Baseline temperatures were taken once every ten min for thirty min before experimental treatment. Temperatures were determined using a Tcat 2df controller rectal thermometer (Physitemp Clifton, NJ) inserted to a 20 mm probe depth. All animals in this study received both an i.p. and an intra-hippocampal injection, with the i.p. injection immediately following the third baseline measurement and intra-hippocampal infusion immediately following i.p. injection. Drug effects were determined 10, 20 and 30 min after injection.

2.6. Ex-vivo hippocampal cell recordings

Whole cell patch clamp recordings of hippocampal CA1 neurons were made from wild type and heterozygous RGSi $G\alpha_{i2}$ knock-in mice (5-8 weeks of age). Mice were anesthetized with isofluorane and brains were rapidly removed and placed in ice-cold oxygenated (95% O_2-5%

CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 200 sucrose, 25 $NaHCO_3$, 12.5 glucose, 1.25 NaH_2PO_4, 3.5 KCl, 1L-ascorbic acid, 0.5 $CaCl_2$, 3 $MgCl_2$, 305 mOsm, pH 7.4. Coronal slices (300 μm) containing the hippocampus were made using a Leica VT1200 vibratory microtome (Leica Biosystems, Buffalo Grove, IL, USA) and allowed to rest in oxygenated aCSF for at least 40 min before recording. For the recording aCSF, $CaCl_2$ was increased to 2.5 mM and $MgCl_2$ was decreased to 1 mM. Patch pipettes were pulled from 1.5 mm borosilicate glass capillaries (WPI, Sarasota, FL) to a resistance of 3–7 MΩ with a horizontal puller (Model P97, Sutter Instruments, Novato, CA, USA) and filled with a solution containing (in mM): 130 K-methanesulfonate, 10 KCl, 0.4 EGTA, 2 $MgCl_2$, 2 Mg^{2+}-ATP, 0.25 Na^{3+}-GTP, and 10 HEPES, pH 7.3, 285 mOsm when performing current clamp experiments. CA1 hippocampal neurons were identified based on their response to current injection (−200 to 140 pA, 10 pA increments, 500 ms). Neuronal excitability was determined by measuring the number of action potentials elicited by each depolarizing current injection. Input resistance (IR) was determined by the change in voltage from 0 pA to -170 pA current injections. Rheobase is defined as the minimum amount of current injection to elicit an action potential.

2.7 Hippocampal membrane preparation

Mice were sacrificed by cervical dislocation followed by decapitation and the brain was removed. The hippocampus was then immediately dissected. Hippocampi from 6-8 mice matched for age, gender and genotype were pooled to obtain sufficient tissue, homogenized in ice cold 50 mM Tri-HCl buffer pH 7.4 and membrane homogenates prepared as described previously (Lester and Traynor 2006). Protein concentration was determined using a BCA assay kit (Thermo Scientific, Rockford, IL).

2.8 5HT1A receptor binding

Hippocampal membrane homogenates (100 μg protein/well) were incubated in 50 mM pH 7.4 Tris-HCl buffer with eight concentrations of the 5HT1A receptor ligand [^{3}H] 8-OH-DPAT ranging in concentration from 0.16 nM to 20 nM. Non-specific binding was determined using 10 μM WAY-100635. The assay was incubated for 60 min at room temperature before filtration through a Whatman GF/C filters using a MLR-24 Brandel harvester. Bound radioactivity was then determined by scintillation counting with a Wallac 1450 Microbeta counter (Perkin Elmer). Each condition was performed in triplicate and independently replicated three times. Data were analyzed using GraphPad Prism 7.0 (GraphPad; La Jolla, CA).

2.9 Western blotting

Hippocampal homogenates containing 20 μg of protein were mixed with sample buffer (63 mM Tris-HCl, pH 6.8, with 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.008% bromophenol blue, and 50 mM dithiothreitol) and 50 mM pH 7.4 Tris-HCl buffer to a total volume of 25 μl. Samples were then separated by SDS-PAGE using polyacrylamide gels and transferred to a nitrocellulose membrane (Pierce). Membranes were probed with primary antibodies against each Gα subtype ($G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, Gβγ (1-6) and RGS19; Santa Cruz Biotechnology). Each membrane was also stripped and re-probed for α-tubulin or β-actin as loading control (Sigma-Aldrich). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibodies were used for chemiluminescent detection in combination with SuperSignal™ West Pico Chemiluminescent Substrate (ThermoFisher Scientific). Signal intensity was determined using Image J software (http://rsbweb.nih.gov/ij/index.html).

2.10. Statistical analysis

GraphPad Prism 7.0 (GraphPad; La Jolla, CA) was used to analyze all reported data. Two-way analysis of variance (2-way ANOVA) was used to analyze data involving two independent drug treatments as well as data involving a genetic variable and a drug treatment. Prior to all parametric comparisons, normality of the data distribution was evaluated using the Shapiro-Wilk normality test. Post-hoc tests were Tukey's or Sidak's multiple comparisons. Experiments involving only two conditions were analyzed by Student's t-test. Threshold for significance was $p < 0.05$ for all experiments. In saturation binding experiments affinity (Kd) and maximal binding (Bmax) were obtained using a one-site saturation binding curve with Hill slope set to 1 as described previously (Lamberts et al., 2013).

3. Results

3.1 Effects of intra-hippocampal WAY100635 on tail suspension test responses in wild type and $G\alpha_{i2}{}^{GS/GS}$ mice

Homozygous $G\alpha_{i2}{}^{GS/GS}$ knock-in mice given saline bilaterally into the hippocampus exhibited less immobility (92.7 ± 27.5 s) in the TST than their wild type littermates (183 ± 7.3 s; Figure 1), confirming that the intra-hippocampal microinjection procedure does not disrupt the previously described antidepressant-like phenotype in these mice. Bilateral intra-hippocampal administration of WAY100635 (3 μg each side) fully reversed the reduction in immobility back to levels seen in wild type littermates (186 ± 13.3 s). WAY100635 similarly administered to wild type littermates had no effect (186 ± 11.2 s; Figure 1). Two-way ANOVA revealed a

significant interaction (WAY100635 x genotype, $F(1,22) = 7.34$, $p = 0.013$) and significant main effects of genotype ($F(1,22) = 9.5$, $P = 0.006$) and WAY100635 ($F(1,22) = 7.05$, $p = 0.01$).

3.2 Effects of intra-hippocampal 8-OH-DPAT on tail suspension test immobility and hypothermia in wild type mice

The reversal of the $G\alpha_{i2}^{GS/GS}$ behavioral phenotype by intra-hippocampal WAY100635 suggests the hippocampus as a primary site of the increased 5-HT1AR signaling and thus of the antidepressant-like phenotype. Therefore, we sought to test if we could mimic this behavioral phenotype by administration of the 5-HT1AR agonist 8-OH-DPAT directly into the hippocampus of wild type $G\alpha_{i2}^{+/+}$ mice. Intra-hippocampal 8-OH-DPAT (3 μg) bilaterally into the hippocampus of $G\alpha_{i2}^{+/+}$ mice resulted in an immobility time of 51.3 ± 11.5 s. This action of 8-OH-DPAT was attenuated by systemic (s.c) administration of 0.1 mg/kg WAY100635 (Figure 2), giving an immobility time of 133 ± 20.6 s (n = 7). This dose of WAY100635 did not affect the behavior of animals given an intra-hippocampal saline infusion (immobility time = 177 ± 20.0 s; in presence of WAY100635 = 201.7 ± 17.2 s). Two-way ANOVA showed significant main effects of 8-OH DPAT ($F(1,20) = 10.6$, $p = 0.004$) and WAY100635 ($F(1,20) = 26.2$, $p = 0.0001$), and a trend towards a significant 8-OH DPAT x WAY100635 interaction ($F(1,20) = 3.4$, $p = 0.079$).

