nature neuroscience

5-HT1A receptors on mature dentate gyrus granule cells are critical for the antidepressant response

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Selective serotonin reuptake inhibitors (SSRIs) are widely used antidepressants, but the mechanisms by which they influence behavior are only partially resolved. Adult hippocampal neurogenesis is necessary for some of the responses to SSRIs, but it is not known whether mature dentate gyrus granule cells (DG GCs) also contribute. We deleted the serotonin 1A receptor (5HT1AR, a receptor required for the SSRI response) specifically from DG GCs and found that the effects of the SSRI fluoxetine on behavior and the hypothalamic-pituitary-adrenal (HPA) axis were abolished. By contrast, mice lacking 5HT1ARs only in young adult-born GCs (abGCs) showed normal fluoxetine responses. Notably, 5HT1AR-deficient mice engineered to express functional 5HT1ARs only in DG GCs responded to fluoxetine, indicating that 5HT1ARs in DG GCs are sufficient to mediate an antidepressant response. Taken together, these data indicate that both mature DG GCs and young abGCs must be engaged for an antidepressant response.

Elucidation of the neurobiological basis of depression and anxiety and identification of improved treatments for patients are two of the foremost challenges in modern psychiatry. Mood disorders affect 7% of the world's population and severe forms of depression affect 2–5% of the US population[1]. In addition, anxiety and depression have a high comorbidity with co-occurrence rates of up to 60% in patients[2]. Imaging and post-mortem studies have implicated several areas, including prefrontal and cingulate cortices, hippocampus, amygdala, and thalamus, in mood disorders[3,4]. Together, these brain regions operate a series of highly interacting circuits that likely mediate the progression of depression and the antidepressant response[4]. Identification of mechanisms in these brain regions should lead to improved therapies.

In the hippocampus, chronic treatment with selective serotonin reuptake inhibitors (SSRIs), the most widely used class of antidepressants, increases the dendritic spine density of pyramidal neurons in CA subfields and stimulates multiple stages of adult neurogenesis in the dentate gyrus (DG)[5,6]. Chronic, but not acute, SSRI treatment results in increased proliferation of dividing neural precursor cells, as well as faster maturation and integration of young abGCs into the DG[6,7]. The young abGCs are highly active for a few weeks after functional integration into the neuronal network[8,9], but eventually become functionally indistinguishable from the mature developmentally born GCs when they are approximately 8 weeks old[9,10]. Focal radiological or genetic strategies to ablate or impair the neurogenic niche result in the loss of some antidepressant-mediated behaviors, demonstrating that young abGCs are required for some antidepressant effects[7,11,12]. A few lines of evidence indicate that mature DG GCs (a population consisting of both developmentally born and adult born neurons that are older than 8 weeks) may also be involved in mediating mood and the antidepressant response. Stress induces marked changes in the DG. The hippocampus is vulnerable to various hormones induced by stress, such as glucocorticoids, and rat adrenalectomy results in the death of most DG GCs[13]. Furthermore, infusion of peptides, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and activin, into the DG result in an antidepressant-like response[14,15]. In addition, optogenetic manipulations of ventral DG GCs demonstrate a role in anxiety-related behaviors[16]. Notably, humans suffering from major depressive disorder have fewer DG GCs than controls[17]. A recent study also found that DG volume substantially decreases as the number of depressive episodes increases[18]. These data suggest that delineation of whether mature DG GCs contribute to depression and the antidepressant response is necessary. A better understanding of how SSRIs modulate neuronal circuitry *in vivo* to confer behavioral changes is crucial for the development of novel, more effective and faster-acting antidepressants. Moreover, approaches that target specific serotonin receptors or downstream pathways, rather than generally elevating serotonin (as SSRIs do), may also lead to improved treatment strategies.

Human genetic and imaging studies have shown that differences in 5HT1AR levels or regulation are associated with depression, anxiety and the response to antidepressants[19,20]. A C(-1019)G polymorphism in the promoter region of the *5HT1AR* (also known as *HTR1A*) gene is associated with mood-related variables, including depression and the response to antidepressant treatment[19,21]. Germline 5HT1AR-deficient mice do not show behavioral or neurogenic responses to fluoxetine[7]. In addition, chronic treatment with the 5HT1AR agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) results in

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Received 8 July; accepted 19 August; published online 21 September 2015; doi:10.1038/nn.4116

increased neurogenesis and decreased anxiety[7]. Taken together, these results demonstrate that 5HT1ARs are a major target of elevated serotonin and are required for the beneficial effects of antidepressant treatment. Thus, tissue-specific deletions of 5HT1AR populations will both determine the subset of 5HT1ARs and help to identify the circuitry that mediates the antidepressant response. In the ventral DG, 5HT1ARs are highly expressed in mature DG GCs[22]. It is unknown whether 5HT1ARs are expressed in neural progenitors or young abGCs in the DG. We sought to examine the independent roles of both mature DG GCs and young abGCs in the antidepressant response by deleting 5HT1AR from both populations using tissue-specific promoters.

RESULTS

Creation of floxed 5HT1AR mice

To study tissue-specific 5HT1AR deficiencies, we created mice with *loxP* sites flanking the single exon and the 3′ untranslated region containing the polyadenylation signal of the *5ht1ar* gene (**Fig. 1a**). We engineered the mice so that, following Cre-mediated excision of the *5ht1ar* exon and the 3′ untranslated region, a yellow fluorescent protein (YPet) would be expressed under control of the *5ht1ar* promoter. Initial experiments, including assessment of the behavioral and neurogenic response to fluoxetine and labeling of 5HT1ARs with the radioactive ligand I-125 MPPI, revealed that mice homozygous for the floxed *5ht1ar* allele (fl1A mice) were indistinguishable from wild-type (WT) littermates (**Supplementary Fig. 1** and data not shown). Thus, we refer to homozygous fl1A mice as control mice throughout the study.

Behavioral effects of fluoxetine require 5HT1ARs on DG GCs

We first investigated the functional roles of 5HT1ARs in all DG GCs. To this end, we crossed the floxed 5HT1AR mice with POMC-Cre mice[16,23], in which Cre is highly and selectively expressed in all GCs of the DG and in the arcuate nucleus of the hypothalamus (**Fig. 1a**). I-125 MPPI autoradiography demonstrated a near complete deletion of 5HT1ARs in the DG (>90%) when bigenic POMC-Cre/fl1A mice were 8 weeks old (**Fig. 1b**,**c**). This deletion was specific to the DG, as 5HT1AR levels were unchanged throughout the rest of the brain, including in the raphe nucleus (**Fig. 1b**,**d**).

We next assessed the behavioral response to antidepressants by treating control and POMC-Cre/fl1A mice with either vehicle or fluoxetine (18 mg per kg per d for 21 d; **Fig. 1e**). First, we tested the mice in novelty suppressed feeding (NSF), which involves a 24-h food deprivation followed by placement into a large, brightly lit, novel arena containing food in the center. Chronic, but not acute, antidepressant administration decreases the latency for mice to enter the center of the anxiogenic arena and bite the food pellet[7,12]. As expected, chronic fluoxetine treatment decreased the latency for control mice to feed ($P < 0.0001$; **Fig. 1f** and **Supplementary Table 1**). However, fluoxetine was ineffective in the POMC-Cre/fl1A mice ($P = 0.5071$; **Fig. 1f**). At baseline, there were no effects of genotype. There were no differences between groups in the percentage of weight lost during the deprivation or the amount of food consumed in the home cage (**Supplementary Fig. 2**). Thus, these data indicate that POMC-Cre/fl1A mice do not respond to fluoxetine in the NSF.

We also tested the behavior of this cohort of mice in the elevated plus maze (EPM; **Fig. 1g**). In the EPM, chronic fluoxetine increases the number of open arm entries and the duration of time spent in the open arms[12]. Two-way ANOVAs revealed significant genotype × treatment interactions for open arm entries ($F_{(1,89)} = 8.120$, $P = 0.0054$) and duration ($F_{(1,89)} = 6.435$, $P = 0.0129$). As expected, fluoxetine significantly increased open arm entries and duration in control mice ($P < 0.0001$ for entries, $P = 0.0003$ for duration). However, fluoxetine was ineffective in POMC-Cre/fl1A mice ($P = 0.9980$ for entries, $P = 0.9859$ for duration). At baseline, there were no effects of genotype. Thus, similar to NSF, fluoxetine was ineffective in mice lacking 5HT1ARs in all GCs.

We also assessed the behavior of these mice in the forced swim test (FST; **Fig. 1h**), which is commonly used to assess antidepressant-like effects[12]. Acute or chronic treatment with antidepressants decrease the time spent immobile in the FST. We found a significant genotype × treatment interaction in the FST ($F_{(1,86)} = 9.769$, $P = 0.0024$). Notably, fluoxetine decreased immobility in control mice ($P < 0.0001$), but did not have any significant effects in POMC-Cre/fl1A mice ($P = 0.2624$). At baseline, there were no effects of genotype. Taken together, these data suggest that 5HT1ARs in DG GCs are critical for the behavioral response to SSRIs.

In addition to DG GCs, the *Pomc* promoter also drives Cre expression in the arcuate nucleus of the hypothalamus. We did not detect any YPet expression in the arcuate nucleus of the hypothalamus (data not shown), and *in situ* hybridizations indicated that there were very low levels of 5HT1AR expression in the arcuate nucleus (**Supplementary Fig. 3**). However, to confirm that the behavioral effects observed in the POMC-Cre/fl1A mice were a result of a loss of 5HT1ARs from DG GCs, we performed a very different manipulation to delete 5HT1ARs specifically from DG GCs. To this end, we bilaterally injected AAV8-CamKII-Cre or a control (AAV8-CamKII-GFP) virus into the DG of 4-week-old fl1A mice, and then, 4 weeks later, treated these mice with vehicle or fluoxetine (**Fig. 2a**). We confirmed that the AAV8-CamKII-Cre virus mediated 5HT1AR deletion in the DG using I-125 MPPI (>80% deletion) and found that the nearby region CA1 was unaffected (**Fig. 2b**–**d**). Notably, we found that fluoxetine was ineffective in the NSF, EPM and FST tasks in fl1A mice injected with the AAV8-CamKII-Cre virus (NSF: control vehicle versus fluoxetine, $P = 0.0004$; AAV8-CamKII-Cre vehicle versus fluoxetine, $P = 0.3345$; EPM open arm entries: two-way ANOVA, $F_{(1,53)} = 13.95$, $P = 0.0005$; EPM open arm duration: two-way ANOVA, $F_{(1,53)} = 13.00$, $P = 0.0007$; FST: two-way ANOVA, $F_{(1,54)} = 5.385$, $P = 0.0241$; **Fig. 2e**–**g**). Thus, two very different manipulations both indicated that 5HT1ARs in DG GCs are necessary for the behavioral response to SSRIs.

