DENTATE GYRUS’S ROLE IN THE RESPONSE TO ANTIDEPRESSANT TREATMENT

By

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Depression is a complex psychiatric disorder that is a major burden on society as 300 million people are diagnosed worldwide. Furthermore, only 33% of depressed patients achieve remission of symptoms upon initial monotherapy with commonly used selective serotonin reuptake inhibitors (SSRI). In preclinical studies using mice, chronic stress paradigms, such as chronic corticosterone and chronic social defeat stress, are used to induce negative valence behaviors. Chronic treatment with the SSRI fluoxetine (FLX) reverses these stress-induced behavioral changes in some, but not all mice, permitting stratification of mice into behavioral responders and non-responders to FLX. Recently, we reported that 5-HT\textsubscript{1A} receptors, which are Gi-coupled inhibitory receptors, on mature granule cells (GCs) in the dentate gyrus (DG), a subfield of the hippocampus, are necessary and sufficient for the behavioral, neurogenic, and neuroendocrine response to chronic SSRI treatment. Since inhibition of mature DG GCs through cell autonomous Gi-coupled receptors is critical for mounting an antidepressant response, we
predicted that behavioral response to FLX would correlate with a decrease in DG GC activation and an increase in adult hippocampal neurogenesis compared to FLX non-responders and stress controls. Additionally, we wanted to assess whether chronic functional manipulation of DG GC activity via the usage of chemogenetics could mimic the effects of antidepressants. Our data shows that response to FLX treatment following chronic stress exposure leads to behavioral responders and non-responders across three distinct negative valence tasks (novelty suppressed feeding, elevated plus maze, and forced swim test). Intriguingly, behavioral responders show decreased DG GC activation (as measured by cFos immunostaining) and increased adult neurogenesis (as measured by Ki67 and DCX immunostaining) relative to stress only controls and non-responders. Furthermore, we show that chronic inhibition of ventral DG GCs (through usage of Gi-DREADDs) results in a decrease in negative valence behaviors. Taken together, these results illustrate that inhibition of DG GCs is a critical component of the response to antidepressants.
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### Table of Contents

Abstract of Thesis................................................................. ii
Acknowledgments........................................................................ iv
List of Figures........................................................................... vi
Introduction.................................................................................. 1
Specific Aims.............................................................................. 5
Materials and Methods............................................................... 10
Results....................................................................................... 17
Discussion................................................................................... 24
References.................................................................................. 29
Figure Legends........................................................................... 32
INTRODUCTION

Mood disorders, such as depression, are prevalent forms of mental illness in today’s society, with major depressive disorder (MDD) affecting more than 300 million people worldwide (Levinstein & Samuels, 2014; Murray & Lopez, 1996; Smith, 2014). MDD is a ubiquitous illness that is characterized by diverse etiologies and an overlapping symptomology with highly comorbid disorders, such as anxiety, which has a co-occurrence rate with MDD of up to 60% (Gorman, 1996; B. A. Samuels et al., 2015; Yohn, Gergues, & Samuels, 2017). Although the individual risk for depression is 40-50% genetic, no specific gene polymorphisms with high penetrance have been identified as causative factors for developing MDD (Adachi, Barrot, Autry, Theobald, & Monteggia, 2008). Today antidepressants such as SSRIs are one of the most frequently prescribed drugs in the world and their usage continues to increase. From 1999 to 2012 the percentage of Americans on antidepressants increased from 6.8% to 13% (Kantor, Rehm, Haas, Chan, & Giovannucci, 2015). However, these commonly prescribed drugs fail to have any effect for many patients and have incomplete effects for many others (Trivedi et al., 2006). Thus, the multifaceted and heterogeneous underlying pathology of depression has hindered the development of effective treatments for depressed individuals.

The dentate gyrus (DG), a subfield of the hippocampus, is an important mediator of the antidepressant response. Chronic SSRI treatment increases multiple stages of adult neurogenesis within the DG, such as proliferation of dividing neural precursor cells, maturation and integration of young adult born granule cells (abGCs) into the DG circuitry (Wang et al., 2008). Although adult hippocampal neurogenesis is important for a variety of behaviors in rodents, including associative learning tasks that require the hippocampus, the formation of trace memories (Shors et al., 2001; Shors, Townsend, Zhao, Kozorovitskiy, & Gould, 2002) and
spatial navigation in the Morris water maze (Kempermann & Gage, 2002), it also appears essential for antidepressant effects. Specifically, ablation of the adult hippocampal neurogenic niche via focal radiological strategies result in loss of antidepressant-mediated behaviors (Airan et al., 2007; David et al., 2009; Santarelli et al., 2003; Yohn et al., 2017).

Recently, Samuels and colleagues (2015) found that deletion of 5-HT$_{1A}$ heteroreceptors from DG mature granule cells (GCs) abolished the behavioral response to SSRIs in the novelty suppressed feeding task (NSF), while attenuating the effects of SSRIs on adult hippocampal neurogenesis. By contrast, when 5-HT$_{1A}$ heteroreceptors were deleted from abGCs in the DG, the SSRI effects on neurogenesis and behavior remained intact. Moreover, through expressing 5-HT$_{1A}$ heteroreceptors in DG GCs on a 5-HT$_{1A}$ deficient background Samuels and colleagues (2015) demonstrated that 5-HT$_{1A}$ heteroreceptors are sufficient to mediate behavioral and neurogenic effects of SSRIs. Functionally, 5-HT$_{1A}$Rs are Gi/o-coupled metabotropic receptors that inhibit neuronal activity by suppressing cyclic adenosine monophosphate (cAMP) levels and activating GIRK channels (Hannon & Hoyer, 2008).

5-HT$_{1A}$ receptors are densely packed in the ventral DG, which is an important mediator in regulating emotional affective states (Fanselow & Dong, 2010; Kheirbek et al., 2013; Yohn et al., 2017). Seeing as DG 5-HT$_{1A}$ receptors are necessary and sufficient in underlying the behavioral effects of SSRIs, their location in the ventral pole positions them to have a direct influence on limbic circuitry that regulates mood-related behaviors (Yohn et al., 2017). For instance, acute optogenetic inhibition of the ventral DG, but not dorsal, alters anxiety-related behaviors in mice (Kheirbek et al., 2013). The DG is extremely susceptible to stress, and alterations in the HPA axis can have profound anatomical changes on the DG. Human postmortem data reveals that untreated MDD patients have fewer DG GCs than controls and as
the number of depressive episodes increase, the DG volume decreases in MDD patients (Boldrini et al., 2013; Treadway et al., 2015). Given that the ventral DG is involved in both regulating emotional affect and the antidepressant response, direct and chronic manipulations of the ventral DG may yield a novel therapeutic avenue in treating mood disorders like depression.