Activation of 5-HT1ARs in the raphe nuclei modulates body temperature (Hillegaart 1991). Doses of 8-OH-DPAT that produce an antidepressant-like effect when administered peripherally (1 mg/kg and 10 mg/kg, i.p., Talbot et al., 2010) produced a lasting hypothermic effect in $G\alpha_{i2}^{+/+}$ animals (Figure 2B). In contrast doses of 8-OH-DPAT that produce an antidepressant-like effect

in the TST when administered into the hippocampus (3μg/side) did not affect body temperature (Figure 2B). There was a significant interaction between time and treatment as determined by two-way ANOVA ($F(9,84) = 5.01$, $p = 0.0001$), and significant main effects of time ($F(3,84) = 20.2$, $p = 0.0001$ and treatment ($F(3,84) = 39.9$, $p = 0.0001$).

3.3. Effect of 8-OH-DPAT on excitability of CA1 hippocampal neurons.

Both 5-HT1AR and $G\alpha_{i2}$ are expressed in mouse hippocampus, in agreement with published results (Laporte 1994; Allen Brain Atlas). Based on the above results and our previous findings (Talbot et al., 2010) we predicted that the decreased immobility in the $G\alpha_{i2}^{GS/GS}$ mice is due to increased activation of hippocampal 5-HT1ARs coupled to $G\alpha_{i2}$. 5-HT1AR activation alters potassium currents and hyperpolarization-activated currents to regulate cell excitability (Ko et al., 2016; Andrade and Nicoll 1987; Colino and Halliwell 1987; Oleskevich 1995). Therefore, to test for increased 5-HT1AR activity, we compared the effect of 8-OH-DPAT between heterozygous ($G\alpha_{i2}^{GS/+}$) and $G\alpha_{i2}^{+/+}$ littermates on the excitability of CA1 hippocampal neurons. Heterozygous mice were used because the homozygous animals show a maximal antidepressant-like effect in the tail suspension test (Talbot et al., 2010) and we have previously shown that this 5-HT1AR agonist has increased potency in the heterozygotes (Talbot et al., 2010). We recorded the responses of CA1 hippocampal neurons to current injection from minus 200 pA to plus 140 pA at 10 pA intervals before and after 5 μM 8-OH-DPAT application in $G\alpha_{i2}^{+/+}$ and $G\alpha_{i2}^{GS/+}$ littermates ($G\alpha_{i2}^{GS/+}$ N= 7 cells from 3 mice; $G\alpha_{i2}^{+/+}$ N= 9 cells from 5 mice for all measures). Application of 8-OH-DPAT did not affect neuronal excitability in $G\alpha_{i2}^{+/+}$ mice (2-way ANOVA, Baseline/8-OH-DPAT x current injection interaction: $F(14,112) = 0.8026$, $p = 0.67$). Example traces are shown in Figure 3C. In contrast, the same 8-OH-DPAT treatment

significantly decreased excitability in slices from the $G\alpha_{i2}^{GS/+}$ mice (Fig 3B: 2-way ANOVA, baseline/8-OH-DPAT x current injection interaction: $F(14,84) = 2.632$, $p = 0.003$); example traces are shown in Fig 2.3D. Thus, consistent with behavioral data above, 5 μM 8-OH-DPAT decreased membrane excitability in hippocampal CA1 neurons from $G\alpha_{i2}^{GS/+}$ mice, but not in neurons from $G\alpha_{i2}^{+/+}$ littermates.

In addition, bath application of 8-OH-DPAT produced a significant decrease in resting membrane potential in cells from the $G\alpha_{i2}^{GS/+}$ mice (-71.7 ± 2.0 mV to -76.4 ± 2.1 mV; $t(8) = 6.9$, $p < 0.001$), but not their $G\alpha_{i2}^{+/+}$ littermates (-74.2 ± 1.1 to -75.6 ± 2.2; $t(8) = 1.02$, $p = 0.34$) and the minimum amount of current needed to reach the firing threshold (the rheobase) was significantly increased by 8-OH-DPAT in cells from the $G\alpha_{i2}^{GS/+}$ mice (71.4 ± 17.1 pA to 95.7 ± 21.3 pA; $t(6) = 4.25$, $p < 0.01$), but not in cells from $G\alpha_{i2}^{+/+}$ mice (84.4 ± 14.7 pA to 104.4 ± 23.0 pA; $t(8) = 1.31$, $p = 0.23$). Overall, the results demonstrate that 5 μM 8-OH-DPAT application caused a decrease in membrane excitability in hippocampal CA1 neurons only from $G\alpha_{i2}^{GS/+}$ mice, but this concentration was ineffective in neurons from $G\alpha_{i2}^{+/+}$ littermates, showing increased activity of the 5-HT1AR agonist in the absence of RGS activity.

3.4. Effect of the RGS19/RGS4 inhibitor, CCG-203769, on tail suspension test responses

We have previously demonstrated that RGS19 acts as a negative modulator of 5-HT1AR signaling in mouse hippocampal neurons *in vitro* (Wang et al., 2014). To examine if this is a critical component of the 5-HT1AR signaling pathway in the hippocampus *in vivo,* we used the RGS19/RGS4 inhibitor CCG-203769 (Blazer et al., 2015). A single, acute intra-hippocampal administration of CCG-203769 (3 μg/side) produced a non-significant trend to antidepressant-

like behavior (data not shown). CCG-203769 is an irreversible inhibitor of RGS19 and RGS4 but may not be inactivating a sufficient level of the RGS protein after a single administration. Therefore, to further inhibit RGS activity we gave three infusions separated by 24 h. Female, but not male, wild type mice showed a reduction in immobility compared to vehicle treated controls. Two-way ANOVA revealed a significant sex x treatment interaction ($F(1,21) = 6.4$, $p = 0.02$) and significant main effects for sex ($F(1,21) = 15.7$, $p = 0.0007$) and treatment ($F(1,21) = 4.4$, $p = 0.05$; Figure 4A). There was no significant difference in the potency of 8-OH-DPAT in the TST between male and female wild type mice (Figure 4B). Hippocampal homogenates from male and female wild type mice did not significantly differ in RGS19 protein expression as determined by western blot (Figure 4C), although there was a trend to less RGS19 in the female mice.

