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5-$HT_{2A/2C}$ receptor blockade regulates progenitor cell proliferation in the adult rat hippocampus

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ABSTRACT

Adult hippocampal neurogenesis is reported to be a target of antidepressants, drugs of abuse and animal models of depression, suggesting a role for this form of structural plasticity in psychopathology. Serotonergic neurotransmission, which is implicated in several psychiatric diseases, has been reported to regulate adult hippocampal neurogenesis. Amongst the serotonergic receptors, the serotonin$_{2A/2C}$ (5-$HT_{2A/2C}$) receptors play an important role in the actions of antidepressants and the effects of hallucinogenic drugs of abuse. We have used the mitotic marker 5′-bromo-2-deoxyuridine to address the effects of the 5-$HT_{2A/2C}$ receptors on the proliferation of adult hippocampal progenitors following acute or chronic treatment with the hallucinogenic partial agonists, (+/−)-2,5-dimethoxy-4-iodoamphetamine (DOI) and lysergic acid diethylamide (LSD) and the antagonist, Ketanserin. Acute, and chronic, DOI and LSD treatments induced a strong behavioral activation, but did not alter adult hippocampal progenitor proliferation. In striking contrast, Ketanserin treatment resulted in a biphasic regulation with a significant decline (22%) in progenitor proliferation following a single treatment, and a robust increase (46%) observed following chronic administration. These results indicate that hallucinogenic drugs that primarily target the 5-$HT_{2A/2C}$ receptors, in contrast to other drugs of abuse, may not alter adult hippocampal neurogenesis. In addition, our results that enhanced adult hippocampal progenitor proliferation results from a sustained blockade of the 5-$HT_{2A/2C}$ receptors suggest that the 5-$HT_{2A/2C}$ receptors may be an important target for the neurogenic effects of antidepressant treatment.

Recent studies indicate that animal models of depression (reviewed in [35]), drugs of abuse [5,7,8,10], as well as therapeutic agents such as antidepressants [17,25] regulate adult hippocampal neurogenesis. While depression models and exposure to drugs of abuse have been reported to cause a decline in hippocampal neurogenesis [5,7,8,10,35], chronic antidepressant treatments enhance this process [17,25]. This has raised the intriguing possibility that decreased hippocampal neurogenesis may contribute to the hippocampal damage observed in depressive disorders and following chronic drug abuse, while enhanced hippocampal neurogenesis may contribute to the therapeutic effects of antidepressants and antipsychotics [35]. Recent evidence indicates that genetic or irradiation-mediated blockade of adult neurogenesis results in a loss of the behavioral effects of chronic treatment with specific antidepressants [25,26]. Although the functional relevance of adult neurogenesis in the hippocampus is not well understood, this process has been implicated in learning, memory and mood regulation [35]. As a consequence there is substantial interest in identifying the pathways that may be recruited to regulate adult hippocampal neurogenesis.

Altered serotonergic neurotransmission, in particular, the 5-$HT_{2A/2C}$ receptors, have been implicated in the pathogenesis of depression [30] and anxiety, and in the actions of antidepressant treatments (reviewed in [4]). In addition, the 5-HT_2 receptors are also thought to contribute to the hallucinogenic effects of drugs of abuse like lysergic acid diethylamide (LSD) [12]. Although serotonin has been reported to regulate adult neurogenesis [3], the role of the 5-$HT_{2A/2C}$ receptors in the regulation of adult hippocampal neurogenesis is not well understood. The focus of the present study was to address the influence of 5-$HT_{2A/2C}$ receptor agonists (and hallucinogens) (+/−)-2,5-dimethoxy-4-iodoamphetamine (DOI) and LSD, and the 5-$HT_{2A/2C}$ receptor antagonist Ketanserin on the proliferation of adult hippocampal progenitors.