Assessment of the antidepressant response in rodents requires manipulations that produce behaviors related to negative affect (i.e. increased anxiety and anhedonia), which can ultimately be reversed by antidepressant treatment. Social defeat stress is one commonly used paradigm that mimics some pathological dimensions of depression, resulting in decreases in sucrose preference (a possible measure of anhedonia), increases in anxiety-related phenotypes, and increased social avoidance (Tsankova et al., 2006). Similar to social defeat stress, chronic corticosterone (CORT) effectively induces some pathological dimensions of comorbid anxiety and depression including increases in anxiety-related phenotypes such as increased latency to emerge into the light in the light/dark test, more time and entries on the open arms of an elevated plus maze (EPM), as well as increased latency to eat in the NSF (David et al., 2009). Although chronic antidepressant treatment significantly reduces the latency to approach the food pellet, a subset of antidepressant treated mice will have a longer latency (Levinstein & Samuels, 2014; B. A. Samuels et al., 2015; Benjamin Adam Samuels & Hen, 2011). Therefore, the bimodal distribution observed in the NSF permits stratification of stress + antidepressant treated animals into responders and non-responders to treatment. Additionally, SSRI treated mice with lower NSF latencies also have lower immobility scores in the forced swim test (FST) (Yohn, Gergues, Levinstein, & Samuels, unpublished; Figure 1).

In this study we seek to further understand the role of the DG in mediating the antidepressant response. Previous work from us and others indicates that inhibition of mature DG
GCs, through both cell autonomous Gi-coupled receptors and the local microcircuitry, may be critical for regulating negative behaviors. Therefore, through functional manipulation of the ventral DG using chemogenetics, we seek to delineate the role of the ventral DG in regulating negative behaviors as well as treatment response.
SPECIFIC AIMS

Aim 1. Relationship between behavioral effects of FLX treatment and mature DG GCs activity.

Inactivation of mature DG GCs via 5-HT_{1A} heteroreceptors activation is important for behavioral responses to SSRIs (Samuels et al., 2015). Therefore, we propose that responders to antidepressant treatment will have lower DG GC activity and higher levels of neurogenesis than non-responders.

1.A Assess behavioral response to FLX in both chronic CORT and Social Defeat stressors

In response to first line antidepressants only about 33% of depressed patients feel a reprieve in their depressed symptoms. Preclinical data can use experimental systems to assist in understanding differences between responders (remitters) and non-responders to antidepressant treatment. We predict that responders to the antidepressant FLX will have a decrease in negative valence behaviors compared to non-responders to FLX. To assess our hypothesis, we created a cohort of corticosterone (CORT) treated mice where after 4-weeks of CORT (5mg/kg/day) treatment mice received either oral dosages of vehicle (VEH) or fluoxetine (FLX) (18mg/kg/day; Figure 2) for 3 weeks (timeline reference Figure 1). Subsequent to 3-weeks of antidepressant treatment, behavioral response to FLX was measured in a subset of the CORT group using the following tests: open field (OF), light/dark test (LD), EPM, forced swim test (FST), and NSF. The FST is another measure that allows for the detection of SSRI activity, with a pharmacological response to SSRIs being indicated by lower immobility times (David et al., 2009).

Additionally, we furthered assessed our hypothesis using the social defeat stress paradigm, to compare differences in treatment response across two valid stress models. To study
the antidepressant response to chronic SSRI treatment (FLX), mice were subjected to chronic defeat stress by an aggressor for 10 consecutive days (reference Figure 2 for timeline). Twenty-four hours following the last defeat, control and defeated mice underwent a social interaction test, where social interaction and avoidance behavior were assessed by time spent in an ‘interaction zone’ around a novel caged aggressor (Golden, Covington, Berton, & Russo, 2011; Tsankova et al., 2006). After characterizing mice into susceptible (SUS; < 100) and resilient (>100) based off their social interaction time percentage, we randomly assigned SUS and control (CON; non-stressed mice) to receive either FLX or VEH for 3 weeks. Following treatment, we conducted the social interaction test for a second time to assess antidepressant impact on social behavior. Additionally, we ran the OF, LD, EPM, and NSF.


Separate cohorts of CORT and Social Defeat mice were created to assess DG activity underlying the behavioral antidepressant response. We hypothesized that responders to chronic antidepressant treatment would have less activation of DG GCs than non-responders. After 40 minutes of exposure to the NSF arena, mice were transcardially perfused and brains were extracted. Coronal serial sections (40uM) were collected and used for immunostaining, with activation of mature DG GCs assessed by staining and counting the number of DG cFos-positive cells in 1 out of every 6 sections throughout the hippocampus.

1.C Evaluate adult hippocampal neurogenesis in responders and non-responders to FLX treatment.

We predicted that responders to antidepressant treatment would have higher levels of neurogenesis and lower levels of DG GC activity. To assess adult hippocampal neurogenesis, we used tissue collected from the cohorts described above in 1.A. We assessed cell proliferation
of young DG GCs by staining and counting the number of DG ki67-positive cells in 1 out of every 6 sections throughout the hippocampus. Additionally, doublecortin (DCX), an early marker of young neurons, was used to assess differentiation and maturation of DG GCs across 1 out of every 6 hippocampal sections. The total number of DCX+ cells were used to assess differentiation into neurons, and for maturation DCX+ cells were subcategorized according to their dendritic morphology: DCX+ cells with no tertiary dendritic processes and DCX+ cells with complex, tertiary dendrites. After counting, we calculated a maturation index, defined as the ratio of DCX+ cells possessing tertiary dendrites over the total DCX+ cells (Samuels et al., 2015).

**AIM 2. Analyze behavioral response in relation to direct modulation of mature DG GCs.**

The ventral DG is important in regulating mood-related behaviors and facilitating responses to antidepressants (Bagot et al., 2015; Kheirbek et al., 2013; Nautiyal et al., 2016). Kheirbek and colleagues (2013) observed that optogenetic inhibition of ventral DG, but not dorsal DG, alters behavior in anxiety-related negative behavioral tasks. Recently, Samuels and colleagues (2015) found that deletion of 5-HT$_{1A}$ heteroreceptors, which are Gi-coupled, from mature DG GCs abolished the behavioral response to SSRIs within the NSF, EPM, and FST while attenuating the neurogenic effects of SSRIs. 5-HT$_{1A}$ heteroreceptors are more enriched in the ventral DG, positioning these receptors to directly influence limbic circuitry in order to regulate mood-related behavior and response to SSRIs (Yohn et al., 2017). Therefore, direct inhibition of ventral DG GCs via Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) may be important for SSRI-mediated behavioral effects.
2.A. Test whether Gi-DREADD mediated silencing of mature ventral DG GCs facilitates an antidepressant response.