3.5 Hippocampal [^{3}H] 8-OH-DPAT binding and G-protein expression in RGSi $G\alpha_{i2}$ knock-in mice.

In order to determine whether the electrophysiological and behavioral changes observed in the RGSi $G\alpha_{i2}$ mice could be explained by compensatory changes in 5-HT1AR expression, we characterized 5-HT1AR ligand binding in the mouse hippocampus. Saturation binding with [^{3}H] 8-OH-DPAT was performed in hippocampal membrane homogenates from wild type and heterozygous (+/GS) RGSi $G\alpha_{i2}$ mice (Figure 5A). Neither the maximal receptor expression (Bmax) nor the affinity (Kd) of [^{3}H] 8-OH-DPAT binding were significantly different between RGSi $G\alpha_{i2}$ and wild type mice (Table 1), indicating no large changes between the genotypes. However, variability in the data could have obscured small differences.

Hippocampal homogenates from wild type and RGSi $G\alpha_{i2}$ knock-in mice were assayed by western blotting for heterotrimeric G-proteins subunits using primary antibodies against $G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$ and $G\beta\gamma$(1-6); Figure 5B). There was a decrease in the level of $G\alpha i_1$ between wild type and the homozygotes and a trend to a decrease in $G\alpha i_2$.

3.6. Effects of peripheral WAY100635 on tail suspension test responses in heterozygous RGSi $G\alpha_o$ knock-in mice

$G\alpha_{i2}$ appears to play a critical role in regulating antidepressant-like behavior *via* the 5-HT1AR as RGSi $G\alpha_{i2}$ knock-in mice have an antidepressant-like phenotype, while $G\alpha_{i2}$ knockout mice exhibit pro-depressant behaviors (Talbot et al., 2010). However, 5-HT1ARs also couple to $G\alpha_o$ especially in the frontal cortex and hippocampus (La Cour et al., 2006). To examine whether loss of RGS control of $G\alpha_o$ similarly affected behavior, we studied mice expressing RGSi $G\alpha_o$ proteins ($G\alpha o^{GS/GS}$; Fu et al., 2006; Lamberts et al., 2013). The homozygous knock-in mice are not viable but heterozygote $Gao^{GS/+}$ mice showed a reduction in immobility compared to wild type littermates in the TST (Figure 6). However, unlike the RGS-$G\alpha_{i2}$ mice, systemic (s.c.) injection of 0.1 mg/kg WAY100635 did not reverse the behavioral phenotype (Figure 6). Two-way ANOVA disclosed a main effect of genotype ($F(1,30) = 16.2$, $p = 0.0004$) but no genotype x WAY100635 interaction ($F(1,30) = 0.086$, $p = 0.8$) or main effect of WAY100635 ($F(1,30) = 0.01$, $p = 0.9$) showing that the low levels of immobility in the $G\alpha o^{GS/+}$ mice are not due to activity in the 5-HT1A receptor system.

4. Discussion

The current results show that the antidepressant-like behavioral phenotype observed in the TST in mice expressing an RGS-insensitive variant of $G\alpha_{i2}$ ($G\alpha_{i2}^{GS/GS}$) was fully reversed by administration of the 5-HT1AR antagonist WAY100635 locally to the hippocampus, suggesting enhanced signaling of endogenous serotonin at this site. The behavioral phenotype observed in the RGSi $G\alpha_{i2}$ mice was accompanied by increased activity of the agonist 8-OH-DPAT on hippocampal slices, such that a concentration of 8-OH-DPAT that was ineffective in slices from wild-type mice caused hyperpolarization in slices from the mutant mice. These data suggest that promoting signaling through the 5-HT1AR/$G\alpha_{i2}$ complex in the hippocampus selectively enhances the antidepressant-like effects of 5-HT1AR agonism and that hippocampal 5-HT1ARs appear to be necessary and sufficient to explain the antidepressant-like behavior in mice expressing RGSi $G\alpha_{i2}$ protein. These conclusions do not appear to be confounded by developmental changes caused by the constitutive knock-in of an RGS-insensitive $G\alpha_{i2.}$ There were no changes in hippocampal 5-HT1ARs, although there was a paradoxical reduction in the level of $G\alpha_{i1}$ and a trend to decreased level of $G\alpha_{i2}$ in the homozygote mutant mice compared to their wild-type littermates. This likely represents a compensatory response in an attempt to reduce the increased activity of 5HT1AR signaling due to the RGSi mutant of the $G\alpha_{i2}$. We have observed a similar compensatory decrease in the level of $G\alpha o$ in mice expressing RGSi $G\alpha o$ (Lamberts et al., 2013). Moreover, the mutant phenotype was mimicked by direct hippocampal administration of the 5-HT1AR agonist 8-OH-DPAT to mice expressing wild type $G\alpha_{i2}$ ($G\alpha_{i2}^{+/+}$), that was in turn fully blocked by systemic WAY100635, and by hippocampal administration of an RGS19/4 inhibitor, although only in female mice.

While it remains difficult to develop drugs that target a specific GPCR bound to a particular G-protein subunit, targeting RGS proteins may provide an additional level of selectivity. RGS family members 2, 7, 8, 10, 14 and 19 are expressed in the hippocampus at high levels, while 4, 5, 11 and 13 are expressed only moderately, and 3, 6, 9 and 16 are expressed at very low levels or are absent (Gold et al., 1997; Grafstein-Dunn et al., 2001). Of these RGS proteins, RGS19 regulates 5-HT1AR function in isolated hippocampal neurons (Wang et al., 2014). These properties make RGS19 an attractive target to selectively enhance hippocampal 5-HT1AR function for potential antidepressant effects while avoiding the drawbacks of activating all 5-HT1ARs expressed in the CNS. Due to the lack of a highly selective RGS19 small molecule inhibitor we tested the effects of CCG-203769, a RGS19/RGS4 dual inhibitor (Blazer et al., 2015). CCG-203769 forms a disulfide bridge with these RGS proteins leading to permanent inactivation (Turner et al., 2011). Repeated (3-day) administration of CCG-203769 bilaterally into the hippocampus was necessary to produce an anti-depressant-like effect, but surprisingly this was only seen in female mice. This sex difference was not explained by differences in hippocampal RGS19 protein expression as levels were similar between male and female mice. In addition, there was no sex difference in the antidepressant-like effects of 8-OH-DPAT suggesting that a differential response to 5-HT1AR activation is not the primary cause. The disparity may be due to a lower potency of CCG-203769 in males compared to females rather than an all-or-none difference or to a greater sensitivity of male mice to motor suppression by CCG-203769 since intra-hippocampal infusion of a single dose of 10 μg/side CCG-203769 produced motor suppression and catatonia in the mice This may be an off-target effect of high doses of CCG-203769 since peripheral administration of this inhibitor has been shown to reverse raclopride-induced suppression of movement (Blazer et al., 2015).