Adult male Wistar rats (225–275 g) were used in all experiments in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The partial agonists at the 5-$HT_{2A/2C}$ receptor, lysergic acid diethylamide (0.5 mg/kg, Sigma, USA) and the phenethylamine hallucinogen, (+/−)-2,5-dimethoxy-4-iodoamphetamine (8 mg/kg, Sigma), and the 5-$HT_{2A/2C}$ receptor antagonist Ketanserin (5 mg/kg, Sigma) were administered through

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intraperitoneal (i.p.) injection ($n = 4$–7 animals per group), either once (acute) or once daily for 7 days (chronic). It is well established that DOI and Ketanserin exhibit high specificity for the 5-$HT_{2A/2C}$ receptors [24,28] and that LSD appears to exert its hallucinogenic effects primarily via 5-HT_2 receptors [12], though it also exerts agonist effects on the 5-HT1A & Dopamine receptors ([2] review, [27]). The control group received vehicle treatment (0.9% saline–DOI, LSD experiment or 10% DMSO–Ketanserin experiment). The doses of the drugs were selected based on prior literature [19,32]. Treatment with DOI and LSD caused a robust behavioral activation including increased locomotor activity, back-muscle contractions, flat body posture and Straub tail [19,22]. A significant increase in the head twitch response was observed following acute DOI treatment (vehicle = 14 ± 3.35, acute DOI = 31 ± 4.83*; vehicle = 10 ± 1.5, acute LSD = 6.2 ± 2.5; results are the mean ± S.E.M. number of head twitches/20 min, $^*p < 0.05$, Student's t-test). To label dividing cells, the mitotic marker 5-bromo-2′-deoxyuridine (BrdU; 200 mg/kg, Sigma) was administered i.p. 30 min after the vehicle or drug treatment, and animals were sacrificed 2 h later in the acute drug treatment paradigms. For the chronic treatment experiments, BrdU was given 2 h after the final vehicle/drug administration and animals were sacrificed 24 h later. BrdU-positive cell number is thought to accurately reflect the proliferative pool of cells in S-phase at the timepoint of BrdU administration, and is unlikely to reflect changes in bioavailability given that even robust activatory paradigms like seizures do not appear to influence BrdU uptake [21]. Animals received an overdose of nembutal and were then transcardially perfused with 4% paraformaldehyde (PFA). Brains were removed and stored in 4% PFA prior to being sectioned.

Serial coronal sections (50 μm) through the rostro-caudal axis of the hippocampus (Bregma: −2.56 to −5.80) [20] were cut on a vibratome (TPI, USA). Every fifth section from the hippocampus was processed for BrdU immunohistochemistry as described previously [13]. In brief, after DNA-denaturation with 50% formamide/2× SSC for 2 h at 65 °C and acid hydrolysis (2N HCl at 37 °C for 30 min), sections were rinsed in 0.1 M boric acid, followed by blocking with 10% normal horse serum. Sections were incubated overnight with Mouse anti-BrdU (1:500; Roche, Switzerland) followed by exposure to biotinylated anti-mouse secondary antibody (1:500; Vector, USA). Signal amplification was performed using the Vectastain Elite Avidin–Biotin system (Vector) and was visualized using diaminobenzidine (Sigma) as the substrate. To address whether there is a regulation of hippocampal neurogenesis, we also addressed effects on the number of Doublecortin (DCX)-positive cells. DCX, a microtubule-associated protein, is an established marker for adult hippocampal neurogenesis [6]. Coronal sections from chronic Ketanserin, DOI and LSD treatments were incubated overnight with goat anti-DCX antibody (Santa Cruz), followed by exposure to a biotinylated anti-goat secondary antibody (1:500; Vector), signal amplification and visualization was performed as described above for BrdU immunohistochemistry.

The number of BrdU-positive cells within the dentate gyrus (DG) was quantitated using a modified, unbiased stereology protocol under blinded conditions [13]. Every fifth hippocampal section was processed for quantitation (10 sections/animal). BrdU-positive cells were considered within the SGZ/GCL if they were present in the GCL or SGZ or directly touching it, and were quantitated as hilar when they were at least two cell depths away from the SGZ. The cell counting was done at 400× using a light microscope (Zeiss Axioskop, Germany). The total number of BrdU-positive cells was estimated by multiplying the total number of BrdU-positive cells per SGZ/GCL and hilus counted from every fifth section by the section periodicity (5), and reported as the total number of BrdU-positive cells per region. The total DCX-positive cells were counted from five uniform random sections/animal and the results were expressed as DCX-positive cells per section. Further, we also classified the total number of DCX-positive cells as those with or without tertiary dendrites based on the presence of tertiary branches on their dendritic arbors [33]. All counting analysis for DCX-positive cells was performed under blinded conditions. The data was subjected to statistical analysis (Student's t-test) using Instat-3 software (Graphpad, USA) with statistical significance determined at $p < 0.05$.