Behavioral effects do not require 5HT1ARs on young abGCs

The POMC-Cre/fl1A mice lacked 5HT1ARs in all DG GCs (both mature DG GCs and young abGCs). Thus, we next examined the functional role of 5HT1ARs specifically in the young abGCs by crossing the fl1A mice with Nestin-CreERT2 mice[24–26]. In this line, 57.5 ± 3.3% of total surviving young abGCs exhibit Cre-mediated recombination[24,25]. To this end, we pretreated 8-week-old Nestin-CreER/fl1A (**Fig. 3a**) or control (**Supplementary Fig. 4**) mice with either tamoxifen (8 weeks) to induce Cre-mediated recombination or vehicle. The YPet expression pattern in the Nestin-CreER/fl1A mice indicated that the *5ht1ar* promoter is not active (and 5HT1AR is not expressed) until young abGCs are 3–4 weeks old (**Supplementary Fig. 5**). Thus, we tried two different treatment protocols. First, we commenced chronic fluoxetine (18 mg per kg per d, fluoxetine 8 weeks) or vehicle administration in 8-week-old mice, concurrent with tamoxifen or vehicle pretreatment (**Fig. 3a**). The timing of this group was driven by the rationale that it takes several weeks for SSRIs to stably increase 5-HT levels in the hippocampus[27], and that the increase in 5-HT levels would therefore align with expression of the 5HT1AR receptors. Second, we commenced fluoxetine (18 mg per kg per d, fluoxetine 11 weeks) administration in 11-week-old mice (3 weeks after pretreatment), around the onset of 5HT1AR expression in young abGCs (**Fig. 3a**).

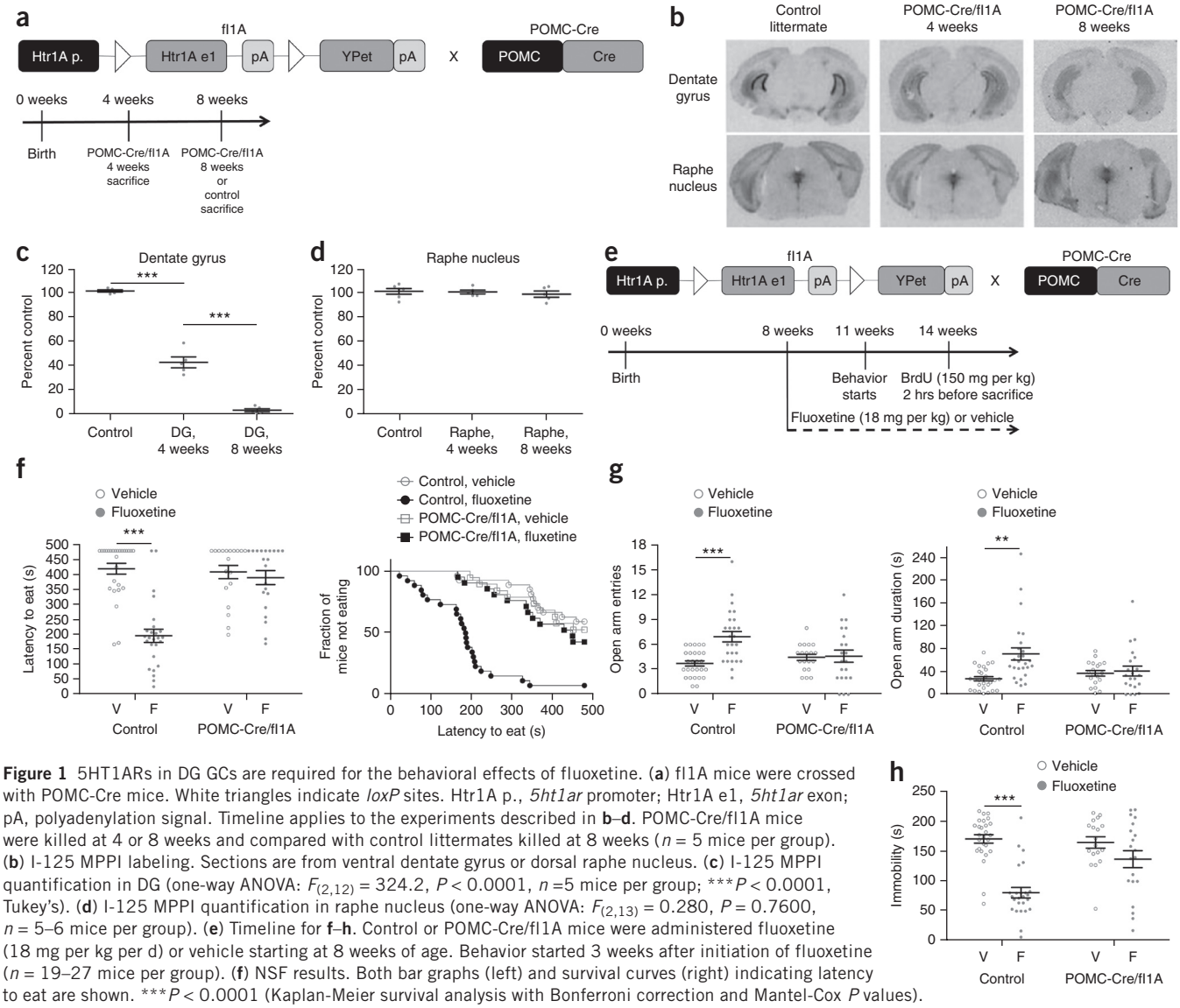

Figure 1 5HT1ARs in DG GCs are required for the behavioral effects of fluoxetine. (**a**) fl1A mice were crossed with POMC-Cre mice. White triangles indicate *loxP* sites. Htr1A p., *5ht1ar* promoter; Htr1A e1, *5ht1ar* exon; pA, polyadenylation signal. Timeline applies to the experiments described in **b**–**d**. POMC-Cre/fl1A mice were killed at 4 or 8 weeks and compared with control littermates killed at 8 weeks ($n = 5$ mice per group). (**b**) I-125 MPPI labeling. Sections are from ventral dentate gyrus or dorsal raphe nucleus. (**c**) I-125 MPPI quantification in DG (one-way ANOVA: $F_{(2,12)} = 324.2$, $P < 0.0001$, $n = 5$ mice per group; $^{***}P < 0.0001$, Tukey's). (**d**) I-125 MPPI quantification in raphe nucleus (one-way ANOVA: $F_{(2,13)} = 0.280$, $P = 0.7600$, $n = 5$–6 mice per group). (**e**) Timeline for **f**–**h**. Control or POMC-Cre/fl1A mice were administered fluoxetine (18 mg per kg per d) or vehicle starting at 8 weeks of age. Behavior started 3 weeks after initiation of fluoxetine ($n = 19$–27 mice per group). (**f**) NSF results. Both bar graphs (left) and survival curves (right) indicating latency to eat are shown. $^{***}P < 0.0001$ (Kaplan-Meier survival analysis with Bonferroni correction and Mantel-Cox P values). (**g**) EPM results. Open arm entries (left) and open arm duration (right) are shown. Both open arm entries ($F_{(1,89)} = 8.120$, $P = 0.0054$) and open arm duration ($F_{(1,89)} = 6.435$, $P = 0.0129$) were analyzed by two-way ANOVA. $^{***}P < 0.0001$ (Tukey's), $^{**}P = 0.0003$ (Tukey's). (**h**) FST results. Immobility duration ($F_{(1,86)} = 9.769$, $P = 0.0024$) was analyzed by two-way ANOVA. $^{***}P < 0.0001$ (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine.

The timing of this second group was driven by the rationale that 5HT1ARs should be expressed when fluoxetine treatment begins. All of these groups of mice were then subjected to NSF, EPM and FST[12] (**Fig. 3** and **Supplementary Fig. 4**).

In NSF, EPM and FST tasks, we found that all groups of mice that were treated with fluoxetine showed a clear and significant decrease in latency to eat, increase in open arm entries and duration, and decrease in immobility, respectively (**Fig. 3b**–**d** and **Supplementary Fig. 4**). In all of the measures there were significant effects of treatment, but there were no significant effects of pretreatment (tamoxifen). Fluoxetine was effective when treatment commenced concurrent with tamoxifen or vehicle pretreatment and when treatment commenced slightly before 5HT1AR expression in young abGCs (**Fig. 3b**–**d**). In the NSF task, there were no differences between groups in the percentage of weight lost during the deprivation or the amount of food consumed in the home cage (**Supplementary Fig. 2**). For the EPM and FST tasks, there were no significant genotype × treatment interactions. These data indicate that 5HT1ARs in young abGCs do not contribute to the behavioral response to SSRIs. This is markedly different from ablation of young abGCs, which results in a partial loss of antidepressant effects[7,12], and may be related to the fact that 5HT1ARs are expressed late in the differentiation process of young abGCs. Together with the results from the POMC-Cre/fl1A mice, these findings strongly suggest that 5HT1ARs in mature DG GCs are required for the behavioral effects of fluoxetine across a battery of anxiety- and depression-related tests and that mature DG GCs have an essential role in mounting an antidepressant response.