Although RGS19 inhibition is a promising candidate for the antidepressant-like phenotype behavior in the $G\alpha_{i2}^{GS/GS}$ mice and for antidepressant-like effect of CCG-203769 in female wild type mice, inhibition of other RGS proteins may play a role. Out of all the RGS proteins tested with CCG-203769, only RGS4 is inhibited more potently than RGS19 (Blazer et al., 2015). Overexpressed RGS4 inhibits 5-HT1AR signaling in the raphe nuclei (Beyer et al., 2004), but RGS4 knockout mice show no change in baseline antidepressant-like behavior compared to their wild-type controls and show a decreased response to a selective serotonin reuptake inhibitor in the forced swim test (Stratinaki et al., 2013) suggesting that RGS4 is a positive modulator. Thus RGS4 inhibition likely cannot explain the antidepressant-like effects seen in the $G\alpha_{i2}^{GS/GS}$ mice or after CCG-203769 treatment. In contrast, RGS6 knockout ($RGS6^{-/-}$) mice do show a 5-HT1AR-mediated baseline antidepressant-like phenotype (Stewart et al., 2014). Whereas this behavioral response is consistent with antidepressant-like effects seen in $G\alpha_{i2}^{GS/GS}$ mice there is no evidence that CCG-203769 interacts with RGS6. RGS6 is a member of the R7 family of RGS proteins that includes RGS6, 7, 9 and 11 (Hollinger and Helper, 2002). CCG-203769 has over 1000-fold selectivity for RGS19 compared to other R7 RGS family members (Blazer et al., 2015) and RGS6 lacks an available cysteine residue to form a covalent interaction with CCG-203769. In addition, the increased phospho-GSK3β levels seen in the $G\alpha_{i2}^{GS/GS}$ mice (Talbot et al., 2010) are not seen in the $RGS6^{-/-}$ mice which instead show an increase in phospho-CREB (Stewart et al., 2014). Thus, the mechanisms underlying the 5-HT1AR-mediated phenotype in the $RGS6^{-/-}$ and $G\alpha i2^{GS/GS}$ mice appear to be different.

The above discussion suggests that the complex of 5-HT1AR/$G\alpha_{i2}$ with a specific RGS protein, possibly RGS19, might provide a suitable target for antidepressant drug therapy. Furthermore, this could offer an explanation for the selectivity of 5-HT1AR ligands, such as F15599, which show a preference for frontal cortex 5-HT1A heteroreceptors compared to raphe nuclei 5-HT1A autoreceptors (Newman-Tancredi et al., 2009) or ligands such as F13640 and F13714 with preference for 5-HT1A autoreceptors compared to other 5-HT1ARs (Buritova et al., 2009). F15599 also stimulates 5-HT1ARs coupled to $G\alpha_i$ more potently and efficaciously than 5-HT1ARs coupled to $G\alpha_o$, while serotonin shows no G-protein preference (Newman-Tancredi et al., 2009), supporting the notion that a small molecule agonist can achieve selectivity.

Surprisingly we found that heterozygous mice expressing RGS-insensitive $G\alpha_o$ protein mice also exhibit an antidepressant-like phenotype. However, this behavioral phenotype was not reversed by the 5-HT1AR antagonist WAY100635, suggesting that the antidepressant-like behaviors displayed by the RGSi $G\alpha_{i2}$ and RGSi $G\alpha_o$ mice are driven by distinct mechanisms. Studies in E. coli suggest that the 5-HT1AR couples more efficiently to $G\alpha_{i2}$ than $G\alpha_o$ proteins (Bertin et al., 1992). Although 5-HT1ARs do couple to other Gα proteins the close association between 5-HT1AR and $G\alpha_{i2}$ is supported by a 5-HT1AR dependent antidepressant-like phenotype in the $G\alpha_{i2}^{GS/GS}$ mice and a pro-depressant phenotype in $G\alpha_{i2}^{-/-}$ mice (Talbot et al., 2010).

When both the therapeutic and detrimental effects of a drug are mediated by the same molecular target developing new therapies is particularly challenging. The 5-HT1AR has long been recognized as one such target, where activation of autoreceptors on serotonergic cells is generally considered detrimental while activating heteroreceptors expressed on cells downstream

of the serotonin neurons produces beneficial effects (Artigas 1993; Blier and Abbott 2001). A considerable amount of effort has thus been spent on identifying strategies to block the 5-HT1A autoreceptors without affecting heteroreceptor activity, or conversely activating heteroreceptors without stimulating autoreceptor activity (Blier et al., 1993; Romero et al., 1996; Rabiner et al., 2000; Newman-Tancredi et al., 2009). The RGSi $G\alpha_{i2}$ mutation appears to accomplish this as evidenced by a promotion of 5-HT1AR dependent antidepressant-like behaviors, but not hypothermic effects, although it is acknowledged that hypothermia is not a direct measure of raphe nuclei 5-HT1AR autoreceptor activity. Nonetheless, a therapeutic that can selectively enhance signaling through 5-HT1A/$G\alpha_{i2}$ complexes, or alternatively selectively inhibit RGS proteins acting at $G\alpha_{i2}$, may dissociate the therapeutic and detrimental effects of 5-HT1A agonism with potential benefits for the neuropsychiatric treatment of depression.

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References

Andrade, R., Nicoll, R.A., 1987. Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro. J. Physiol. 394, 99–124.

Artigas, F., 1993. 5-HT and antidepressants: new views from microdialysis studies. Trends Pharmacol. Sci. 14, 262.

Berman, D.M., Wilkie, T.M., Gilman, A.G., 1996. GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. Cell 86, 445–52.

Bertin, B., Freissmuth, M., Breyer, R.M., Schütz, W., Strosberg, A.D., Marullo, S., 1992. Functional expression of the human serotonin 5-HT1A receptor in Escherichia coli. Ligand binding properties and interaction with recombinant G protein alpha-subunits. J. Biol. Chem. 267, 8200–6.

Beyer, C.E., Ghavami, A., Lin, Q., Sung, A., Rhodes, K.J., Dawson, L.A., Schechter, L.E., Young, K.H., 2004. Regulators of G-protein signaling 4: modulation of 5-HT1A-mediated neurotransmitter release in vivo. Brain Res. 1022, 214–220.

Blazer, L.L., Storaska, A.J., Jutkiewicz, E.M., Turner, E.M., Calcagno, M., Wade, S.M., Wang, Q., Huang, X.-P., Traynor, J.R., Husbands, S.M., Morari, M., Neubig, R.R., 2015. Selectivity and Anti-Parkinson's Potential of Thiadiazolidinone RGS4 Inhibitors. ACS Chem. Neurosci. 6, 911–919.

Blier, P., Abbott, F. V, 2001. Putative mechanisms of action of antidepressant drugs in affective and anxiety disorders and pain. J. Psychiatry Neurosci. 26, 37–43.

Blier, P., Lista, A., De Montigny, C., 1993. Differential properties of pre- and postsynaptic 5-hydroxytryptamine1A receptors in the dorsal raphe and hippocampus: I. Effect of spiperone. J. Pharmacol. Exp. Ther. 265, 7–15.

Buritova, J., Berrichon, G., Cathala, C., Colpaert, F., Cussac, D., 2009. Region-specific changes in 5-HT1A agonist-induced Extracellular signal-Regulated Kinases 1/2 phosphorylation in rat brain: A quantitative ELISA study. Neuropharmacology 56, 350–361.

Celada, P., Bortolozzi, A., Artigas, F., 2013. Serotonin 5-HT1A Receptors as Targets for Agents to Treat Psychiatric Disorders: Rationale and Current Status of Research. CNS Drugs 27, 703–716.