The hallucinogenic 5-$HT_{2A/2C}$ partial agonists, DOI and LSD did not alter the proliferation of adult hippocampal progenitors

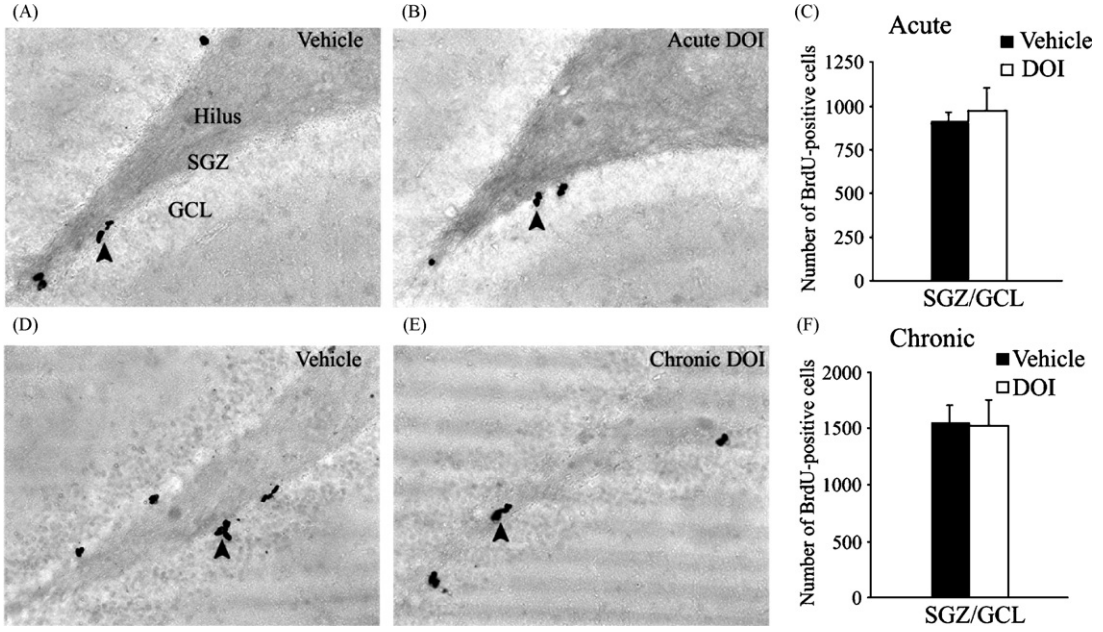

Fig. 1. Effect of the 5-$HT_{2A/2C}$ agonist, DOI, on adult hippocampal progenitor proliferation in the dentate gyrus. Representative photomicrographs of BrdU-positive cells from vehicle (A, acute; D, chronic) and DOI (B, acute; E, chronic) treated animals are shown. Arrowheads indicate BrdU-positive cells observed in clusters in the subgranular zone (SGZ), at the border of the hilus and the granule cell layer (GCL). DOI treatment did not alter the number of BrdU-positive cells in the SGZ/GCL. Results are expressed as mean ± S.E.M. of BrdU-positive cells in the dentate gyrus ($n = 6$–7 per group; acute paradigm; $n = 4$–5 per group; chronic paradigm).

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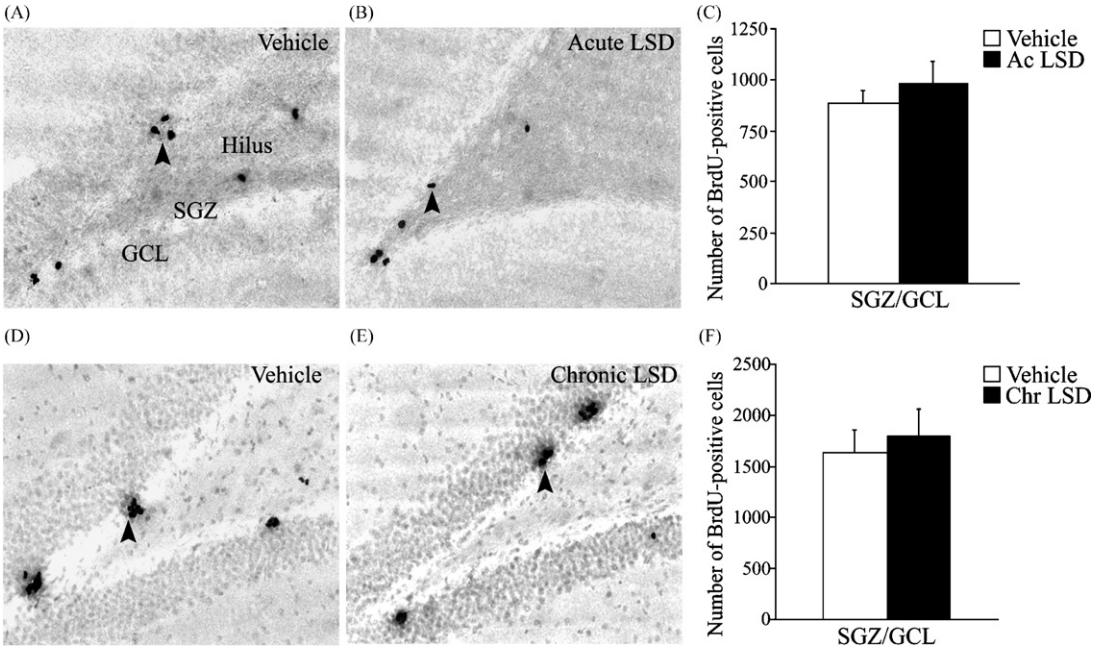