5HT1ARs on DG GCs are sufficient for an antidepressant response

Since the behavioral data in the Nestin-CreER/fl1A, POMC-Cre/fl1A and virus-injected mice demonstrate the necessity of 5HT1ARs in mature DG GCs, we also wanted to determine if these receptors are sufficient to mediate an antidepressant response. These experiments could help determine whether DG GC 5HT1ARs are a potential

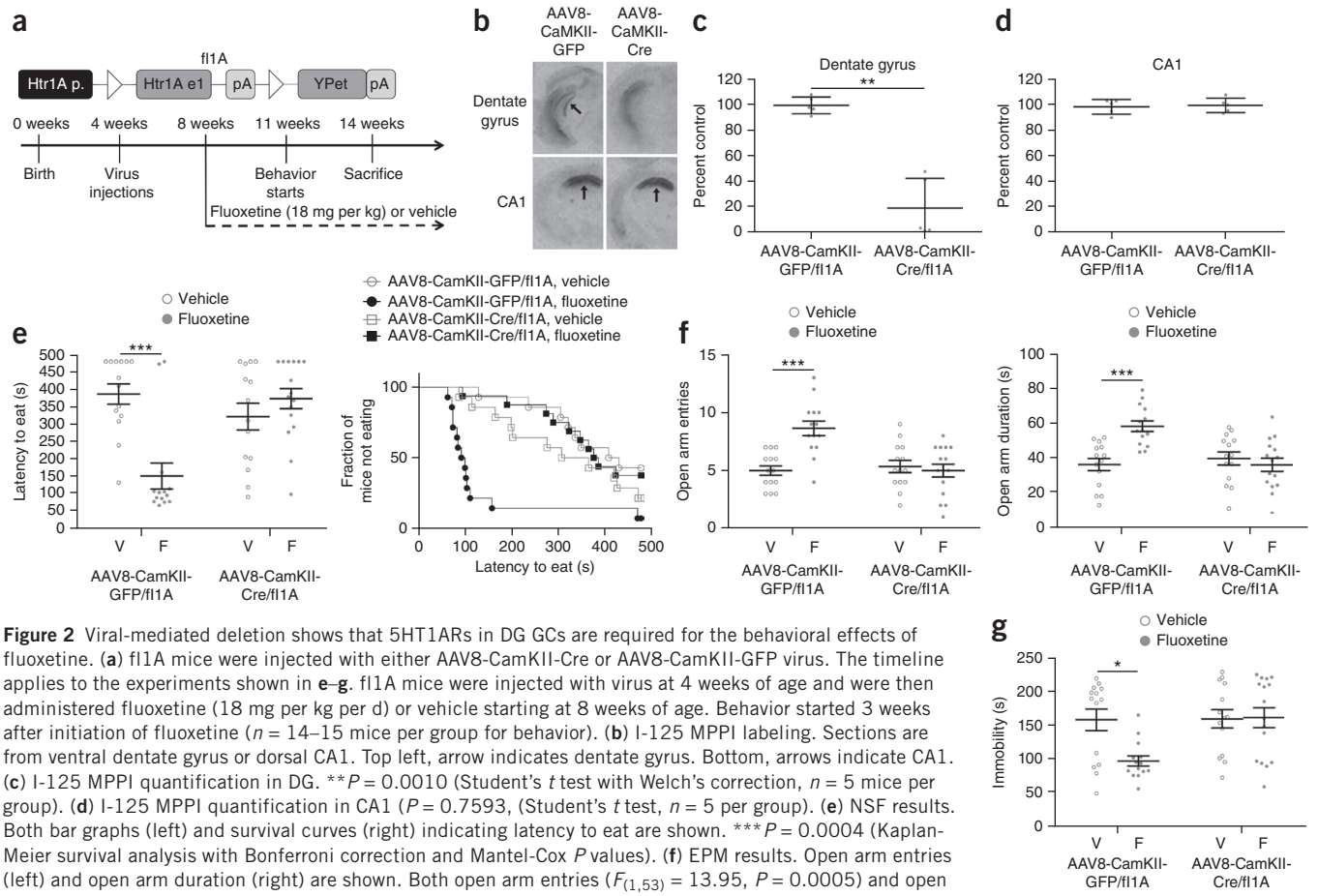

Figure 2 Viral-mediated deletion shows that 5HT1ARs in DG GCs are required for the behavioral effects of fluoxetine. (**a**) fl1A mice were injected with either AAV8-CamKII-Cre or AAV8-CamKII-GFP virus. The timeline applies to the experiments shown in **e**–**g**. fl1A mice were injected with virus at 4 weeks of age and were then administered fluoxetine (18 mg per kg per d) or vehicle starting at 8 weeks of age. Behavior started 3 weeks after initiation of fluoxetine (n = 14–15 mice per group for behavior). (**b**) I-125 MPPI labeling. Sections are from ventral dentate gyrus or dorsal CA1. Top left, arrow indicates dentate gyrus. Bottom, arrows indicate CA1. (**c**) I-125 MPPI quantification in DG. $^{**}P = 0.0010$ (Student's t test with Welch's correction, n = 5 mice per group). (**d**) I-125 MPPI quantification in CA1 ($P = 0.7593$, (Student's t test, n = 5 per group). (**e**) NSF results. Both bar graphs (left) and survival curves (right) indicating latency to eat are shown. $^{***}P = 0.0004$ (Kaplan-Meier survival analysis with Bonferroni correction and Mantel-Cox P values). (**f**) EPM results. Open arm entries (left) and open arm duration (right) are shown. Both open arm entries ($F_{(1,53)} = 13.95$, $P = 0.0005$) and open arm duration ($F_{(1,53)} = 13.00$, $P = 0.0007$) were analyzed by two-way ANOVA. $^{***}P < 0.0001$ for open arm entries and $P = 0.0004$ for open arm duration (Tukey's). (**g**) FST results. Immobility duration ($F_{(1,54)} = 5.385$, $P = 0.0241$) was analyzed by two-way ANOVA. $^{*}P = 0.0142$ (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine.

target for more specific treatments. To this end, we used transgenic mice that express 5HT1ARs in the DG under the control of the *Nrip2* promoter (**Fig. 4a**)[28]. As previously reported, when these transgenic mice are crossed with mice that are germline deficient for 5HT1AR (termed 1A KO)[29], then the resulting mice (termed DG-1A+) express 5HT1ARs at high levels in the DG and at low levels in the central nucleus of the amygdala (CeA)[28]. Slice recordings show that, although DG GCs in these mice have a robust response to serotonin, the CeA neurons do not[28]. Thus, functional 5HT1ARs are expressed specifically in DG GCs in the DG-1A+ mice. I-125 MPPI experiments confirmed that 5HT1AR was expressed in the DG and not in other brain regions such as the raphe nucleus in DG-1A+ mice (**Fig. 4b** and **Supplementary Fig. 6**)[28].

As expected, fluoxetine was ineffective in the NSF, EPM and FST in 1A KO mice (**Fig. 4c**–**f** and **Supplementary Fig. 7**)[7]. By contrast, DG-1A+ mice treated with fluoxetine showed a significant decrease in latency to eat in the NSF, increase in open arm entries and duration in the EPM, and decrease in immobility in the FST (NSF: 1A KO vehicle versus fluoxetine, $P = 0.7412$; DG-1A+ vehicle versus fluoxetine, $P = 0.0079$; EPM open arm entries: two-way ANOVA, $F_{(1,58)} = 7.204$, $P = 0.0095$; EPM open arm duration: two-way ANOVA, $F_{(1,58)} = 6.773$, $P = 0.0117$; FST: two-way ANOVA, $F_{(1,58)} = 4.848$, $P = 0.0317$; **Fig. 4c**–**f**). There were no effects of genotype (between 1A KO and DG-1A+ mice) at baseline. Taken together, these results strongly suggest that DG 5HT1ARs are sufficient for mediating the behavioral effects of fluoxetine across several anxiety- and depression-related tests and further demonstrate that DG GCs have a critical role in the antidepressant response.

DG GC 5HT1ARs regulate fluoxetine-induced neurogenesis

We found that DG GC 5HT1ARs are critical for mediating the behavioral response to SSRIs. We therefore wanted to examine whether mature DG GCs also mediate other effects of antidepressants, such as increased adult hippocampal neurogenesis[6,7]. Data from germline 5HT1AR-deficient mice revealed that 5HT1ARs are also required for the effects of fluoxetine on neurogenesis (**Supplementary Fig. 7**) (ref. 7). Thus, we assessed several neurogenesis measures in all groups of mice that were behaviorally tested (**Fig. 5**).

We first assessed brain sections from POMC-Cre/fl1A and control mice to determine whether 5HT1ARs in all DG GCs are necessary for mediating the effects of fluoxetine on neurogenesis (**Fig. 5a**–**d**). We found significant genotype × treatment interactions for the number of proliferating cells as measured by 5-bromo-2′-deoxyuridine (BrdU) incorporation ($F_{(1,28)} = 10.66$, $P = 0.0029$; **Fig. 5b**), the number of young abGCs as measured by the number of Doublecortin (Dcx)-expressing cells ($F_{(1,28)} = 5.292$, $P = 0.0291$; **Fig. 5c**) and maturation of young abGCs as measured by the number of Dcx-positive cells with tertiary dendrites ($F_{(1,28)} = 4.954$, $P = 0.0343$; **Fig. 5d**). Notably, the effects of fluoxetine were attenuated in POMC-Cre/fl1A mice relative to control mice for all measures (control vehicle versus fluoxetine, $P < 0.0001$ for BrdU, Dcx and Dcx with tertiary dendrites; POMC-Cre/fl1A vehicle versus fluoxetine, $P < 0.0001$ for BrdU, $P < 0.0001$ for