Chen, F.S., Shim, H., Morhardt, D., Dallman, R., Krahn, E., McWhinney, L., Rao, A., Gold, S.J., Chen, C.-K., 2010. Functional redundancy of R7 RGS proteins in ON-bipolar cell dendrites. Invest. Ophthalmol. Vis. Sci. 51, 686–93.

Cho, H., Kozasa, T., Takekoshi, K., De Gunzburg, J., Kehrl, J.H., 2000. RGS14, a GTPase-activating protein for Gialpha, attenuates Gialpha- and G13alpha-mediated signaling pathways. Mol. Pharmacol. 58, 569–76.

Colino, A., Halliwell, J. V., 1987. Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. Nature 328, 73–77.

Council, N.R. (2011) Guide for the care and use of laboratory animals, Washington DC, National Academic Press.

Czyrak, A., Maćkowiak, M., Chocyk, A., Fijał, K., Tokarski, K., Bijak, M., Wędzony, K., 2002. Prolonged corticosterone treatment alters the responsiveness of 5-HT 1A receptors to 8-OH-DPAT in rat CA1 hippocampal neurons. Naunyn. Schmiedebergs. Arch. Pharmacol. 366, 357–367.

Dong, M.Q., Chase, D., Patikoglou, G.A., Koelle, M.R., 2000. Multiple RGS proteins alter neural G protein signaling to allow C. elegans to rapidly change behavior when fed. Genes Dev. 14, 2003–14.

Doupnik, C.A., Xu, T., Shinaman, J.M., 2001. Profile of RGS expression in single rat atrial myocytes. Biochim. Biophys. Acta 1522, 97–107.

Fu, Y., Huang, X., Zhong, H., Mortensen, R.M., D'Alecy, L.G., Neubig, R.R., 2006. Endogenous RGS Proteins and G Subtypes Differentially Control Muscarinic and Adenosine-Mediated Chronotropic Effects. Circ. Res. 98, 659–666.

Fu, Y., Zhong, H., Nanamori, M., Mortensen, R.M., Huang, X., Lan, K., Neubig, R.R., 2004. RGS-Insensitive G-Protein Mutations to Study the Role of Endogenous RGS Proteins, in: Methods in Enzymology. pp. 229–243.

Ghavami, A., Hunt, R.A., Olsen, M.A., Zhang, J., Smith, D.L., Kalgaonkar, S., Rahman, Z., Young, K.H., 2004. Differential effects of regulator of G protein signaling (RGS) proteins on serotonin 5-HT1A, 5-HT2A, and dopamine D2 receptor-mediated signaling and adenylyl cyclase activity. Cell. Signal. 16, 711–721.

Gold, S.J., Ni, Y.G., Dohlman, H.G., Nestler, E.J., 1997. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. J. Neurosci. 17, 8024–37.

Goldenstein, B.L., Nelson, B.W., Xu, K., Luger, E.J., Pribula, J.A., Wald, J.M., O'Shea, L.A., Weinshenker, D., Charbeneau, R.A., Huang, X., Neubig, R.R., Doze, V.A., 2009. Regulator

of G protein signaling protein suppression of Galphao protein-mediated alpha2A adrenergic receptor inhibition of mouse hippocampal CA3 epileptiform activity. Mol. Pharmacol. 75, 1222–30.

Grafstein-Dunn, E., Young, K.H., Cockett, M.I., Khawaja, X.Z., 2001. Regional distribution of regulators of G-protein signaling (RGS) 1, 2, 13, 14, 16, and GAIP messenger ribonucleic acids by in situ hybridization in rat brain. Brain Res. Mol. Brain Res. 88, 113–23.

Hillegaart, V., 1991. Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on core temperature in the rat. Psychopharmacology (Berl). 103, 291–6.

Hjorth, S., Bengtsson, H.J., Kullberg, A., Carlzon, D., Peilot, H., Auerbach, S.B., 2000. Serotonin autoreceptor function and antidepressant drug action. J. Psychopharmacol. 14, 177–185.

Hollinger, S., Hepler, J.R., 2002. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol. Rev. 54, 527–59.

Huang, X., Fu, Y., Charbeneau, R.A., Saunders, T.L., Taylor, D.K., Hankenson, K.D., Russell, M.W., D'Alecy, L.G., Neubig, R.R., 2006. Pleiotropic Phenotype of a Genomic Knock-In of an RGS-Insensitive G184S Gnai2 Allele. Mol. Cell. Biol. 26, 6870–6879.

Ito, H., Halldin, C., Farde, L., 1999. Localization of 5-HT1A receptors in the living human brain using [carbonyl-11C]WAY-100635: PET with anatomic standardization technique. J. Nucl. Med. 40, 102–9.

Ko, K.W., Rasband, M.N., Meseguer, V., Kramer, R.H., Golding, N.L., 2016. Serotonin modulates spike probability in the axon initial segment through HCN channels. Nat. Neurosci. 19, 826–834.

la Cour, C.M., El Mestikawy, S., Hanoun, N., Hamon, M., Lanfumey, L., 2006. Regional Differences in the Coupling of 5-Hydroxytryptamine-1A Receptors to G Proteins in the Rat Brain. Mol. Pharmacol. 70, 1013–1021.

Lamberts, J.T., Smith, C.E., Li, M.-H., Ingram, S.L., Neubig, R.R., Traynor, J.R., 2013. Differential control of opioid antinociception to thermal stimuli in a knock-in mouse expressing regulator of G-protein signaling-insensitive Gαo protein. J. Neurosci. 33, 4369–77.

Lan, K.L., Sarvazyan, N.A., Taussig, R., Mackenzie, R.G., DiBello, P.R., Dohlman, H.G., Neubig, R.R., 1998. A point mutation in Galphao and Galphai1 blocks interaction with regulator of G protein signaling proteins. J. Biol. Chem. 273, 12794–7.

Laporte, A.M., Lima, L., Gozlan, H., Hamon, M., 1994. Selective in vivo labelling of brain 5-HT1A receptors by [3H]WAY 100635 in the mouse. Eur. J. Pharmacol. 271, 505–14.

Lester, P.A., Traynor, J.R., 2006. Comparison of the in vitro efficacy of μ, δ, κ and ORL1 receptor agonists and non-selective opioid agonists in dog brain membranes. Brain Res. 1073–1074, 290–296.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. 20, 9104–10.

Marazziti, D., Marracci, S., Palego, L., Rotondo, A., Mazzanti, C., Nardi, I., Ladinsky, H., Giraldo, E., Borsini, F., Cassano, G.B., 1994. Localization and gene expression of serotonin 1A (5HT1A) receptors in human brain postmortem. Brain Res. 658, 55–9.

Newman-Tancredi, A., Martel, J.-C., Assié, M.-B., Buritova, J., Lauressergues, E., Cosi, C., Heusler, P., Bruins Slot, L., Colpaert, F.C., Vacher, B., Cussac, D., 2009. Signal transduction and functional selectivity of F15599, a preferential post-synaptic 5-HT1A receptor agonist. Br. J. Pharmacol. 156, 338–53.