Fig. 2. Effect of acute and chronic treatment with the potent hallucinogen LSD on adult hippocampal progenitor proliferation in the dentate gyrus. Photomicrographs of the BrdU-positive cells within the dentate gyrus of representative vehicle (A, acute; D, chronic) and LSD (B, acute; E, chronic) treated animals are shown with arrowheads indicating clusters of BrdU-positive cells. LSD treatments did not influence the number of BrdU-positive cells in the SGZ/GCL. Results are expressed as mean ± S.E.M. of BrdU-positive cells in the dentate gyrus ($n = 5$ per group; acute paradigm; $n = 3$–5 per group; chronic paradigm).

following either acute or chronic administration. Hippocampal progenitors undergoing proliferation were labeled with the mitotic marker BrdU, and BrdU-positive cells were predominantly observed in clusters localized at the border of the GCL and the hilus within the SGZ. Quantitative analysis revealed that the number of BrdU-positive cells within the SGZ in animals that received acute and chronic treatment with DOI (Fig. 1) or LSD (Fig. 2) was not significantly different from vehicle treated controls. In addition, no change was seen in hilar BrdU-positive cell number following acute or chronic treatments with DOI or LSD (data not shown). Similar to previous reports the doses of DOI and LSD [19,22,32] used in our study also resulted in robust behavioral effects (serotonin syndrome, wet dog shakes and in the case of DOI-head twitch behavior) seen within 30 min.

The proliferation of adult hippocampal progenitors was found to be differentially regulated by acute and chronic treatment with the 5-$HT_{2A/2C}$ antagonist, Ketanserin. While a single administration of Ketanserin resulted in a 22% decline in the number of BrdU-positive cells within the SGZ (Fig. 3A–C), chronic administration of Ketanserin for 7 days caused a significant increase (46%) in BrdU-positive cell number in the SGZ (Fig. 3D–F). The number of BrdU-positive cells in the hilar region of the dentate gyrus was enhanced following chronic Ketanserin treatment (vehicle = 661 ± 74, chronic Ketanserin = 922 ± 51; results are the mean ± S.E.M., $^*p < 0.05$, Student's t-test) and was unchanged after a single administration of Ketanserin (vehicle = 718 ± 65, acute Ketanserin = 640 ± 63; results are the mean ± S.E.M.).

Further, we examined whether chronic treatment with Ketanserin, DOI and LSD alters the number of DCX-positive cells, another marker of adult neurogenesis [6]. One week of chronic treatment with Ketanserin did not significantly alter the number of DCX-positive cells (Fig. 4). Further, chronic Ketanserin treatment did not modify the numbers of DCX-positive cells with tertiary dendrites, a feature characteristic of their maturation [33] (Fig. 4). The number of DCX-positive cells was not changed by chronic treatments with either DOI or LSD (vehicle = 124.25 ± 3.26; DOI = 135.9 ± 16.25; LSD = 134.91 ± 4.5; results are the mean ± S.E.M. numbers of the DCX-positive cells/section, $p > 0.05$, Student's t-test).