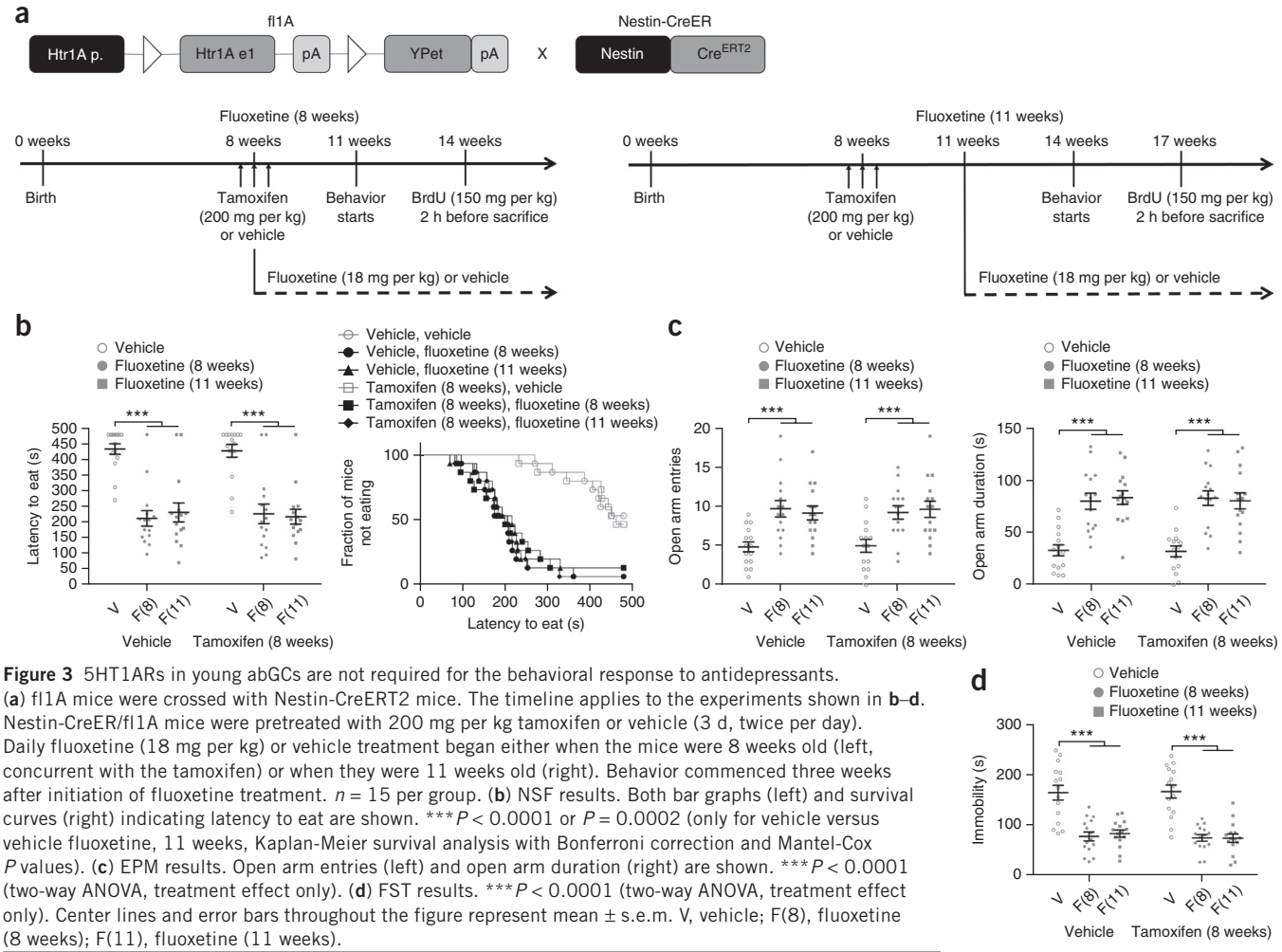

Figure 3 5HT1ARs in young abGCs are not required for the behavioral response to antidepressants. (**a**) fl1A mice were crossed with Nestin-CreERT2 mice. The timeline applies to the experiments shown in **b**–**d**. Nestin-CreER/fl1A mice were pretreated with 200 mg per kg tamoxifen or vehicle (3 d, twice per day). Daily fluoxetine (18 mg per kg) or vehicle treatment began either when the mice were 8 weeks old (left, concurrent with the tamoxifen) or when they were 11 weeks old (right). Behavior commenced three weeks after initiation of fluoxetine treatment. $n = 15$ per group. (**b**) NSF results. Both bar graphs (left) and survival curves (right) indicating latency to eat are shown. $^{***}P < 0.0001$ or $P = 0.0002$ (only for vehicle versus vehicle fluoxetine, 11 weeks, Kaplan-Meier survival analysis with Bonferroni correction and Mantel-Cox P values). (**c**) EPM results. Open arm entries (left) and open arm duration (right) are shown. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). (**d**) FST results. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F(8), fluoxetine (8 weeks); F(11), fluoxetine (11 weeks).

Dcx and $P = 0.0015$ for Dcx with tertiary dendrites; control fluoxetine versus POMC-Cre/fl1A fluoxetine, $P = 0.0007$ for BrdU, $P = 0.0247$ for Dcx and $P = 0.0212$ for Dcx with tertiary dendrites). There were no genotype effects at baseline for any measures. Taken together, these results suggest that DG 5HT1AR is necessary for mediating the complete effects of fluoxetine on adult hippocampal neurogenesis.

We next assessed Nestin-CreER/fl1A and control mice to determine the importance of young abGC 5HT1ARs in mediating the effects of fluoxetine on neurogenesis (**Fig. 5e–h** and **Supplementary Fig. 4**). In both Nestin-CreER/fl1A and control mice, we found that fluoxetine increased the number of proliferating cells, the number of young abGCs and maturation of young abGCs (**Fig. 5f–h** and **Supplementary Fig. 4**). There were no effects of pretreatment (tamoxifen) and no pretreatment × treatment interactions for any measures. Furthermore, fluoxetine was effective in all measures when treatment commenced concurrent with tamoxifen or vehicle pretreatment and when treatment commenced slightly before 5HT1AR expression in young neurons. Thus, the 5HT1AR-mediated effects of fluoxetine on adult neurogenesis do not require 5HT1AR expression in young abGCs and therefore most likely occur in a non-cell autonomous fashion. These data, in combination with the data from the POMC-Cre/fl1A mice, demonstrate that mature DG GCs are important regulators of the neurogenic response to antidepressants.

Finally, we assessed the effects of fluoxetine on adult hippocampal neurogenesis in 1A KO and DG-1A+ mice to determine whether DG GC 5HT1AR is sufficient to mediate the neurogenic response to antidepressants (**Fig. 5i–l**). We found significant genotype × treatment interactions for the number of proliferating cells ($F_{(1,28)} = 23.7$, $P < 0.0001$; **Fig. 5j**), the number of young neurons ($F_{(1,28)} = 6.311$, $P = 0.0180$; **Fig. 5k**) and the maturation of young neurons ($F_{(1,28)} = 8.031$, $P = 0.0084$; **Fig. 5l**). As expected, 1A KO mice did not show a response to chronic fluoxetine treatment in any of the measures[7]. By contrast, DG-1A+ mice treated with fluoxetine had an increased number of proliferating cells ($P < 0.0001$; **Fig. 5j**), an increased number of young neurons ($P = 0.0014$; **Fig. 5k**) and increased maturation of young neurons ($P = 0.0075$; **Fig. 5l**). There were no genotype effects at baseline for any of the measures. Taken together, these data demonstrate that DG GC 5HT1ARs are sufficient for mediating the neurogenic response to fluoxetine and further demonstrate that mature DG GCs are critical mediators of the antidepressant response.

DG GC 5HT1ARs regulate the neuroendocrine fluoxetine response

To define the mechanism underlying why mature DG GCs are necessary for the antidepressant response, we next assessed the effects of 5HT1AR deletions on the HPA axis response to stress. The major neuroendocrine response to stress is activation of the HPA axis, which results in production of corticosterone in the adrenal glands[30]. Alterations in the HPA axis can lead to depressive illness in humans and behavioral phenotypes in anxiety- and depression-related behavioral tasks in rodents[12,31]. Thus, we assessed the responses of the HPA

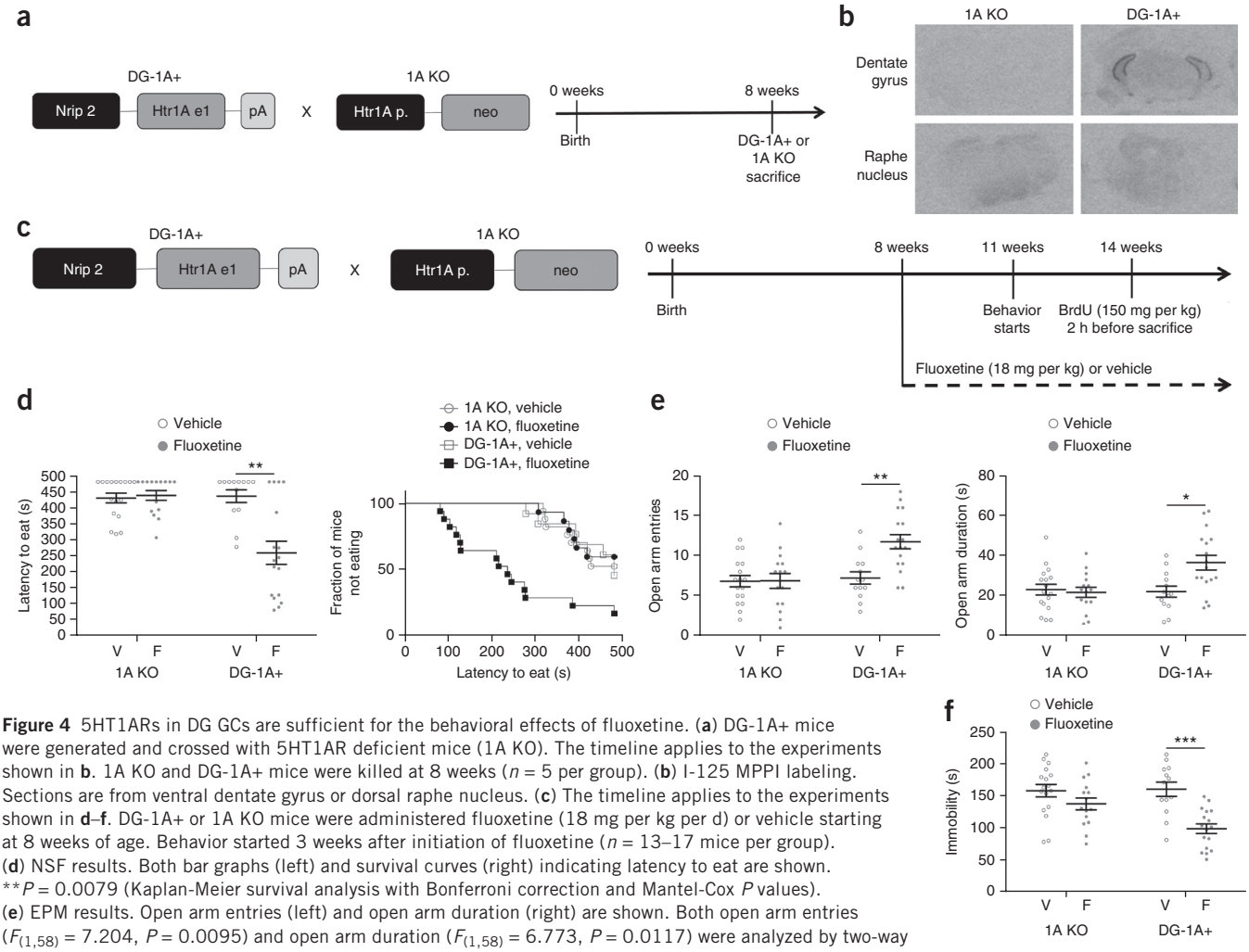

Figure 4 5HT1ARs in DG GCs are sufficient for the behavioral effects of fluoxetine. (**a**) DG-1A+ mice were generated and crossed with 5HT1AR deficient mice (1A KO). The timeline applies to the experiments shown in **b**. 1A KO and DG-1A+ mice were killed at 8 weeks ($n = 5$ per group). (**b**) I-125 MPPI labeling. Sections are from ventral dentate gyrus or dorsal raphe nucleus. (**c**) The timeline applies to the experiments shown in **d**–**f**. DG-1A+ or 1A KO mice were administered fluoxetine (18 mg per kg per d) or vehicle starting at 8 weeks of age. Behavior started 3 weeks after initiation of fluoxetine ($n = 13$–17 mice per group). (**d**) NSF results. Both bar graphs (left) and survival curves (right) indicating latency to eat are shown. $^{**}P = 0.0079$ (Kaplan-Meier survival analysis with Bonferroni correction and Mantel-Cox P values). (**e**) EPM results. Open arm entries (left) and open arm duration (right) are shown. Both open arm entries ($F_{(1,58)} = 7.204$, $P = 0.0095$) and open arm duration ($F_{(1,58)} = 6.773$, $P = 0.0117$) were analyzed by two-way ANOVA. $^{**}P = 0.0022$, $^{*}P = 0.0086$ (Tukey's). (**f**) FST results. Immobility duration ($F_{(1,58)} = 4.848$, $P = 0.0317$) was analyzed by two-way ANOVA. $^{***}P = 0.0001$ (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine.

axis to chronic fluoxetine when mice were in their home cage and then again 1 week later, right after the same cohorts of mice were exposed to the EPM (**Fig. 6**).