Oleskevich, S., 1995. G alpha o1 decapeptide modulates the hippocampal 5-HT1A potassium current. J. Neurophysiol. 74, 2189–2193.

Rabiner, E., Gunn, R.N., Castro, M.E., Sargent, P.A., Cowen, P.J., Koepp, M.J., Meyer, J.H., Bench, C.J., Harrison, P.J., Pazos, A., Sharp, T., Grasby, P.M., 2000. β-blocker Binding to Human 5-HT1A Receptors in vivo and in vitro Implications for Antidepressant Therapy. Neuropsychopharmacology 23, 285–293.

Romero, L., Bel, N., Artigas, F., de Montigny, C., Blier, P., 1996. Effect of Pindolol on the Function of Pre- and Postsynaptic 5-HT1A Receptors: In Vivo Microdialysis and Electrophysiological Studies in the Rat Brain. Neuropsychopharmacology 15, 349–360.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl). 85, 367–70.

Stewart, A., Maity, B., Wunsch, A.M., Meng, F., Wu, Q., Wemmie, J.A., Fisher, R.A., 2014. Regulator of G-protein signaling 6 (RGS6) promotes anxiety and depression by attenuating

serotonin-mediated activation of the 5-HT1A receptor-adenylyl cyclase axis. FASEB J. 28, 1735–1744.

Stratinaki, M., Varidaki, A., Mitsi, V., Ghose, S., Magida, J., Dias, C., Russo, S.J., Vialou, V., Caldarone, B.J., Tamminga, C.A., Nestler, E.J., Zachariou, V., 2013. Regulator of G protein signaling 4 is a crucial modulator of antidepressant drug action in depression and neuropathic pain models. Proc. Natl. Acad. Sci. 110, 8254–8259.

Talbot, J.N., Jutkiewicz, E.M., Graves, S.M., Clemans, C.F., Nicol, M.R., Mortensen, R.M., Huang, X., Neubig, R.R., Traynor, J.R., 2010. RGS inhibition at G(alpha)i2 selectively potentiates 5-HT1A-mediated antidepressant effects. Proc. Natl. Acad. Sci. U. S. A. 107, 11086–91.

Tesmer, J.J., Berman, D.M., Gilman, A.G., Sprang, S.R., 1997. Structure of RGS4 bound to AlF4--activated G(i alpha1): stabilization of the transition state for GTP hydrolysis. Cell 89, 251–61.

Tsai, S.-J., Liou, Y.-J., Hong, C.-J., Yu, Y.W.-Y., Chen, T.-J., 2008. Glycogen synthase kinase-3beta gene is associated with antidepressant treatment response in Chinese major depressive disorder. Pharmacogenomics J. 8, 384–90.

Turner, E.M., Blazer, L.L., Neubig, R.R., Husbands, S.M., 2012. Small Molecule Inhibitors of Regulators of G Protein Signaling (RGS) Proteins. ACS Med. Chem. Lett. 3, 146–150.

Wang, Q., Terauchi, A., Yee, C.H., Umemori, H., Traynor, J.R., 2014. 5-HT1A receptor-mediated phosphorylation of extracellular signal-regulated kinases (ERK1/2) is modulated by regulator of G protein signaling protein 19. Cell. Signal. 26, 1846–1852.

Wang, Q., Traynor, J.R., 2013. Modulation of μ-opioid receptor signaling by RGS19 in SH-SY5Y cells. Mol. Pharmacol. 83, 512–20.

Table 1. Binding of [^{3}H] 8-OH-DPAT to hippocampal membrane homogenates

	Bmax ± SEM (fmols/mg protein)	K_D ± SEM (nM)
Wild Type	142 ± 29.5	6.4 ± 3.0
+/GS	109 ± 17.4	3.8 ± 1.6

Data were derived from the binding of [^{3}H] 8-OH-DPAT to membrane homogenates pooled from 6-8 mice. Each experiment was repeated three-times in triplicate and analyzed using GraphPrism 7.0, as described in the materials and methods section.

Figure 1. Effect of the 5-HT1A antagonist WAY100635 on spontaneous antidepressant-like behavior in mice expressing RGS-insensitive Gαi2 (Gαi2 $^{GS/GS}$). Antidepressant-like behavior was measured using the TST. Intra-hippocampal WAY-100635 (WAY; 3μg/side) reversed the baseline activity in the RGSi $G\alpha_{i2}$ expressing mice (GS/GS) with no action in wild-type (WT) littermates. Each column depicts the mean immobility score ± SEM of 6-7 mice. ** p <0.01 compared to saline treated wild type mice; ## p < 0.01 compared to saline treated RGSi $G\alpha_{i2}$ expressing mice.

Figure 2. Effect of intrahippocampal 8-OH-DPAT administration in wild type mice. (A) Intra-hippocampal (intra-HPC) administration of 8-OH-DPAT (DPAT) produces an antidepressant-like effect in the TST in wild type mice that is reversed by WAY-100635 (WAY; 0.1 mg/kg s.c.). Each column depicts the mean immobility score ± SEM of 6 mice. *** p < 0.001); between saline and 8-OH-DPAT; ## p < 0.01 between s.c. WAY100635 and s.c. saline. (B) Intra-hippocampal 8-OH-DPAT (3 μg/side) does not produce a hypothermic effect at doses capable of producing antidepressant-like effects. Each line represents the mean temperature ± SEM recorded from 6-7 mice at each time; **, *** and **** (p < 0.01, p < 0.001 and p < 0.0001 respectively) indicate a difference between 8-OH-DPAT and vehicle treated animals. Animals receiving 3μg/side 8-OH-DPAT by intra-hippocampal infusion were not significantly different from vehicle treated animals at any time point.

Figure 3. Effect of 8-OH-DPAT on slices from wild type and RGSi Gαi2 knock-in mice. (A) Application of 8-OH-DPAT did not affect neuronal excitability in wild type mice (Gαi2 $^{+/+}$). **(B)** Application of 8-OH-DPAT decreased neuronal excitability in heterozygous RGSi $G\alpha_{i2}$ mice

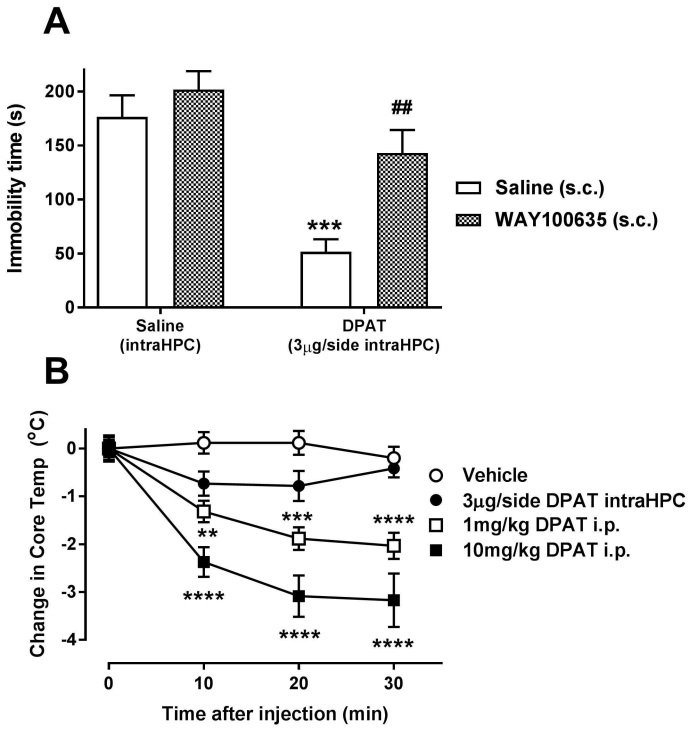
($G\alpha_{i2}^{GS/+}$). **(C)** Example traces of data quantified in 3A. **(D)** Example traces of data quantified in 3B, n= 9 cells recorded for each measure.