The use of DREADDs, a chemogenetic tool, allows for cell-specific in vivo functional manipulation of neuronal and non-neuronal signal transduction (Roth, 2016). We predicted that Gi-DREADD-mediated inhibition of ventral DG GCs would mimic an antidepressant behavioral response. To test the behavioral effects of Gi-DREADD silencing of ventral DG GCs, we bilaterally injected either a AAV8-CamKIIa-hm4D(Gi)-mCherry or AAV8-CamKIIa-EGFP into the ventral DG of 6 week old mice: 3.5mm, +/-2.8mm relative to the bregma line and midline respectively at a depth of 3.6 mm from the skull. Two weeks after surgeries mice were either treated with CORT or VEH. Four weeks after CORT or VEH treatment, mice received either CNO (5mg/kg/day), which activated the Gi-DREADDs (Gomez et al., 2017), or VEH in their drinking water. Thus four groups per virus were created, VEH+VEH, VEH+CNO, CORT+VEH, CORT+CNO. After three weeks of CNO or vehicle treatment we assessed behavioral effects of Gi-DREADD mediated silencing of ventral DG GCs in the OF, LD, EPM, and NSF. Mice were transcardially perfused 40 minutes following NSF to assess DREADD activation. While counting cFos-positive cells within the ventral DG, we also confirmed our viral injections targeted the ventral DG. In counting cFos expression we controlled for the optical intensity of the virus to ensure that each section counted had a similar expression of the virus.

2.B. Test whether Gq-DREADD mediated excitation of mature ventral DG GC facilitates an increase in negative behaviors

We predicted that Gq-DREADD-mediated activation of ventral DG GCs would increase anxiety- and depressive-related behaviors in the OF, LD, EPM, and NSF. To test behavioral differences, we bilaterally injected either a AAV8-CamKIIa-hm3D(Gq)-mCherry or AAV8-
CamKIIa-EGFP into ventral DG of 6 week old mice: 3.5mm, +/-2.8mm relative to the bregma line and midline respectively at a depth of 3.6 mm from the skull. Similar to the aim 2.A, mice were treated with CORT or VEH for four weeks and then either CNO or VEH for three weeks; with four groups per virus. Subsequent to three weeks of CNO or VEH treatment, negative behaviors within the OF, LD, EPM, NSF, and FST were assessed. We assessed Gq-DREADD-mediated excitation of ventral DG GCs by counting cFos positive cells, similar to the methods discussed in aim 2.A.
MATERIALS AND METHODS

Subjects: Adult male C57BL mice were purchased from Jackson Laboratories for both specific aims. All mice were maintained on a 12L:12D schedule with food and water provided *ad libitum*. Across both aims behavioral testing for OF, EPM, light dark (LD), NSF, FST, and social interaction was conducted during the light phase. All testing was conducted in compliance with the NIH laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Protocol # 17-015; 15-070).

Stress Paradigms:

*Chronic Corticosterone:* Adult male C57BL mice ranging between 7-8 weeks old were randomly assigned to either vehicle or corticosterone treatment. Before treatment all mice were weighed, with weights measured once per week during treatments. Corticosterone (35 ug/mL, equivalent to 5 mg/kg/day) was dissolved in 0.45% beta-cyclodextrine (Sigma) and delivered in opaque bottles to protect it from light, where it was available *ad libitum* in the drinking water of corticosterone treated mice (David et al., 2009). Mice within the control group received 0.45% beta-cyclodextrine within their drinking bottles. Corticosterone treatment lasted roughly 10 weeks, with corticosterone remaining in the drinking water throughout antidepressant treatment (see Figure 1 for timeline).

*Social Defeat Stress:* Defeat stress was carried out using similar methods to those already published (Tsankova et al., 2006; Golden, Covington, Berton, Russo, 2011). Prior to social defeat stress, male retired breeder CD1 mice (Charles River Labs) were screened for aggression. CD1 mice that attack screener mice (C57/BL6) for two consecutive days under 60 seconds were selected as aggressors (Golden, et al., 2011). After selection of aggressors, experimental mice were exposed to a different CD1 aggressor mouse each day for 5 min over 10 days. After
contact, experimental mice were inspected for injury and separated from the aggressor and placed in an adjacent compartment of the same cage as the CD1 mouse, separated by a plastic divider with holes. Control test mice were housed in equivalent cages but with members of the same strain, which were changed daily. Twenty-four hours after the last session, all mice were housed individually for the remainder of the study.

**Viral Injections:** To assess antidepressant-like effects of inactivation or activation of ventral DG GC one of three DREADD viruses AAV8-CamKIIa-hm4D(Gi)-mCherry, AAV8-CamKIIa-hm3D(Gq)-mCherry, AAV8-CamKIIa-EGFP-mCherry were injected into the ventral DG of 6 week old mice: 3.5mm, +/-2.8mm relative to the bregma line and midline respectively at a depth of 3.6 mm from the skull at the bregma.

**Drugs:**

*Fluoxetine:* Fluoxetine (18 mg/kg/day in deionized water) or vehicle (deionized water) were delivered by oral gavage following 4 weeks of chronic CORT treatment or social defeat stress for three weeks prior to behavior testing. The oral gavage of fluoxetine or vehicle were continued throughout the behavior testing and to experimental endpoints. On behavioral testing days, fluoxetine or vehicle administrations were conducted after the mice completed the testing in order to avoid any acute effects.

*CNO:* Water bottles were filled on alternating days with 0.05 mg/ml CNO. CNO was dissolved in DMSO, for a final concentration of 0.25% DMSO, and added to 0.5% saccharine water. Based off of mice weights ranging between 30–35 g and water consumption approximately 3 ml/day, each mouse received an approximate dose of 5 mg/kg CNO daily.

**Behavioral Tests:**
**Open Field:** Motor activity was quantified in five Plexiglass open field boxes 43 x 43 cm² (Kinder Scientific). The recording of x-y ambulatory movements was recorded by two sets of 16 pulse-modulated infrared photobeams placed on opposite walls 2.5 cm apart to. As previously described (David et al., 2009), activity chambers were computer interfaced for data sampling at 100ms resolution. The computer software predefines grid lines that divide each open field chamber into center and periphery regions, with the center being a square 11cm from the wall. The number of entries, distance traveled, and total time spent in the center were recorded, as well as percent of distance traveled in the center defined as center distance divided by total distance traveled (Supplemental Figure 1A). To measure overall motor activity total distance (cm) was quantified.