The present study reveals that the hallucinogens LSD and DOI, which act primarily via the 5-$HT_{2A/2C}$ receptor, do not regulate adult hippocampal progenitor proliferation. On the other hand, the 5-$HT_{2A/2C}$ receptor antagonist, Ketanserin, shows a biphasic effect on hippocampal progenitor turnover, with a decline observed after acute treatment and a significant increase in the number of proliferating progenitors seen following repeated administration for 1 week. Thus far a single study has examined the effects of an acute treatment with DOI or Ketanserin on hippocampal progenitor proliferation [3], and our results are in agreement with this previous report. However, this previous study did not assess the effects of chronic DOI and Ketanserin treatment on hippocampal progenitor turnover, and our results clearly indicate that sustained blockade of the 5-$HT_{2A/2C}$ receptor with Ketanserin, opposite to the changes seen with acute treatment, results in a robust increase in progenitor proliferation.

Previous studies clearly indicate that chronic treatment with most drugs of abuse including opiates, alcohol, cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and phencyclidine (PCP) causes a reduction in hippocampal progenitor proliferation [5,7,8,10]. There are few exceptions to the general hypothesis that chronic exposure to drugs of abuse exerts an inhibitory influence on adult progenitor turnover in the hippocampus. These include moderate alcohol administration that has been reported to enhance progenitor proliferation [1] and chronic treatment with Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana that does not appear to influence progenitor cell division [15]. Our results suggest that amongst the exceptions is the hallucinogenic drug LSD that targets the 5-$HT_{2A/2C}$ receptors, which induces robust behavioral changes but does not appear to perturb cell division of adult hippocampal progenitors. In this context it is interesting to note that unlike other classes of drugs of abuse that are reported to deteriorate hippocampal-dependent learning, LSD

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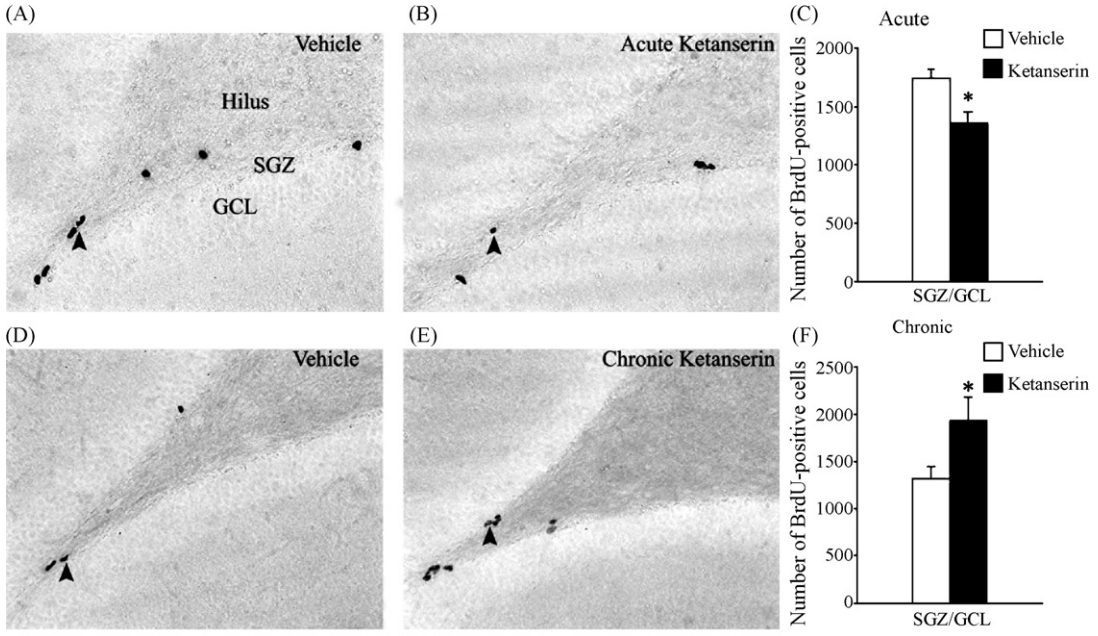

Fig. 3. Influence of the 5-$HT_{2A/2C}$ receptor antagonist Ketanserin on adult hippocampal progenitor proliferation in the dentate gyrus. Shown are representative photomicrographs from vehicle (A, acute; D, chronic) and Ketanserin (B, acute; E, chronic) treated animals with the BrDU-positive cell clusters indicated by arrowheads. Quantitative analysis of BrdU-positive cells in the SGZ at the border of the hilus and GCL revealed a significant decline in the number of BrdU-positive cells following acute Ketanserin treatment and a significant increase following chronic Ketanserin treatment. Results are expressed as mean ± S.E.M. of BrdU-positive cells (n = 6–7 per group; acute Ketanserin experiment; n = 4–6 per group; chronic Ketanserin experiment). *p < 0.05 compared to control (Student's t-test).

has been associated with an increase in learning performance [14]. It has been speculated that the decline in neurogenesis following chronic treatment with most drugs of abuse may underlie the cognitive and learning impairments associated with these agents. In this regard, the fact that hallucinogenic drugs that act at 5-$HT_{2A/2C}$ receptors do not reduce hippocampal progenitor turnover may be relevant to the differences in effects on learning that exist between this class of drugs of abuse and others.