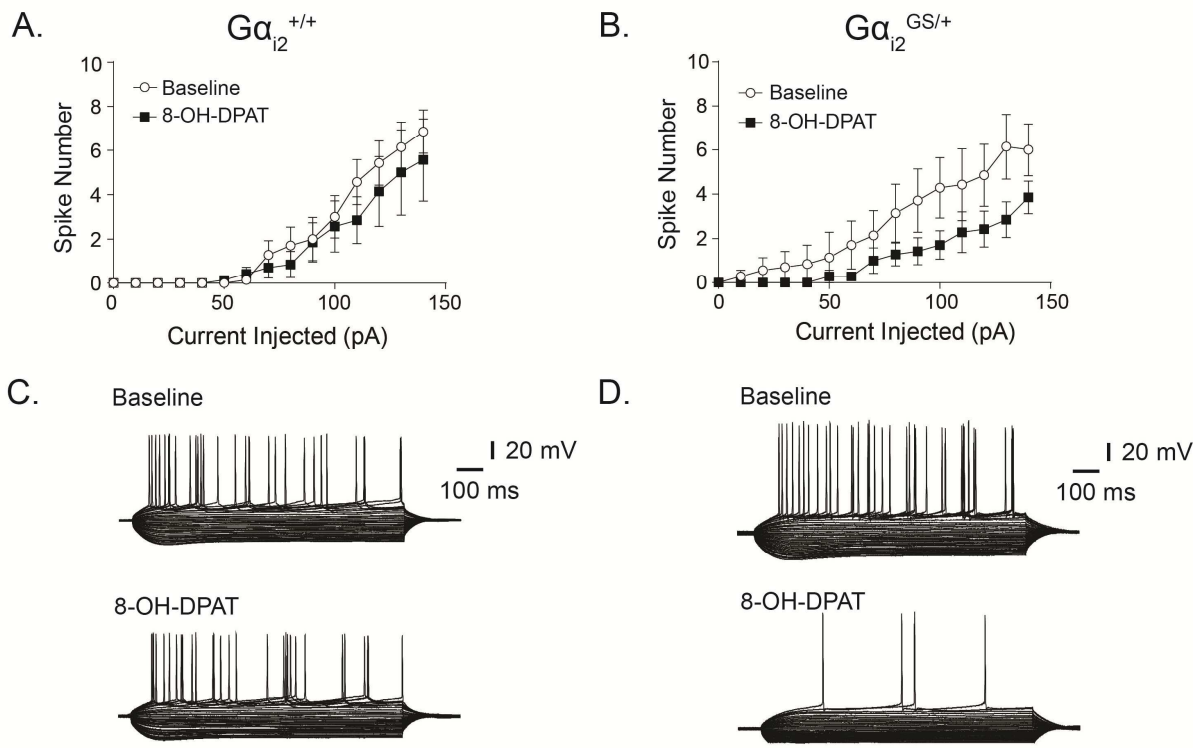
Figure 4. Intra-hippocampal administration of the RGS4/19 inhibitor CCG-203769 produces an antidepressant-like effect in female mice. (A) Once daily administration of CCG-203769 (CCG; 3µg/side) for three days into the hippocampus produces an antidepressant-like effect in female (* $p < 0.05$), but not male wild type mice. Each column depicts the mean immobility score ± SEM of 6-7 mice. (B) No sex difference is seen in the sensitivity to 8-OH-DPAT as determined by the mean immobility score ± SEM of 6-7 mice. (C) Hippocampal RGS19 expression was determined by western blot, normalized to α-Tubulin expression and the ratio of G-protein/α-Tubulin expression was averaged across three independent experiments ± SEM. No significant difference was seen in RGS19 signal intensity between male and female animals, unpaired t-test). Blot shows RGS19 bands detected at ~25kDa for hippocampal homogenates from 3 different female mice (lanes 1-3) and 3 different male mice (lanes 4-6).

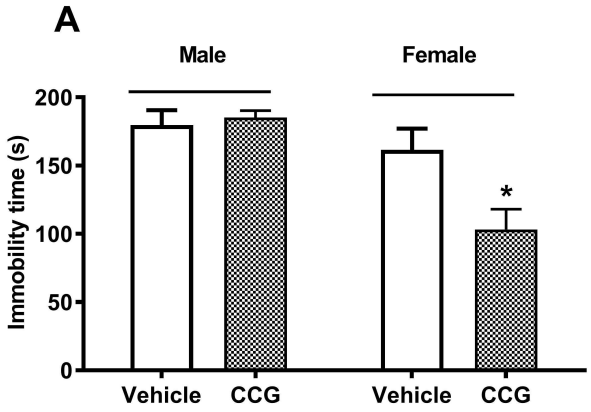
Figure 5. Hippocampal [^{3}H]8-OH-DPAT binding and G-protein expression in heterozygous RGSi $G\alpha_{i2}$ knock-in mice. (A) Specific binding of 8-OH-DPAT: Each point represents the mean specific binding ± SEM from three independent experiments (best fit lines for Kd and Bmax calculation were determined using a one-site saturation binding fit with Hill slopes set to 1 and fitted to mean results averaged across experiments; Bmax and Kd compared between WT and +/GS using unpaired t-test, showed no significant differences. (B) G-protein was measured by western blot and signal intensity was normalized to actin expression; mean G-protein/actin expression ± SEM was compared for hippocampi from three animals at each G-

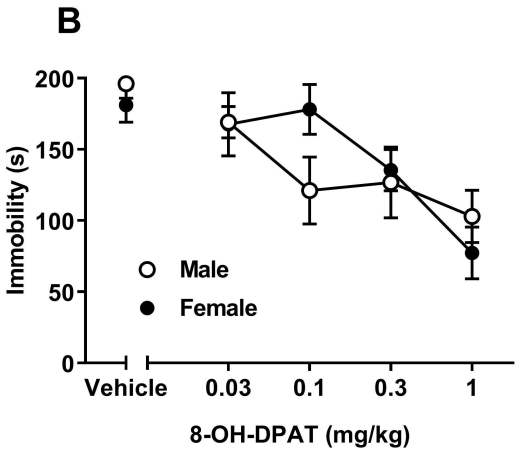
protein subunit. * $p < 0.05$, 1-Way ANOVA with Tukey's posthoc test. (C) Western blots of G proteins in hippocampal homogenates from mouse RGSi $G\alpha i_2$ genotypes.