We began by examining the effects of chronic fluoxetine on the HPA axis in a new cohort of POMC-Cre/fl1A and control mice (**Fig. 6a–c**). When blood was collected from mice in their home cage, there were no significant differences in plasma corticosterone levels (**Fig. 6b**). However, 45 min after mice were exposed to the EPM, we found a significant genotype × treatment interaction for plasma corticosterone levels ($F_{(1,20)} = 5.585$, $P = 0.0284$; **Fig. 6c**). In control mice, fluoxetine significantly attenuated the elevation in plasma corticosterone levels ($P = 0.0117$). However, fluoxetine did not alter plasma corticosterone levels in POMC-Cre/fl1A mice ($P = 0.9991$). There were no baseline differences between genotypes after exposure to the EPM. These data indicate that DG 5HT1ARs are necessary for mediating the effects of fluoxetine on the HPA axis.

We next assessed the effects of chronic fluoxetine on the HPA axis in a new cohort of Nestin-CreER/fl1A mice (**Fig. 6d–f**). There were no differences in plasma corticosterone levels when mice were in their home cage (**Fig. 6e**), but exposure to the EPM markedly increased plasma corticosterone levels. Fluoxetine attenuated this elevation in plasma corticosterone levels in all of the treated groups (**Fig. 6f**). There were no effects of pretreatment (tamoxifen) and no pretreatment × treatment interactions for plasma corticosterone levels. Furthermore, fluoxetine effectively attenuated the elevation in plasma corticosterone levels when treatment commenced concurrent with tamoxifen or vehicle pretreatment and when treatment commenced slightly before 5HT1AR expression in young neurons. Thus, mice lacking 5HT1ARs in young abGCs showed a normal HPA axis response to stress and fluoxetine treatment. Taken together with the data from the POMC-Cre/fl1A mice, these results suggest that deletion of 5HT1ARs from mature DG GCs prevents the ability of fluoxetine to attenuate the stress response. This finding suggests that a critical role of mature DG GCs during the antidepressant response is to modulate HPA axis function.

To further assess the role of DG 5HT1ARs in mediating the effects of fluoxetine on the HPA axis, we next assessed plasma corticosterone levels in a new cohort of 1A KO and DG-1A+ mice (**Fig. 6g–i**). There were no differences in plasma corticosterone levels when mice were in their home cage (**Fig. 6h**), but, after exposure to the EPM, we found a significant genotype × treatment interaction for plasma corticosterone levels ($F_{(1,20)} = 8.878$, $P = 0.0074$; **Fig. 6i**). In 1A KO mice, fluoxetine did not affect the EPM-induced elevation in plasma corticosterone levels ($P = 0.9427$). By contrast, fluoxetine significantly attenuated the elevation in plasma corticosterone levels in DG-1A+ mice ($P = 0.0079$). Taken together, these data demonstrate that 5HT1ARs in DG GCs are sufficient for mediating a HPA axis

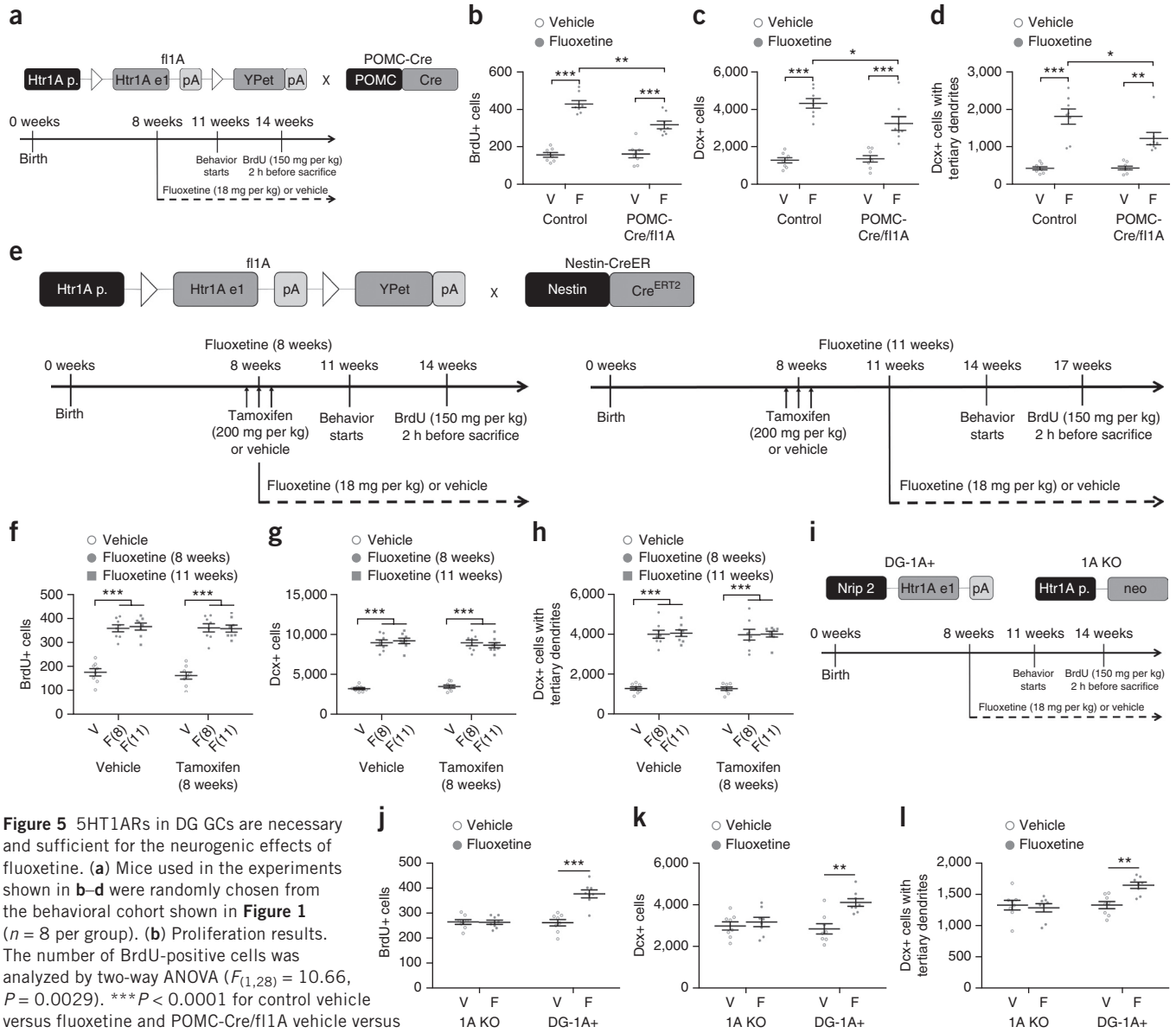

Figure 5 5HT1ARs in DG GCs are necessary and sufficient for the neurogenic effects of fluoxetine. (**a**) Mice used in the experiments shown in **b**–**d** were randomly chosen from the behavioral cohort shown in **Figure 1** ($n = 8$ per group). (**b**) Proliferation results. The number of BrdU-positive cells was analyzed by two-way ANOVA ($F_{(1,28)} = 10.66$, $P = 0.0029$). $^{***}P < 0.0001$ for control vehicle versus fluoxetine and POMC-Cre/fl1A vehicle versus fluoxetine; $^{**}P = 0.0007$ for control fluoxetine versus POMC-Cre/fl1A fluoxetine (Tukey's). (**c**) The number of young abGCs. The number of Dcx-positive cells was analyzed by two-way ANOVA ($F_{(1,28)} = 5.292$, $P = 0.0291$). $^{***}P < 0.0001$, and $^{*}P = 0.0247$ for control fluoxetine versus POMC-Cre/fl1A fluoxetine (Tukey's). (**d**) The number of young abGCs with tertiary dendrites. The number of Dcx-positive cells with tertiary dendrites was analyzed by two-way ANOVA ($F_{(1,28)} = 4.954$, $P = 0.0343$). $^{***}P < .0001$, $^{**}P = 0.0015$ and $^{*}P = 0.0212$ (Tukey's). (**e**) Mice used in the experiments shown in **f**–**h** were randomly chosen from the behavioral cohort shown in **Figure 3** ($n = 8$ per group). (**f**) Proliferation results. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). (**g**) The number of young abGCs. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). (**h**) The number of young abGCs with tertiary dendrites. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). (**i**) Mice used in the experiments shown in **j**–**l** were randomly chosen from the behavioral cohort in **Figure 4** ($n = 8$ per group). (**j**) Proliferation results. The number of BrdU-positive cells was analyzed by two-way ANOVA ($F_{(1,28)} = 23.7$, $P < .0001$). $^{***}P < 0.0001$ (Tukey's). (**k**) The number of young abGCs. The number of Dcx-positive cells was analyzed by two-way ANOVA ($F_{(1,28)} = 6.311$, $P = 0.0180$). $^{**}P = 0.0014$ (Tukey's). (**l**) The number of young abGCs with tertiary dendrites. The number of Dcx-positive cells with tertiary dendrites was analyzed by two-way ANOVA ($F_{(1,28)} = 8.031$, $P = 0.0084$). $^{**}P = 0.0075$ (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine; F(8), fluoxetine (8 weeks); F(11), fluoxetine (11 weeks).

response to fluoxetine and further suggest that mature DG GCs are critical mediators of the antidepressant response.