**Novelty Suppressed Feeding (NSF):** After undergoing 18 hours of food deprivation within their home cage, mice were placed in the corner of a testing apparatus (50x50x20 cm) filled with approximately 2 cm of corncob bedding and a single pellet of food attached to a white platform in the center of the box. The center of the box was illuminated at 1500 lux. The NSF test lasted 6 minutes, where the latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed/recorded. If a mouse did not consume food during the NSF a latency of 360secs was recorded. Immediately afterwards, the mice were transferred to their home cage to assess home cage feeding behavior for 5 minutes. During this task, latency to eat and amount of food consumed was measured as a control for feeding behavior observed in the NSF task. Each mouse was weighed before food deprivation and after home cage feeding to assess the percentage of body weight loss. Following home cage feeding, mice were placed in a new home cage with cage mates and returned to the colony room.
**Light Dark:** The light/dark test was conducted in an open field chamber measuring 43.2 x 43.2 cm (Kinder Scientific, USA), with a clear floor and walls. To divide the open field into separate light and dark compartments, a dark plastic box that covered one third of the chamber was inserted. The dark box was opaque to visible light, but transparent to infrared light, and contained an opening that allowed passage between the light and dark compartments (David et al., 2009). The light compartment was brightly illuminated at 1000 lux. At the beginning of each 5-minute test, mice were placed in the dark compartment. An observer blind to treatment groups recorded the latency to emerge into the light (Supplemental Figure 1C). Using Activity Monitor (Kinder Scientific, USA) software, total time in the light and ambulatory distance in both compartments was analyzed. To calculate percent distance traveled in the light, distance traveled in the light was divided by total distance traveled (Supplemental Figure 1B).

**Elevated Plus Maze (EPM):** The EPM test consisted of a plus-shaped apparatus with two open and two closed arms (side walls), elevated 2 feet from the floor. During the five-minute test, the mouse’s behavior was recorded from a video camera mounted above each EPM arena. EthoVision (Noldus) software was then used to score time spent in the open arms, entries, and distance traveled in both open and closed arms. By dividing total open arm distance traveled by total distance traveled, we were able to analyze percent distance traveled in open arms.

**Forced swim test (FST):** A modified FST procedure suitable for mice was used (David et al., 2009). Individual cylinders (46 cm tall x 32 cm in diameter x 30 cm deep) were filled with room-temperature water (25-26°C) before placing each mouse into the cylinder. Two sets of photobeams were mounted on opposite sides of the cylinder (Kinder Scientific, USA) to allow for the recording of swimming behavior during the 6-minute test. Immobility times (measured by
beam breaks over 5-second intervals) were assessed during the last 4-minutes of the test since mice are habituating to the task during the initial 2-minutes of the test.

**Social interaction:** Experimental mice that underwent the social defeat stress underwent the social interaction test twice (pre and post antidepressant treatment) to assess social interaction and avoidance behavior in the presence of a novel aggressor (CD1 mouse). Mice were placed in an open field arena for two trials running 150 seconds each. The first trial was in the absence of the aggressor, while the second trial was in presence of the aggressor confined in a wire mess container within the social interaction zone (Tsankova et al., 2006; Golden, Covington, Berton, Russo, 2011). The open field arena was divided into zones by the computer software (Etho Vision) with the corner zones (9 cm x 9 cm) on the opposite side of the arena from the social interaction zone (14 cm x 24 cm). Behavior was recorded by overhead cameras, where time spent in the interaction and corner zones were measured. Social avoidant and interaction behavior was measured by time spent in the interaction zone during the first (aggressor absent) and second (aggressor present) trials. An interaction ratio was calculated by the following equation: 100 x ((interaction time, aggressor absent) / (interaction time, aggressor present)). General motor activity was measured by overall distance traveled within the arena.

**Immunohistochemistry:**

**cFos staining:** The effects of a chronic corticosterone treatment in presence or absence of fluoxetine treatment on DG GC activity was assessed. Forty minutes after the NSF task, when cFos peaks in expression, animals were anesthesized with ketamine and xylazine (100 mg/ml ketamine; 20 mg/ml xylazine), and perfused transcardially (cold saline for 2 min, followed by 4% cold paraformaldehyde at 4°C). The brains were removed and stored in 4% paraformaldehyde overnight at 4°C. Next brains were cryoprotected in 30% sucrose, 1% sodium
azide and stored at 4°C. Serial sections (40 µM) were cut on a cryostat and collected through the entire hippocampus (Franklin and Paxinos, 1997). Sections were collected in wells and wet mounted prior to staining. Sections were washed in 1% Triton X-100 PBS for 5 minutes before undergoing three PBS washes. Slides were incubated in warm citrate buffer for 30 minutes. After washing with PBS, slides were blocked for 1 hour in 10% normal goat serum (NGS) before being incubated overnight at 4°C in anti-rabbit cFos (1:750; Cell Signal). Next, slides were washed with PBS then incubated at room temperature for two hours in the secondary antibody (CY-5 goat anti-rabbit; 1:1000). Following the incubation slides were washed with PBS then counterstained with DAPI (1:15000) for 15 minutes. Finally, slides were washed with PBS and cover slipped using the mounting medium prolong diamond. Fluorescent images were taken using an inverted microscope (ThermoFisher), where cFos positive cells overlayed with DAPI across the 12 sections of hippocampus will be counted.

**Ki67 labeling for cell proliferation:** The effects of a chronic corticosterone treatment in presence or absence of fluoxetine treatment on cell proliferation was assessed. Sections were either collected on superfrost plus slides and stored at -20°C or in wells containing 30% sucrose, 0.1% NaN₃ in PBS stored at 4°C. Using a similar protocol as the above cFos section, sections were incubated overnight at 4°C in anti-rabbit ki67 (1:500; abCam) and anti-guinea pig NeuN (1:1000; Invitrogen). Next, slides were washed with PBS then incubated at room temperature for two hours in CY-5 goat anti-rabbit (1:1000) and Alexa 594 goat anti-guinea pig (1:1000). Finally, slides were washed with PBS and cover slipped using the mounting medium prolong diamond. Fluorescent images were taken using an inverted microscope (ThermoFisher), where ki67 positive cells across the 12 sections of hippocampus will be counted.
Doublecortin (DCX) labeling for maturation: For doublecortin (DCX) staining, the procedure consisted of the following steps (Wang et al., 2008): sections were rinsed in PBS, treated with 1% H2O2 in 1:1 PBS and methanol for 15 min to quench endogenous peroxidase activity (and to enhance dendritic staining), incubated in citrate buffer for 30 minutes then in 10% normal donkey serum and 0.3% Triton X-100 for one hour. Following the one-hour incubation, sections were incubated overnight at 4°C in primary antibody for doublecortin anti-goat (1:500; ThermoScientific). The secondary antibody biotinylated donkey anti-goat (1:500) (Jackson ImmunoResearch, PA, USA) was washed in PBS for 2 hr at room temperature. Sections were developed using avidin-biotin complex (Vector, CA, USA) and DAB kit. Bright-field images were taken with an inverted microscope. Stereological procedure were used to quantify labeled cells (Wang et al., 2008). DCX+ cells were subcategorized according to their dendritic morphology: DCX+ cells with no tertiary dendritic processes and DCX+ cells with complex, tertiary dendrites. The maturation index was defined as the ratio of DCX+ cells possessing tertiary dendrites over the total DCX+ cells.
RESULTS

*CORT+FLX animals show a bimodal distribution of responding to antidepressant*

To assess our hypothesis that chronic CORT induces depressive-like behavior that can be reversed by SSRI treatment, we measured depressive and anxiety-like behavior across an array of behaviors. A log-rank Mantel-Cox test revealed that CORT+VEH animals have an increased latency to eat as compared to VEH animals ($\chi^2_{(1)} = 8.50, p = 0.004$) and CORT+FLX animals ($\chi^2_{(1)} = 17.2, p < 0.0001$; Figure 1B). Despite fluoxetine administration reducing overall latency to feed among most individual animals (“Responders”), there is still a subpopulation who do not show this reduction (“Non-responders”).