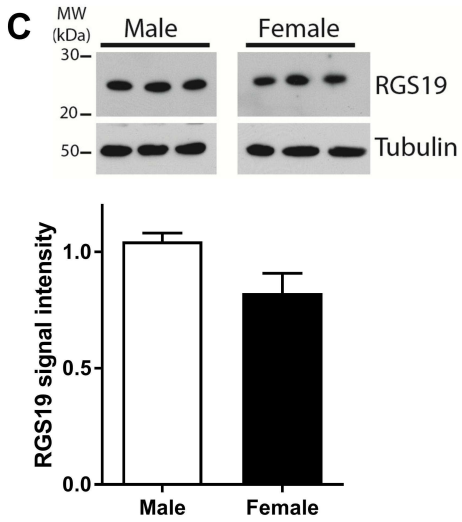
Figure 6. Spontaneous antidepressant-like behavior in mice expressing RGSi $G\alpha_o$ is not reversed by the 5HT1A antagonist WAY100635. Antidepressant-like behavior in heterozygous RGSi $G\alpha_o$ (+/GS) expressing mice in TST is not reversed by WAY-100635 (s.c.). Each column depicts the mean immobility score ± SEM of 4-13 mice, * $p < 0.05$ compared to wild-type mice.

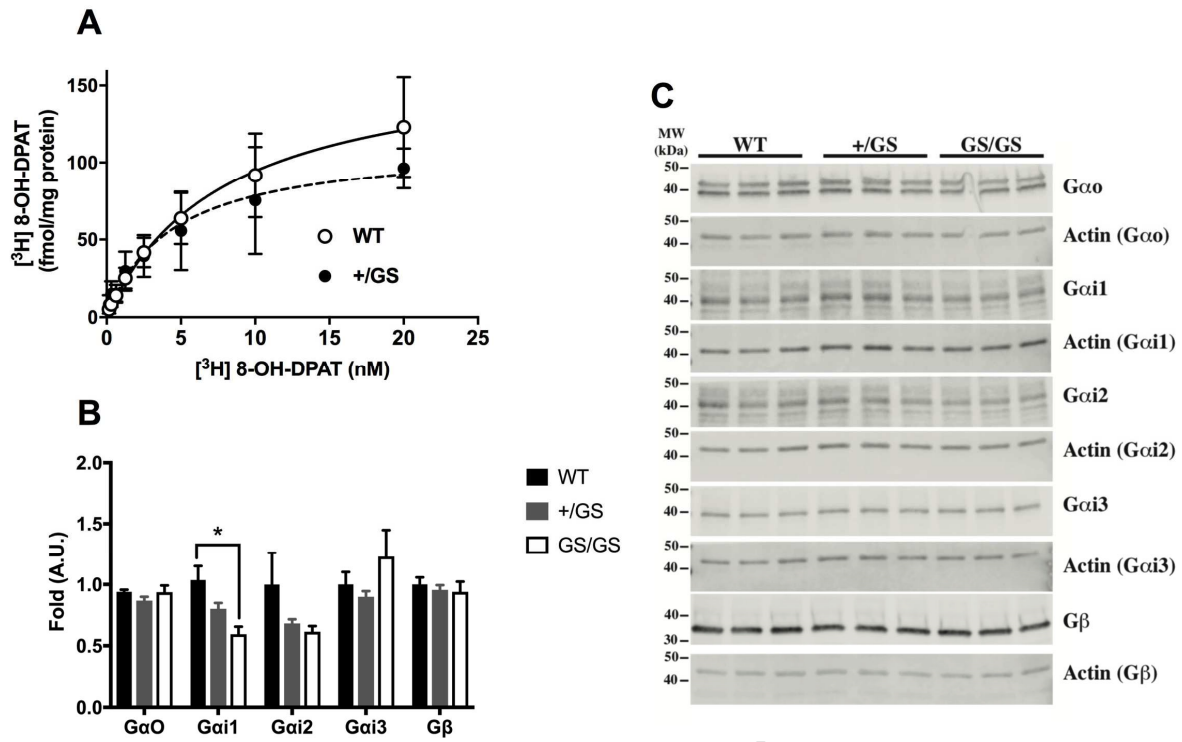


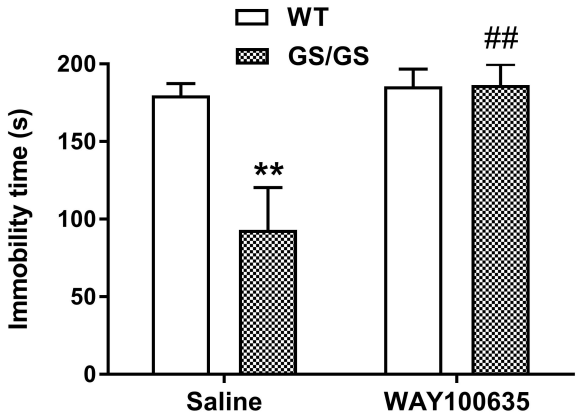


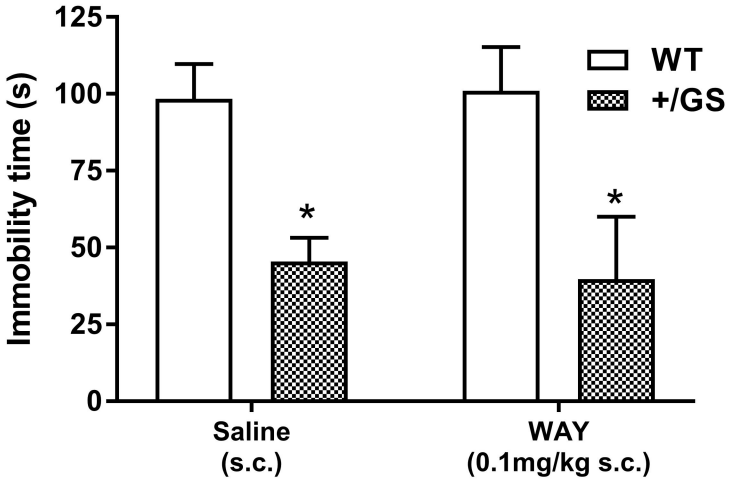

A
Male
Female
Immobility time (s)
200
150
100
50
0
*
Vehicle
CCG
Vehicle
CCG


B
Immobility (s)
200
150
100
50
0
Male
Female
Vehicle
0.03
0.1
0.3
1
8-OH-DPAT (mg/kg)


C
MW
(kDa)
Male
Female
30
20
50
RGS19
Tubulin
RGS19 signal intensity
1.0
0.5
0.0
Male
Female







Role of hippocampal 5-HT1A receptors in the antidepressant-like phenotype of mice expressing RGS-insensitive Gαi2 protein

Highlights

- Mice expressing RGS-insensitive Gαi2 have an antidepressant-like phenotype.
- Antidepressant behavior is reversed by 5HT1A receptor antagonism in the hippocampus.
- 5HT1A agonist in the hippocampus affords antidepressant-like behavior.
- Hippocampal 5HT1A agonism is increased in mice expressing RGS-insensitive Gai2.
- Hippocampal RGS inhibition produces antidepressant-like behavior in female mice.