5HT1ARs on DG GCs regulate growth factor induction

To determine how loss of 5HT1ARs from DG GCs might affect adult hippocampal neurogenesis, we next determined RNA expression levels of the growth factors BDNF and VEGF in the DG of Control and POMC-Cre/fl1A mice treated with vehicle or fluoxetine (**Fig. 7**). BDNF and VEGF expression levels are increased by fluoxetine treatment, and these increases are necessary for the effects of fluoxetine on behavior and adult hippocampal neurogenesis[14,15,32–34]. Furthermore, a selective 5HT1AR agonist increases, whereas a selective 5HT1AR antagonist decreases, VEGF levels in the hippocampus[32]. Two-way ANOVAs revealed significant genotype × treatment interactions for BDNF ($F_{(1,8)} = 10.68$, $P = 0.0114$) and VEGF ($F_{(1,8)} = 6.749$, $P = 0.0317$) RNA expression levels. As expected, chronic fluoxetine treatment

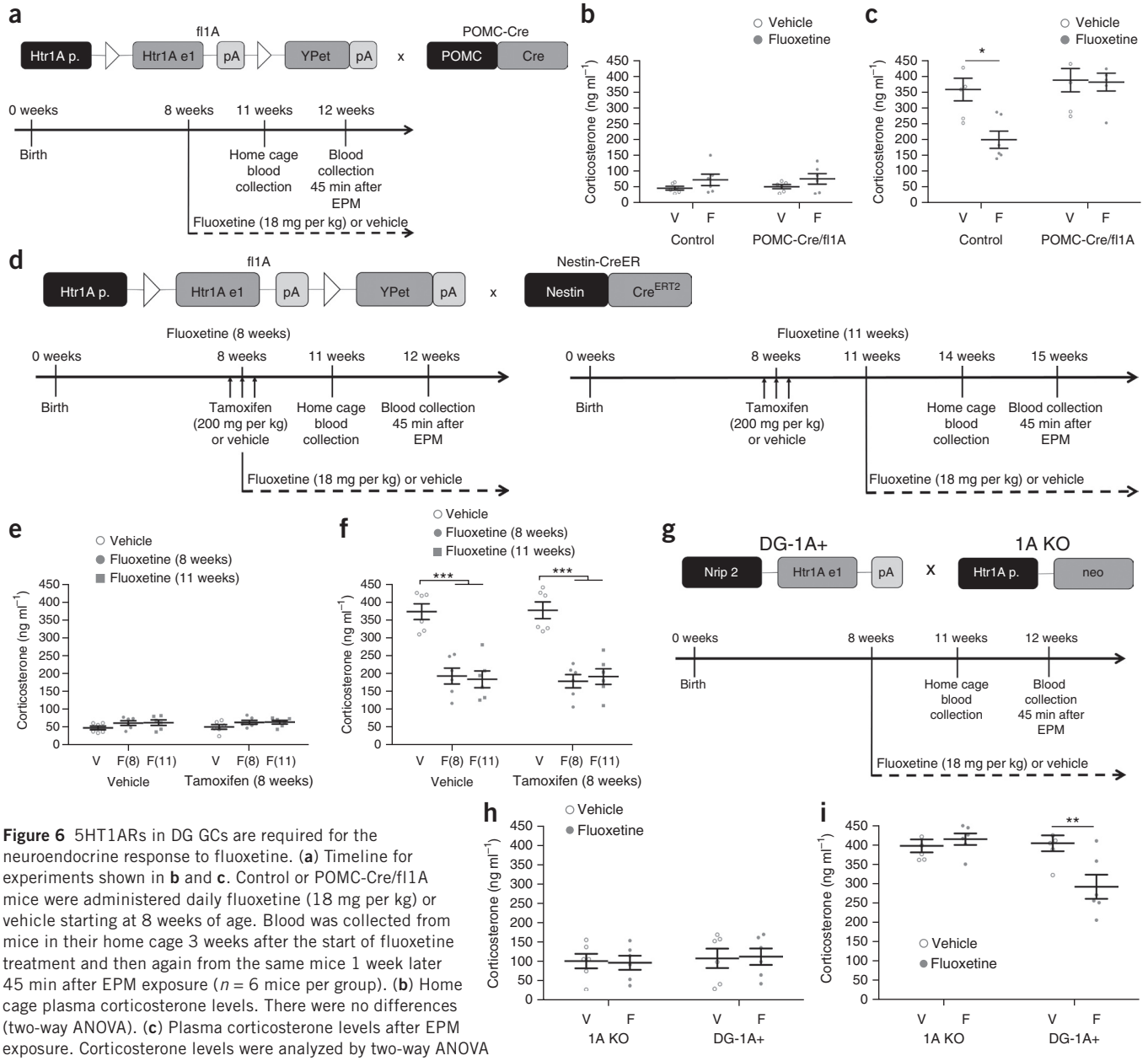

Figure 6 5HT1ARs in DG GCs are required for the neuroendocrine response to fluoxetine. (**a**) Timeline for experiments shown in **b** and **c**. Control or POMC-Cre/fl1A mice were administered daily fluoxetine (18 mg per kg) or vehicle starting at 8 weeks of age. Blood was collected from mice in their home cage 3 weeks after the start of fluoxetine treatment and then again from the same mice 1 week later 45 min after EPM exposure ($n = 6$ mice per group). (**b**) Home cage plasma corticosterone levels. There were no differences (two-way ANOVA). (**c**) Plasma corticosterone levels after EPM exposure. Corticosterone levels were analyzed by two-way ANOVA ($F_{(1,20)} = 5.585$, $P = 0.0284$). $^{*}P = 0.0117$ (Tukey's). (**d**) Timeline for experiments shown in **e** and **f**. Nestin-CreER/fl1A mice were pretreated with 200 mg per kg tamoxifen or vehicle (3 d, twice per day). Daily fluoxetine (18 mg per kg) or vehicle treatment began either when the mice were 8 weeks old (left, concurrent with the tamoxifen) or when they were 11 weeks old (right). Blood was collected from mice in their home cage 3 weeks after the start of fluoxetine treatment and then again from the same mice 1 week later 45 min after EPM exposure ($n = 6$ mice per group. (**e**) Home cage plasma corticosterone levels. There were no differences (two-way ANOVA). (**f**) Plasma corticosterone levels after EPM exposure. $^{***}P < 0.0001$ (two-way ANOVA, treatment effect only). (**g**) Timeline for experiments shown in **h** and **i**. 1A KO or DG-1A+ mice were administered daily fluoxetine (18 mg per kg) or vehicle starting at 8 weeks of age. Blood was collected from mice in their home cage 3 weeks after the start of fluoxetine treatment and then again from the same mice 1 week later 45 min after EPM exposure ($n = 6$ mice per group. (**h**) Home cage plasma corticosterone levels. There were no differences (two-way ANOVA). (**i**) Plasma corticosterone levels after EPM exposure. Corticosterone levels were analyzed by two-way ANOVA ($F_{(1,20)} = 8.878$, $P = 0.0074$). $^{**}P = 0.0079$ (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine; F(8), fluoxetine (8 weeks); F(11), fluoxetine (11 weeks).

increased RNA expression levels of BDNF ($P = 0.0004$) and VEGF ($P = 0.0003$) in the DG of control mice (**Fig. 7b**,**c**). By contrast, the fluoxetine-induced increase in RNA expression levels of BDNF and VEGF were abolished or attenuated in POMC-Cre/fl1A mice (BDNF: POMC-Cre/fl1A vehicle versus fluoxetine, $P = 0.1038$; VEGF: POMC-Cre/fl1A vehicle versus fluoxetine, $P = 0.0210$; control fluoxetine versus POMC-Cre/fl1A fluoxetine, $P = 0.0266$; **Fig. 7b**,**c**). These data indicate that DG 5HT1ARs are necessary for mediating the fluoxetine-induced increase in BDNF and VEGF RNA expression levels.

DISCUSSION

5HT1ARs on mature DG GCs mediate effects of fluoxetine

We found that mature DG GC 5HT1ARs are necessary and sufficient for the effects of fluoxetine on behavior, neurogenesis and the

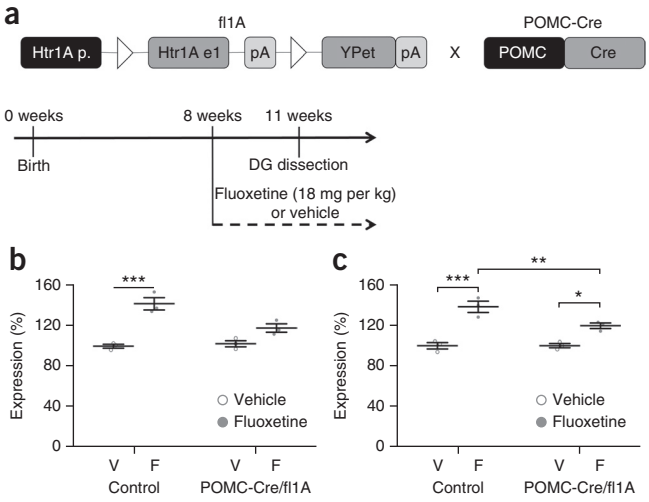

Figure 7 Fluoxetine-induced increases in BDNF and VEGF in the DG are attenuated in mice lacking 5HT1ARs in DG GCs. (**a**) Timeline for experiments shown in **b** and **c**. Control or POMC-Cre/fl1A mice were administered daily fluoxetine (18 mg per kg) or vehicle starting at 8 weeks of age. DG was dissected and RNA was prepared 3 weeks after the start of fluoxetine treatment ($n = 3$ mice per group). (**b**) DG BDNF RNA expression levels were analyzed by two-way ANOVA ($F_{(1,8)} = 10.68$, $P = 0.0114$). $^{***}P = 0.0004$ (Tukey's). (**c**) DG VEGF RNA expression levels were analyzed by two-way ANOVA ($F_{(1,8)} = 6.749$, $P = 0.0317$). $^{***}P = 0.0003$, $^{*}P = 0.0210$ for POMC-Cre/fl1A vehicle versus fluoxetine, and $^{**}P = 0.0266$ for control fluoxetine versus POMC-Cre/fl1A fluoxetine (Tukey's). Center lines and error bars throughout the figure represent mean ± s.e.m. V, vehicle; F, fluoxetine.

neuroendocrine system. Notably, serum fluoxetine levels were similar across all of the genotypes and pretreatments tested (**Supplementary Fig. 8**). SSRIs block serotonin reuptake and increase serotonin levels throughout the brain. Although anxiety behavior is influenced by 5HT1ARs, these effects are largely mediated during discrete developmental windows by the autoreceptor population of 5HT1ARs that are present on the serotonergic projections of the raphe nucleus[27,29,35,36]. Mice that are germline deficient in 5HT1ARs do not show a behavioral or neurogenic response to chronic fluoxetine treatment, but these mice lack both autoreceptors and heteroreceptors throughout life[7,29]. The POMC-Cre/fl1A and AAV8-CamKII-Cre/fl1A mice had a specific deletion of DG GC 5HT1A heteroreceptors limited to adulthood and the DG-1A+ mice only expressed functional 5HT1ARs in DG GCs. The data from these lines demonstrate that 5HT1ARs expressed in the DG are necessary and sufficient for the behavioral, neurogenic and neuroendocrine effects of fluoxetine. Furthermore, given that deleting 5HT1ARs from young abGCs did not affect the fluoxetine response, we can conclude that 5HT1ARs in mature DG GCs are critical for the effects of fluoxetine on behavior, neurogenesis and the neuroendocrine system.