Within the EPM, a one-way ANOVA revealed significant group differences ($F_{(4,65)} = 16.2, p < 0.0001$) with Bonferroni post-hoc comparisons indicating that CORT+VEH animals have less open arm entries than VEH mice ($p = 0.0006$) and responders to fluoxetine (CORT+FLX+R; $p < 0.0001$). CORT+FLX+R had more open arm entries than non-responders to fluoxetine (CORT+FLX+NR, $p < 0.0001$; Figure 1C). Similarly, a One-way ANOVA showed significant group differences in time spent on the open arms of the EPM ($F_{(4,65)} = 15.3, p < 0.0001$; Figure 1C). Planned post-hoc comparisons, Bonferroni corrected, indicated that CORT mice spent less time on the open arms than VEH ($p = 0.0036$) and CORT+FLX+R ($p < 0.0001$). Also, CORT+FLX+R spent more time on the open arms than CORT+FLX+NR ($p < 0.0001$).

To assess pharmacological action of FLX we examined immobility duration in the FST during the last 4 minutes. A one-way ANOVA revealed significant group differences ($F_{(4,65)} = 13.4, P < 0.0001$; Figure 1D), with planned-post hoc comparisons showing that VEH+FLX mice were less immobile than VEH mice ($p = 0.003$). Additionally, CORT+VEH mice spend more time immobile than VEH mice ($p < 0.0001$) and CORT+FLX+R ($p < 0.0001$), with
CORT+FLX+NR also spending more time immobile than CORT+FLX+R mice ($p = 0.0001$)

To further analyze behavioral response to antidepressants across these negative valence tests, we assessed the relationship between NSF latency to feed and behavioral performance within the EPM and FST. Positive relationships emerged between NSF latency to feed and open arm time (Pearson $r = -0.786$, $P < 0.0001$; Figure 1F), as well as NSF latency to feed and immobility duration (Pearson $r = 0.773$, $P < 0.0001$). To characterize these relationships further we ran two separate linear regressions, with NSF latency and open arm time having a linear regression line ($y = -6.02x + 780$, $F_{(1,21)} = 33.9$, $P < 0.0001$), and FST linear regression line ($y = 4.63x - 415$, $F_{(1,21)} = 31.1$, $P < 0.0001$).

**Susceptible+FLX mice show a bimodal distribution of responding to antidepressant**

To further characterize behavioral response to antidepressants, we used a second valid stress paradigm social defeat stress (SDS) to assess treatment response to FLX. Before administrating FLX, we analyzed response to SDS using the social interaction test (SIT), where an interaction time percent above 100% is deemed resilient (RES) and a percent below 100% is deemed susceptible (SUS) to the SDS. A one-way ANOVA revealed significant differences in social interaction time percent’s $F_{(2,60)} = 50.28$, $p < 0.001$ (Figure 2B), with post-hoc comparisons showing that SUS mice have lower interaction time percent’s than control (CON) mice ($p < 0.001$, Bonferroni-corrected) and RES mice ($p < 0.001$, Bonferroni-corrected).

Specifically, a repeated measures ANOVA revealed a significant interaction effect between group and trials where a social target was either absent or present ($F_{(2,54)} = 30.25$, $p < 0.001$; Figure 2C). Planned comparisons revealed that CON ($p = 0.18$) and RES mice ($p = 0.002$) spent more time in the interaction zone when the social target was present than absent (Figure 2C). On the other hand, SUS mice spent significantly less time in the social interaction zone when the
social target was present (p < 0.001). The repeated measures ANOVA also showed a significant
group effect \( F_{(2,54)} = 11.2, p < 0.001 \), with planned post-hoc comparisons indicating that SUS
mice spend less time in the social interaction zone when the social target is present compared to
both CON (p<0.001) and RES (p <0.001) mice. Following characterization of response to SDS
as RES or SUS, only SUS and CON mice were used for the remainder of the experiments.

Subsequent to 3 weeks of FLX or VEH treatment mice were exposed to an array of
negative valence behaviors, OF, LD, EPM, NSF, and re-exposed to the SIT to assess effects of
FLX on interaction and avoidant behaviors. A log-rank Mantel-Cox test revealed that group
differences emerged in the NSF \( \chi^2_{(3)} = 11.98, p = 0.007; \) Figure 2J. SUS mice had a longer
latency to eat as compared to CON mouse \( \chi^2_{(1)} = 6.62, p = 0.01 \) and SUS+FLX mouse \( \chi^2_{(1)} =
3.99, p = 0.04; \) Figure 2J. Despite fluoxetine administration reducing overall latency to feed
among most individual SUS mice (“Responders”), there is still a subpopulation of SUS+FLX
mice that do not show this reduction (“Non-responders”), as seen in the CORT paradigm.

In assessing effects of FLX on avoidance behavior we conducted the SIT for a second
time, with a repeated-measures ANOVA revealing a significant interaction effect between time
(pre/post) and treatment \( F_{(4,44)} = 3.98, p = 0.007 \) and a significant treatment effect \( F_{(4,44)} =
15.48, p < 0.0001 \). Planned comparisons revealed that interaction times for SUS+FLX+R were
significantly different between pre-FLX and post-FLX (p = 0.004) treatment (Figure 2D). A one-
way ANOVA revealed significant group differences in percent distance traveled in the light
during the LD test \( F_{(2,44)} = 7.96, p < 0.001 \). Planned post-hoc comparisons showed that
SUS+VEH mice had traveled less distance in the light than CON (p < 0.05) and SUS+FLX+R (p
< 0.001) mice (Figure 2E). SUS+FLX+NR mice also traveled less distance in the light than
SUS+FLX+R (p < 0.001; Figure 2E). Similar group differences were seen in latency to emerge
into the light ($F_{(2,44)} = 19.6, p < 0.001$), with SUS+VEH mice having a longer latency to emerge than CON ($p < 0.05$) and SUS+FLX+R ($p < 0.001$) mice (Figure 2F). SUS+FLX+NR mice also had a longer latency to emerge into the light than SUS+FLX+R ($p < 0.001$). Separate one-way ANOVAs revealed significant group differences in time spent ($F_{(2,44)} = 6.81, p < 0.001$) and distance traveled ($F_{(2,44)} = 6.1, p < 0.001$) on the open arms of the EPM. Planned post-hoc comparisons showed that SUS+VEH mice travel less (Figure 2G) and spend less time (Figure 2H) on the EPM open arms than CON ($p = 0.029$ distance; $p = 0.039$ time) and SUS+FLX+R mice ($p = 0.005$ distance; $p = 0.024$ time). SUS+FLX+NR mice also travel less distance ($p = 0.017$; Figure 2G) and spend less time ($p = 0.003$; Figure 2H) than SUS+FLX+R on the open arms of the EPM.

**Differences in activity of DG GCs between FLX Responders and Non-responders**

To assess our hypothesis that response to antidepressants decreases activation of DG GCs, we collected tissue from a separate cohort of mice 40 minutes following NSF. We stained for cFos in 1 out of every 6 sections of the DG. A one-way ANOVA revealed significant group differences $F_{(4,36)} = 18.47, p < 0.001$ (Figure 3B, 3D), with planned post-hoc comparisons showing VEH mice have more cFos expression within the DG than VEH+FLX mice ($p < 0.001$). Additionally, CORT+FLX+R have less DG cFos$^+$ cells than CORT mice ($p < 0.001$) as well as CORT+FLX+NR ($p = 0.021$; Figure 3B, 3D).

Similar activation of DG GCs was observed in the SDS paradigm, with a one-way ANOVA revealing significant group differences ($F_{(3,21)} = 15.45, p <0.001$; Figure 4B, 4D) in DG cFos expression. Specifically, planned-comparisons revealed that SUS+FLX+R mice had significantly less cFos$^+$ cells within the DG than SUS+VEH ($p<0.001$; Figure 4B, 4D) and SUS+FLX+NR ($p<0.001$) mice.
**FLX responders have higher levels of DG neurogenesis than FLX non-responders**

Antidepressant treatment is known to increase all stages of adult hippocampal neurogenesis (David et al., 2009), therefore, we predicted that responders to antidepressant treatment would have higher levels of neurogenesis than non-responders to FLX. In staining 1 out of every 6 section for Ki67, a marker of proliferating cells, a one-way ANOVA revealed significant group differences $F_{(4,36)} = 18.79, p < 0.001$. Planned post-hoc comparisons showed that VEH mice had less ki67$^+$ cells than VEH+FLX mice ($p = 0.011$; Figure 3C, 3E), but more ki67$^+$ cells than CORT mice ($p = 0.02$). CORT+FLX+R mice had higher expression of DG ki67$^+$ cells than CORT mice ($p < 0.001$) or CORT+FLX+NR ($p < 0.001$; Figure 3C, 3E). In assessing group differences in expression of immature neurons, a one-way ANOVA revealed significant group differences ($F_{(4,36)} = 17, p < 0.001$) in DG DCX$^+$ cells (Figure 3F-G). Planned post-hoc comparisons showed that VEH mice had less DCX$^+$ cells than VEH+FLX mice ($p = 0.015$), but more DCX$^+$ cells than CORT mice ($p = 0.02$; Figure 3F-G). CORT+FLX+R mice had higher expression of DG ki67$^+$ cells than CORT mice ($p < 0.001$) or CORT+FLX+NR ($p < 0.001$).

Lastly, a One-way ANOVA showed significant group differences in mature neurons (DCX$^+$ with tertiary dendrites), $F_{(4,36)} = 20.9, p < 0.001$ (Figure 3H). Post-hoc comparisons revealed that VEH mice had less mature neurons than VEH+FLX mice ($p = 0.019$). Moreover, CORT+FLX+R had more mature neurons than CORT mice ($p < 0.001$) and CORT+FLX+NR ($p < 0.001$; Figure 3H).

Within the SDS mice we detected similar differences in neurogenesis, with a significant group difference in expression of ki67$^+$ cells within the DG ($F_{(3,21)} = 22.54, p < 0.001$; Figure 4, 4E). Planned-comparisons revealed that SUS+FLX+R mice had significantly more ki67$^+$ cells within the DG than SUS+VEH ($p<0.001$) and SUS+FLX+NR ($p<0.001$) mice (Figure 4C, 4E). Similar group differences emerged in expression of DG DCX$^+$ cells ($F_{(3,21)} = 4.92, p <0.001$;
Figure 4F-G), with SUS+FLX+R mice having more DG DCX$^+$ cells than SUS+VEH ($p = 0.048$) and SUS+FLX+NR ($p = 0.035$). Lastly, similar group differences were observed in expression of mature neurons ($F_{(3,21)} = 5.18, p=0.008$) with SUS+FLX+R mice having more mature neurons cells than SUS+VEH ($p = 0.007$) and SUS+FLX+NR ($p = 0.025$; Figure 4H).

**Inhibition of DG GCs facilitates antidepressant response**

To assess our hypothesis that inhibition of DG GCs facilitates behavioral response to antidepressants, we injected either an inhibitory (Gi DREADD), excitatory (Gq DREADD), or control virus into the ventral DG. In order to analyze the impact of each virus on behavior, four groups were created: VEH, VEH+CNO, CORT, CORT+CNO (Figure 5A). Subsequent to 3 weeks of CNO treatment mice were exposed to OF, LD, EPM, and NSF to assess effects of ventral DG inhibition or excitation on negative valence behaviors. A log-rank Mantel-Cox test revealed that treatment differences within the Gi DREADD group emerged in the NSF ($\chi^2_{(3)} = 20.53, p = 0.0001$; Figure 5E, 5G). Specifically, Gi DREADD CORT+CNO mice had a shorter latency to eat than CORT mice ($\chi^2_{(1)} = 12.64, p = 0.0004$). DREADD mediated excitation, Gq DREADD+CNO, resulted in an increase in latency to eat compared to Gq DREADD+VEH ($\chi^2_{(3)} = 19.8, p = 0.0002$; Figure 5F, 5G).