There are 14 distinct serotonin receptors, so the neuromodulatory effects of SSRI-enhanced serotonin levels could be mediated by several different subtypes[37]. 5HT1AR and 5HT4R are the most prominently expressed serotonin receptor subtypes in the DG[22]. Notably, 5HT4R agonists display antidepressant-like activities[38,39] and fluoxetine is ineffective in several behavioral tasks in germline-deficient 5HT4R mice[40]. 5HT1ARs inhibit cAMP signaling through coupling to Gi/o, whereas 5HT4Rs stimulate cAMP formation through coupling to Gs[37]. Given that these two receptors have opposing effects on intracellular signaling, the effects of antidepressants on mature DG GCs may require a balance that is only achieved when both receptors are present. Increased signaling through either 5HT4R (as seen here in the case of 5HT1AR deficiency) or through 5HT1AR (in the case of 5HT4R deficiency) may yield improper fluoxetine-mediated modulation of mature DG GCs that has consequences for the hippocampal circuitry. Future studies should further address this hypothesis by investigating mice with specific deletion of 5HT4Rs in the DG. In addition, the behavioral and neurogenic effects of fluoxetine in DG-1A+ mice were not as prominent as what was seen in a distinct cohort of WT mice (**Supplementary Fig. 7**). Thus, we cannot rule out that 5HT1ARs in other brain regions may also be important for the antidepressant response.

Mature DG GCs are critical to the antidepressant response

One potential mechanism of how the mature DG GCs may modulate young abGCs is through highly regulated secretion of growth factors such as BDNF and VEGF[14,15,32–34,41]. Acute pharmacological manipulations of 5HT1ARs influence VEGF expression levels in the DG[32]. Notably, we found that fluoxetine-induced increases in BDNF and VEGF expression levels in the DG were attenuated in mice lacking 5HT1ARs in DG GCs. Although there is a precedent for 5HT1AR-mediated regulation of VEGF in the DG[32], these results are still somewhat surprising given that 5HT1AR is an inhibitory receptor and that both BDNF and VEGF are induced by activity. Thus, it is possible that DG 5HT1ARs mediate the effects of chronic fluoxetine on BDNF and VEGF expression levels via distinct 5HT1AR-related signaling cascades or through an indirect mechanism. Future work is necessary to further delineate these possibilities.

Complete ablation of young abGCs by methods such as focal irradiation consistently leads to alterations in the antidepressant response in only one behavioral test (NSF)[7,12]. By contrast, deletion of 5HT1ARs from all DG GCs led to alterations in the antidepressant response in several behavioral tests (NSF, EPM and FST). Thus, complete ablation of young abGCs has much milder effects than modulation of all DG GCs. Enhancing adult neurogenesis through genetic methods does not result in an antidepressant-like response, indicating that increasing the number of young abGCs is not sufficient for mediating a change in mood-related behavior, at least in baseline conditions[25]. Our data suggests that the mature population of GCs must also be engaged for the antidepressant response, and that the young abGCs may work in concert with the mature population of GCs (**Supplementary Fig. 9**).

We propose that, during a response to fluoxetine treatment, 5HT1ARs on mature DG GCs respond to the increase in serotonin levels by activating signaling cascades that result in the secretion of growth factors, such as BDNF and VEGF, that enhance adult neurogenesis (**Supplementary Fig. 9**)[14,15]. In turn, the young abGCs, which are more plastic than the mature DG GCs, may modulate the activity of the mature DG GCs by acting on the local microcircuit (**Supplementary Fig. 9**)[42–44]. The resulting combined activity of the both the mature DG GCs and the young abGCs is then required for the antidepressant response.

Action on the HPA axis is through mature and young abGCs

Although it remains unknown how the DG mediates the behavioral effects of antidepressants, a candidate mechanism can be found in the downstream circuitry. Stress profoundly regulates the DG, but the hippocampus also provides negative feedback regulation to the HPA axis. Ventral hippocampal outputs to the ventral subiculum influence the HPA axis through GABAergic projections of areas such as the bed

nucleus of the stria terminalis (BNST)[45]. Our data indicate that deletion of 5HT1ARs from DG GCs blocks the effects of fluoxetine on the HPA axis. Other studies have found that manipulations of adult neurogenesis result in altered plasma levels of corticosterone[46,47]. In addition, unpredictable chronic mild stress in mice reduces hippocampal neurogenesis and dampens the relationship between the hippocampus and the HPA axis[48]. This relationship can be restored by treatment with fluoxetine in a neurogenesis-dependent manner[48]. Thus, our data and previous studies suggest that antidepressants regulate the HPA axis through modulation of both mature DG GCs and young abGCs. The output of the DG into this circuit is defined by the interaction between these two populations of cells. Since fluoxetine regulates both mature DG GCs and young abGCs, the DG provides an entry point for antidepressants to modulate a mood-related circuit and, ultimately, behavior.

Ventral DG as a target for new therapies

The hippocampus shows highly distinct afferent and efferent connectivity along its dorsoventral axis. The dorsal hippocampus connects with associational cortical regions that are important for cognitive functions, whereas the ventral hippocampus connects with regions that mediate emotional affect such as the prefrontal cortex, nucleus accumbens, hypothalamus and BNST[49]. Lesions of the dorsal hippocampus affect spatial memory, whereas lesions of the ventral pole affect anxiety[16,49]. We found that 5HT1ARs in mature DG GCs are necessary and sufficient for the behavioral effects of antidepressants. Given that the vast majority of 5HT1ARs in the DG are expressed in the ventral pole[22], 5HT1ARs are positioned to exhibit a specific influence on the limbic system and the HPA axis and thereby regulate mood- and anxiety-related behavior. Clinical trials with drugs that target 5HT1ARs, such as pindolol, have yielded disappointing results[50]. However, genetic methods that manipulate specific populations of 5HT1ARs are showing that distinct populations of 5HT1ARs have extremely different functional roles[27,35]. This is especially evident when comparing autoreceptors with heteroreceptors. Thus, specific manipulation of 5HT1ARs or downstream intracellular effectors in the ventral DG may provide a more precise method for controlling mood and anxiety circuitry than drugs that generally increase serotonin throughout the brain or that target all populations of 5HT1ARs. Future work is necessary to delineate these potential targets for new antidepressants. Our data also indicates that mature DG GCs are critical mediators of the antidepressant response. Notably, optogenetic elevation of activity in ventral DG GCs has anxiolytic effects[16]. Thus, we propose that pharmacological or electrical (such as deep brain stimulation) manipulations of the ventral DG are also potential treatments for mood and anxiety disorders.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

The authors thank K. Win and D. Tora for technical support, and M. Kheirbek and D. Leonardo for discussions. This work was supported by NIMH R37MH068542 (R.H.), NIMH R01MH083862 (R.H.), HDRF MPPN8883 (R.H.), NYSTEM C029157 (R.H.), NIMH K01MH098188 (B.A.S.), BBRF NARSAD Young Investigator 19658 (B.A.S.), a Charles H. Revson fellowship (B.A.S.), a German Research Foundation (DFG) postdoctoral fellowship (C.A.), NIMH T32MH015144 (Z.R.D.), NIMH R01MH01844 (A.D.), funds from EMBL (C.T.G.), and an EC Marie Curie Fellowship (N.M.).

AUTHOR CONTRIBUTIONS

B.A.S. conceived and performed the experiments, analyzed the results, and wrote the manuscript. C.A. contributed to virus injection experiments. A.H. and M.R.L. performed mouse husbandry and contributed to most of the experiments. A.P., Z.R.D. and L.J.D. contributed to experiments and analysis. T.T., N.M. and C.T.G. performed mouse husbandry for 1A KO and DG-1A+ mice and carried out the experiments shown in **Supplementary Figure 6**. A.D. performed mouse husbandry for Nestin-CreER mice and contributed to analysis. K.F.T. made the fl1A mice and performed the experiments shown in **Supplementary Figure 3**. Most experiments were performed in the laboratory of R.H., who also conceived the experiments and contributed to the analysis and writing of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Mice. *Creation of fl1A mice.* The targeting vector for the fl1A mice consisted of a 5′ homology arm (−4,993 to 4,009, numbers correspond to the translation initiation site of *5ht1ar*) with *loxP*, FRT, PGK-EM7-Neo minigene, FRT, *loxP*, YPet cDNA (YPet is a modified yellow fluorescent protein[51]), SV40 polyadenylation signal, 3′ homology arm (4,010 to 5,569, 1.6 kb), and diphteria toxin A subunit (DTA). The first *loxP* site was inserted just upstream of the coding region (1 to 1,266) and the second *loxP* site was inserted after the *Htr1a* polyadenylation signal (3,848 to 3,853). The linearized targeting vector was electroporated into embryonic stem cells derived from the 129S6 strain (line CSL3) and G418 resistant clones were selected. Southern blots were then performed to verify the homologous recombination. To this end, genemic DNA was digested with BamHI and a ^{32}P-labeled probe (7040-7879) was used. This probe detected a 13-kb band for the WT allele and 9-kb band for the knock-in allele. The recombinant embryonic stem cells were then injected into blastocysts from the C57BL/6 strain and chimeric mice were obtained. Germline transmitted mice were then crossed with ROSA-Flpe mice[52] to remove the PGK-EM7-Neo minigene through FLP-FRT recombination. The offspring of these crosses were established as the fl1A mice. Nestin-CreER, POMC-Cre, 1A KO and DG-1A+ mice were previously described[7,16,23–25,28,29].

Husbandry. Mice were housed in groups of three to five per cage and had *ad libitum* access to food and water. Mice were maintained on a 12:12-h light/dark schedule; all testing was conducted during the light period. Mouse protocols were approved by the Institutional Animal Care and Use Committee of Columbia University and the Research Foundation for Mental Hygiene and were conducted in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Mice. Care was taken to minimize the number of mice used and their suffering.

Experimental mice. Only male mice were used throughout the manuscript. All cohorts entailed littermates from several breeding cages. Mice of different genotypes, pretreatments and treatments were all housed in the same cages.