Within the OF, a 3x4 ANOVA revealed a significant treatment effect ($F_{(3,60)} = 3.13, p = 0.032$; Figure 5H) on time spent within the center of the OF arena. Within group post-hoc comparisons showed that Gi DREADD+CORT mice spent less time in the OF center than Gi DREADD+CORT+CNO ($p = 0.018$). Between virus and treatment groups, a 3x4 ANOVA revealed a significant interaction effect ($F_{(6,60)} = 3.53, p = 0.005$, Figure 5I). Within the Gi DREADD virus group post-hoc comparisons illustrated Gi DREADD+VEH+CNO mice travel more distance on the open arms than Gi DREADD+VEH mice ($p = 0.011$; Figure 5I). Similarly,
activation of the Gi DREADD on a stress background resulted in similar differences with Gi DREADD+CORT+CNO mice having more open arm distance than Gi DREADD+CORT (p<0.001). In the LD test a 3x4 ANOVA showed a significant interaction effect between virus and treatment (F_{(6,60)} = 4.64, p < 0.001; Figure 5J) on percent distance traveled within the light. Post-hoc comparisons showed Gi DREADD+VEH+CNO mice traveled more distance in the light compared to Gi DREADD+VEH mice (p = 0.005). Additionally, Gi DREADD+CORT+CNO mice traveled more within the light compartment of the LD test than Gi DREADD+CORT mice (p=0.006).

To evaluate whether CNO administration lead to the inhibition of vDG GCs we analyzed cFos expression in mice sacrificed 40 minutes post NSF. We counted cFos expression within sections of the vDG that had viral expression, with viral expression (optical intensity) controlled for across all sections counted. A 2x3 ANOVA revealed a significant effect of virus (F_{(2,18)} = 3.95, p = 0.038; Figure 5B, 5C) and treatment (VEH and VEH+CNO; F_{(1,18)} = 8.67, p = 0.009, Figure 5C), with a significant interaction between virus groups and treatment (F_{(2,18)} = 4.21, p = 0.032) emerging. Post-hoc comparisons revealed that within VEH+CNO virus group differences emerged with Gi DREADD mice having less cFos expression than control (GFP; p = 0.007; Figure 5) or Gq DREADD (p = 0.009) mice. Additionally, Gi DREADD VEH+CNO mice had less cFos expression than Gi DREADD VEH mice (p = 0.002). No differences in optical intensity were detected across viral or treatment groups.
DISCUSSION

In the current study we illustrate the importance of the DG in mediating the behavioral response to antidepressant treatment. Across two valid chronic stress experimental systems, we find that behavioral response to FLX results in a decrease in negative valence behaviors. Behavioral response to FLX is associated with a decrease in DG GC activity and an increase in adult hippocampal neurogenesis. Furthermore, we illustrate that chemogenetic silencing of DG GCs via DREADDs facilitates a decrease in negative valence behaviors. Together these results suggest that DG GCs inhibition is a critical component of the behavioral response to FLX.

Behavioral response to FLX

Within the United States, 16% of the population will experience an episode of major depression in their lifetime (Smith, 2014). Although commonly used treatments, such as SSRIs, are readily prescribed to reprieve depressed patients of their symptoms, only a subset of patients (~33%) achieve remission with initial treatment (Trivedi et al., 2006). In using two valid chronic stress experimental systems, we show a bimodal distribution in both stress+FLX groups, with roughly 26% of the stress+FLX population showing no behavioral response to antidepressants. Across both chronic CORT and social defeat stress paradigms, we illustrate that behavioral response to FLX (CORT+FLX R and SUS+FLX R) results in a decrease in negative valence behaviors. Additionally, within the social defeat stress paradigm SUS mice that are responders to FLX show a decrease in social avoidant behaviors during the second social interaction test as compared to both SUS+VEH and SUS+FLX NR mice. Thus, our results add to the literature that FLX leads to a decrease in negative valence (David et al., 2009) and social avoidant behaviors. In assessing the relationship in behavioral response to FLX across the negative valence tests, our results highlight that lower latency to eat within the NSF is related to both lower FST immobility.
times (Figure 1E) as well as more time spent on the open arms of the EPM (Figure 1F).

Moreover, our data highlights that a non-responsive phenotype to FLX is persistent, since CORT FLX+NR mice tested up to 6 months had no change in latency to eat over time.

Our preclinical data highlights a growing dilemma in modern psychiatry, with a subset of the population non-responsive to first line pharmacotherapies. Within the clinical population, studies have tried to address this issue by switching non-remitters to SSRIs to a second antidepressant. Specifically, Rush and colleagues (2006) switched citalopram (SSRI) non-remitters to either bupropion (norepinephrine dopamine reuptake inhibitor), venlafaxine (serotonin norepinephrine reuptake inhibitor), or sertraline (SSRI) and measured their response to these second line antidepressants. Surprisingly, Rush and colleagues (2006) found that similar remittance profiles still persisted in these patients, despite these drugs acting on different monoaminergic neurotransmitters. These results highlight the need for a better understanding in the molecular and cellular action of antidepressants as well as novel pharmacotherapies that address the heterogeneous nature of depression.

Our data illustrates that the behavioral response to FLX is related to a decrease in DG GC activity. In the current study we aimed to delineate whether it is excitation or inhibition of the DG that may mediate the behavioral response to FLX. Both CORT+FLX R (Figure 3B) and SUS+FLX R (Figure 4B) mice had lower cFos expression than their stress and FLX NR counterparts. Therefore, behavioral response to FLX may be related to a decrease in DG GC activity, since response to FLX resulted in lower cFos expression within the DG.

**Chronic Inhibition of the DG mimics antidepressant behavioral response**

Research suggests that inhibition of the DG appears to be a critical component of the behavioral response to SSRIs. Specifically, we show that behavioral responders to FLX have less
DG GC activity than non-responders to FLX. Additionally, Samuels and colleagues (2015) showed that behavioral response to FLX is related to 5-HT$_{1A}$ heteroreceptors inhibition of mature DG GCs within the ventral DG. Using DREADDs we assessed whether chronic inhibition of DG GCs would mimic an antidepressant behavioral response. Compared to Gi DREADD+VEH mice, we show that chronic inhibition of ventral DG GCs within Gi DREADD+CNO mice results in a reduction of negative valence behaviors. On a chronic CORT background, we showed an interaction between chronic stress and chronic inhibition, with Gi DREADD+CORT+CNO mice exhibiting a decrease in negative valence behaviors compared to Gi DREADD+CORT mice. Additionally, we show no behavioral differences between Gq DREADD+CORT and Gq DREADD+CORT+CNO mice, illustrating that chronic excitation of ventral DG GCs on a chronic stress background does not mimic an antidepressant response. Moreover, we show no behavioral differences between GFP+CNO and GFP+VEH mice controlling for the behavioral effects of CNO being reversed-metabolized to clozapine in vitro (Webster et al., 2018). However, we show that GFP+CORT+CNO mice have a reduction in latency to eat compared to GFP+CORT mice, indicating that CNO conversion to clozapine in vitro may facilitate antidepressant-like behavioral response on a stress background.