Receptor autoradiography. Quantitative measurements of receptor autoradiography were performed as previously described[35]. Briefly, mice were killed by cervical dislocation and decapitation. Extracted brains were frozen immediately on crushed dry ice (−75 °C) and maintained at −80 °C until sectioning. Brains were cryosectioned at a thickness of 18 µm and sections were thaw-mounted on Superfrost slides (Fisher Scientific). Sections were maintained at −80 °C until processing. Mounted sections were processed for 4-(2′-methoxyphenyl)-1-[2′-(n-2″-pyridinyl)-p-[^{125}I]iodobenzamido]ethylpiperazine (^{125}I-MPPI) autoradiography and receptor levels were quantified as described[27].

Drugs. *Tamoxifen administration.* 8-week-old Control or Nestin-CreER/fl1A mice were administered either vehicle or tamoxifen pretreatment as indicated in the figure timelines. Tamoxifen (Sigma) was suspended in a 50% honey/50% water mixture and was administered by oral gavage. 200 mg per kg of tamoxifen were administered twice per day to the mice when they were 54, 55 and 56 d old (a total of six administrations). Vehicle pretreated mice received a 50% honey/50% water mixture that did not contain tamoxifen.

Fluoxetine administration. Fluoxetine (18 mg per kg per d in deionized water) or vehicle (deionized water) was delivered by oral gavage for 3 weeks before behavior testing. The oral gavage of fluoxetine or vehicle continued throughout the behavior testing and to experimental endpoints. On the days when mice were subjected to behavioral testing, fluoxetine or vehicle administrations were conducted after the mice completed the testing in order to avoid any acute effects.

Behavioral testing. The behavioral testing was conducted in the following order: EPM, NSF and then FST. Mice were given 3 d between exposures to different behavioral tests. All behavioral testing was performed between 10 a.m. and 3 p.m.

Elevated plus maze. EPM was performed as previously described[12]. Briefly, mice were placed into the central area facing one closed arm and allowed to explore the maze for 5 min. Testing occurred in bright ambient light conditions (800–900 lx). The maze was cleaned with disinfecting wipes and paper towels between each run. Data were scored using TopScan software (CleverSys).

Novelty-suppressed feeding. NSF was performed as described[12,53]. The testing apparatus consisted of a plastic box (50 × 50 × 20 cm), the floor of which was covered with approximately 2 cm of wooden bedding. 24 h before behavioral testing, all food was removed from the home cage. At the time of testing, a single pellet of food was placed on a white paper platform in the center of the box. A mouse was placed in a corner of the box, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. Mice were in the testing arena for a total of 8 min. Immediately after the testing period, the mice were transferred to their home cages, and the amount of food consumed by the mouse in the subsequent 5 min was measured. Each mouse was weighed before food deprivation and before testing to assess the percentage of body weight loss.

Forced swim test. FST was performed as previously described[12]. Briefly, mice were placed into clear plastic buckets 20 cm in diameter and 23 cm deep, filled two-thirds of the way up with 26 °C water and were videotaped. Mice were in the forced swim buckets for 6 min, but only the last 4 min were scored. Scoring was automated using Videotrack software (ViewPoint).

Virus injections. Mice were anesthetized with sodium pentobarbital (diluted 1:10 from stock of 50 mg ml^{-1} and injected at a volume of 10 ml kg^{-1}) and positioned in a stereotaxic frame (Stoelting) over a heated pad. Using a stereomicroscope (Leica), the head was shaved and disinfected. An incision was made in the scalp, exposing the skull. Craniotomies were opened bilaterally with a dental drill at −2.0 mm, ±1.4 mm from the bregma line and midline, respectively, and a stainless steel 33 gauge blunt needle attached to a 10-µl syringe (World Precision Instruments) was inserted to 2.1-mm depth from the top of the brain at injection craniotomy, corresponding to the dorsal dentate gyrus. For ventral dentate gyrus targeting, additional craniotomies were made at −3.5 mm, ±2.8 mm from the bregma line and midline, respectively, and injections were done at a depth of 3.6 mm from the skull at bregma. All mice received bilateral dorsal and ventral dentate gyrus injections (a total of four injections per mouse; 0.2 µl over 2 min for dorsal and 0.3 µl over 3 min for ventral) of either AAV8-CamKII-mCherry-Cre or AAV8-CamKII-eGFP (both obtained from UNC Vector core). The needle was left *in situ* for an additional 5 min to aid diffusion from the needle tip and prevent backflow. The needle was then slowly retracted and the scalp incision closed with Vetbond 3M. Each animal was monitored and received carprofen (subcutaneous, 5 mg per kg) for pain management for 3 d. The mice were house for 4 weeks postoperatively before beginning of drug administration.

Immunohistochemistry. *Perfusions and sectioning.* Mice were anesthetized between 12 and 2 p.m. with ketamine and xylazine (100 mg ml^{-1} ketamine, 20 mg ml^{-1} xylazine) and were then perfused transcardially (cold saline for 2 min, followed by 4% cold paraformaldehyde (wt/vol) in saline at 4 °C). The brains were then dissected and cryoprotected in 30% sucrose (wt/vol) with 0.1% NaN_3 (wt/vol) and stored at 4 °C. Serial sections (35 µM) were cut through the entire hippocampus on a cryostat and stored in phosphate-buffered saline (PBS) with 0.1% NaN_3.

BrdU administration and immunostaining. Mice were administered BrdU (150 mg per kg, intraperitoneal, dissolved in saline), 2 h before sacrifice to assess cell proliferation. For the immunohistochemistry experiments, the sections were washed three times for 10 min in PBS, mounted onto slides, dried and then exposed to citrate buffer (10 mM citric acid, pH 6.0 at 95 °C) for 2 h. After a brief 1 min wash in PBS, the slides were then incubated overnight at 21–26 °C with the primary antibody (Anti-BrdU rat monoclonal, BU1/75 (ICR1), Serotec, 1:100). The next day, the slides were washed two times for 5 min with PBS and incubated for 1 h with the secondary antibody (Cy3-conjugated goat anti-rat, A10522, Molecular Probes, 1:200). The slides were then washed three times for 10 min in PBS and coverslipped.

Dcx immunostaining. For doublecortin staining, sections were rinsed in PBS, treated with 1% H_2O_2 (wt/vol) in 1:1 PBS and methanol for 15 min to quench endogenous peroxidase activity (and to enhance dendritic staining), incubated in 10% normal donkey serum (wt/vol) and 0.3% Triton X-100 (wt/vol) for 30 min, and then incubated overnight at 4 °C in primary antibody for doublecortin (goat, C-18, 1:500, Santa Cruz Biotechnology). The next day, the sections were exposed to biotinylated donkey anti-goat (1:500) seconday antibody (705-005-003, Jackson ImmunoResearch) in PBS for 2 h at 21–26 °C. The immunostaining was then developed using an avidin-biotin complex (Vector) and a DAB kit, and the sections were then mounted onto slides and coverslipped.

Cell counting. Cells were counted on a Zeiss Axioplan-2 upright microscope[12,54]. One of every six sections through the hippocampus (12 total sections) was counted for each mouse.

Blood collection and corticosterone level measurements. Blood was collected by submandibular venipuncture[55]. The blood was collected into Eppendorf tubes that contained 10 µl of 0.5 M EDTA to prevent coagulation. The blood-EDTA mixture was then mixed by inversion and was placed on ice for no more than 15 min. The mixture was then centrifuged for 5 min at 1,800*g*. Plasma was collected and stored at −80° C. Blood was collected from mice in their home cage and then again from the same mice one week later, 45 min after exposure to the EPM. All blood collection occurred between 12–2 p.m. to minimize any potential effects of the diurnal corticosterone variation. The plasma corticosterone levels were then assessed using an enzyme immunoassay (ELISA) kit (Arbor Assays). The manufacturer's instructions for the ELISA kit were followed.

Quantitative PCR. DG was dissected and then RNA was extracted using a RNA/DNA Purification kit (Norgen Biotek). Conversion of total RNA into first strand cDNA was then accomplished by using Superscript III enzyme (Invitrogen). Quantitative PCR was carried out in 6.5-µl reactions using Taqman Fast Advanced Mastermix and Taqman probes for BDNF, VEGF and the housekeeping gene Rn18s (Life Technologies) on a StepOne Plus Real-Time PCR System (Applied Biosystems). Triplicate cycle thresholds (Ct values) were obtained for each dentate gyrus and averaged. The values for Rn18s were then used to normalize the expression values of BDNF and VEGF with the delta Ct method. After converting delta Ct values to percentage of the control group (the mean of control + vehicle was assigned a value of 100%), the mean and s.e.m. of each group was calculated.

***In situ* hybridizations.** *In situ* hybridizations were performed as described previously[22]. The probe for 5HT1AR was also previously described[22] (bases 2,114–4,089 in NM_008308). The probe for POMC corresponded to bases 35–945 in NM_008895. Briefly, after a paraformaldehyde treatment, a series of washes, and an acetylation step, prehybridization was carried out for 5 h at 21–26 °C in hybridization buffer, consisting of 50 per cent formamide (Roche), 5× SSC (saline sodium citrate buffer), 5× Denhardts (Sigma), 0.25 mg ml^{-1} yeast tRNA (Ambion) and 0.4 mg ml^{-1} Salmon Sperm DNA (Stratagene). Sections were then incubated in hybridization buffer containing digoxigenin (DIG)-labeled cRNA probe at 60 °C overnight. After another series of washes and a blocking step, the sections were incubated with alkaline phosphatase–conjugated anti-DIG antibody (1:5,000 dilution, 11093274910, Roche) for 90 min at 21–26 °C. After another series of washes to remove unbound antibody, the sections were incubated with freshly prepared nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate p-toluidine salt (NBT/BCIP) color substrate (Roche) for up to 16 h at 21–26 °C, after which the reaction was stopped by immersion into PBS. After ISH staining, the sections were counterstained with Nuclear Fast Red (Vectastain).

Statistics. All statistics are presented in **Supplementary Table 1**. Two-way ANOVA assessing pretreatment × treatment (for Nestin-CreER/fl1A mice), genotype × treatment (for control, POMC-Cre/fl1A, 1A KO, and DG-1A+ mice) or virus × treatment (for AAV8 injected mice) were used to assess EPM, FST, neurogenesis, and corticosterone levels as indicated in the figures. $P < 0.05$ was considered significant. If significant interactions were found, then Tukey's Method was used as the *post hoc* test to compare between individual groups. Since NSF does not show a regular distribution of data points, Kaplan-Meier Survival Analysis was used to assess the latency to eat results[53]. Multiple comparisons were subjected to a Bonferroni correction to determine significance. Student's *t* test and one-way ANOVA were also used to assess I-125 MPPI results and serum fluoxetine levels when appropriate.

A **Supplementary Methods Checklist** is available.

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doi:10.1038/nn.4116