Hypothesis of DG microcircuitry

Our results corroborate the findings that the DG is critical for the beneficial behavioral, neurogenic, and neuroendocrine effects of fluoxetine (Samuels et al 2015). In the current study we showed that behavioral response to antidepressants is related to an inhibition of DG GCs supported by 1. FLX Rs having lower DG cFos expression than FLX NRs and 2. DREADD mediated chronic inhibition of ventral DG GCs mimics a behavioral antidepressant response. In addition to these findings we show that response to FLX is also related to increases in adult
hippocampal neurogenesis, with both CORT+FLX R (Figure 3D-I) and SUS+FLX R (Figure 4D-I) having more young abGCs (proliferating cells ki67), and higher expression of both immature and mature neurons (DCX). These results support studies showing that antidepressant treatment increases hippocampal neurogenesis (Wang et al., 2008; David et al., 2009). Samuels and colleagues (2015) illustrated that deletion of 5-HT\textsubscript{1A} heteroreceptors from mature DG GCs, not abGCs, abolished the behavioral response to SSRIs and attenuated the effects of SSRIs on adult hippocampal neurogenesis. Moreover, Anacker and colleagues (2018) showed that increased neurogenesis results in a decrease in ventral DG mature GC activity, with decreases in ventral DG activity facilitating resilience to stress. Our data in combination with these studies illustrates that decreases in mature DG GCs activity facilitates a behavioral response to FLX. Mature DG GCs may be inhibited by young abGCs acting on the local microcircuitry or through feedback inhibition mechanisms (Drew et al., 2016; Ikrar et al., 2013; Lacefield, Itskov, Reardon, Hen, & Gordon, 2012; Samuels et al., 2015). Specifically, young abGCs can evoke strong inhibitory input to mature DG GCs via activation of GABAergic interneurons (Ikrar et al., 2013). After ablating abGCs, Burghardt and colleagues (2012) observed an increase in behaviorally induced immediate early gene (IEG) expression in mature DG GCs. Behavioral responses to SSRIs may also be mediated by inhibition of GABA release from DG cholecystokinin (CCK) neurons (Medrihan et al., 2017). Specifically, by inhibiting the inhibitory DG CCK neurons, parvalbumin (PV) interneurons are disinhibited, leading to an inactivation of DG GCs. Altogether, behavioral response to FLX is related to an increase in hippocampal neurogenesis and a decrease in mature DG GC activity, through the active DG microcircuitry.
**Conclusion**

The current study illustrates that behavioral response to SSRIs is related to a decrease in DG GCs activity and an increase in adult hippocampal neurogenesis. Further we show that DREADD mediated inhibition of DG GCs mimics an antidepressant behavioral response. Direct modulation as well as inhibition of DG GCs through 5HT$_{1a}$ heteroreceptors and via local microcircuit is critical in mounting behavioral effects of antidepressants. Delineating the role of the DG GCs in mediating the behavioral response to antidepressants could lead to the development of novel pharmacotherapies.
REFERENCES


FIGURE LEGENDS

Figure 1. CORT+FLX animals show a bimodal distribution of responding to antidepressant. Timeline representing experimental design and time points behavior was conducted (A). CORT+FLX animals have a bimodal distribution in the NSF task, with CORT+FLX+R having a lower latency to eat than CORT+FLX+NR (B). Within the EPM stress alone (CORT) reduces time and entries on the open arms; with CORT+FLX+R spending more time and having more entries on the open arms than CORT and CORT+FLX+NR (C). In assessing pharmacological response to FLX we find CORT+FLX+R are less immobile than CORT+FLX+NR and CORT mice (D). Latency to eat in the NSF is significantly related to behavioral performance in the FST (E) and EPM (F). The CORT+FLX+NR phenotype is persistent, with mice remaining behavioral non-responders up to 6 months after initial NSF assessment (G).

Figure 2. Social Defeat stress warrants similar behavior phenotypes in response to FLX treatment as chronic CORT paradigm. Timeline and schematic drawing representing chronic social defeat stress is depicted (A). Following 10 days of social defeat stress, social interaction test was run to assess behavioral response to stress, with resilient (RES) mice having a higher social interaction percentage than susceptible (SUS) mice (B). Further, SUS mice spend less time in social interaction zone when the social target is absent than present (C). After 3 weeks of FLX treatment, social interaction was run again with SUS+FLX+R having a decrease in social avoidant behaviors following FLX treatment (D). SUS+FLX+R mice also have a lower latency to emerge into the light (E), travel more in the light (F) and on open arms of EPM (G) as well as are more immobile (H) than SUS and SUS+FLX+NR mice. Within the NSF there is a bimodal
distribution in the SUS+FLX group with SUS+FLX+R having a lower latency to eat then SUS+FLX+NR.

Figure 3. Differences in DG GC activity and neurogenesis between CORT+FLX responders and non-responders. Mice were sacrificed 40 minutes following behavioral task (A). CORT+FLX+R have less cFos expression within the DG than CORT and CORT+FLX+NR (B). Response to FLX results in higher expression of ki67+ (C) and DCX+ (F-G) within the DG compared to non-responders and CORT mice. Images representing differences (D, E, H).

Figure 4. Differences in DG GC activity and neurogenesis between SUS+FLX responders and non-responders. Mice were sacrificed 40 minutes following behavioral task (A). SUS+FLX+R have less cFos expression within the DG than SUS and SUS+FLX+NR (B). Response to FLX results in higher expression of ki67+ (C) and DCX+ (F-G) within the DG compared to non-responders and SUS mice. Images representing differences (D, E, H).

Figure 5. Direct inhibition of DG GCs facilitates antidepressant-like behavioral response. Mice had 1 of 3 viruses (Control, Gi DREADD, Gq DREADD) expressed within ventral DG (A). Within each viral group 4 groups emerged: VEH, VEH+CNO, CORT, CORT+CNO. Following 3 weeks of CNO or VEH treatment mice experienced negative valence behaviors. To assess DREADD mediated DG GC silencing cFos counts were used (B-D). Gi DREADD+VEH mice have more cFos expression than Gi DREADD+CNO mice (C). Latency to eat in NSF is decreased in Gi DREADD+CORT+CNO mice compared to their stress counterparts (E, G). Administration of CNO in Gq DREADD mice results in an increase in latency to eat compared
to Gq DREADD+VEH mice (F, G). Inhibition of the DG GCs (Gi DREADD+CNO and Gi DREADD+CORT+CNO) resulted in an increase in center time in the open field (H), increase in distance traveled on open arms (I), and distance traveled in the light of the light/dark task (J).
Figure 1. CORT+FLX animals show a bimodal distribution of responding to antidepressant.
Figure 2. Social Defeat stress warrants similar behavior phenotypes in response to FLX treatment as chronic CORT paradigm.
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Figure 4. Differences in DG GC activity and neurogenesis between SUS+FLX responders and non-responders.
Figure 5. Direct inhibition of DG GCs facilitates antidepressant-like behavioral response.