While chronic Ketanserin treatment does enhance hippocampal progenitor turnover within a week as reflected by an increase in BrdU-positive cell number, a commensurate change in DCX-positive immature neurons is not seen within this duration. Further, chronic ketanserin treatment does not appear to influence the dendritic maturation of DCX-positive newborn neurons, as the fraction of DCX-positive cells with tertiary dendrites is unaltered. Given the biphasic effects of Ketanserin treatment on hippocampal progenitor proliferation it is possible that at the time-point of sacrifice (7 days) enhanced proliferative changes have not yet translated into a significant increase in DCX-positive cell number. Future experiments need to address whether the effects of chronic Ketanserin eventually result in increased new neuron addition or whether there is an influence on glial fate choice. At present the mechanisms that underlie the opposite effects of acute and chronic treatment with Ketanserin on adult hippocampal progenitor proliferation are unknown. Chronic, but not acute, treatment with 5-$HT_{2A/2C}$ antagonists is known to decrease the expression of the 5-HT_2 receptor [31] raising the possibility that changes in receptor expression or coupling may underlie the differences seen with acute and chronic treatment. Further studies are required to address if the effects of 5-$HT_{2A/2C}$ receptors on adult hippocampal progenitors

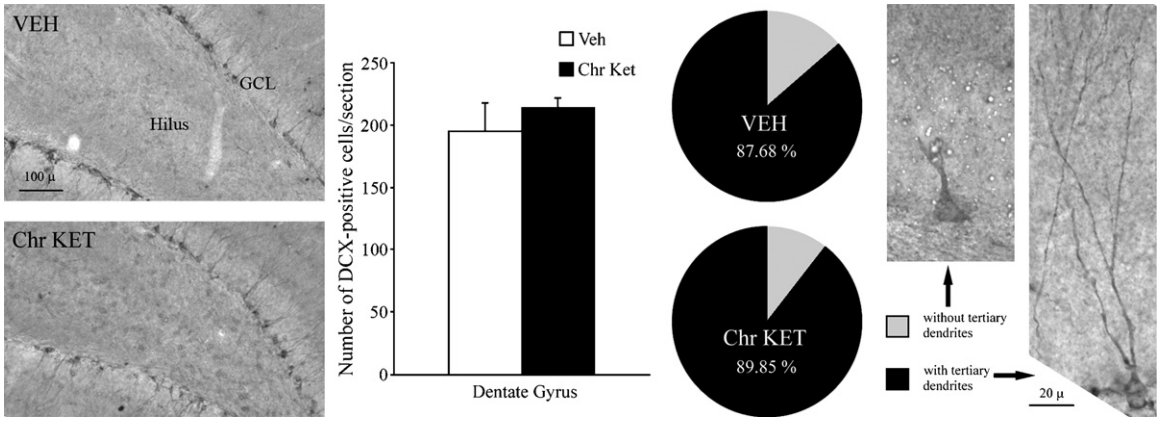

Fig. 4. Influence of chronic treatment with the 5-$HT_{2A/2C}$ receptor antagonist Ketanserin on the number of Doublecortin (DCX) positive cells in the dentate gyrus. Shown are representative photomicrographs of DCX immuno-positive cells from vehicle (VEH) and chronic Ketanserin (Chr KET) treated animals. Quantitative analysis indicated no change in the numbers of DCX-positive cells/section following chronic Ketanserin treatment. Results are expressed as mean ± S.E.M. of DCX-positive cells in the dentate gyrus (n = 5 per group). The percentage of DCX-positive cells with or without tertiary dendrites was not altered following chronic Ketanserin treatment. Shown are representative images of a DCX-positive cell with and without tertiary dendrites.

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are mediated through direct effects at the level of the progenitor or through changes in the hippocampal neurogenic milieu. Given that in the hippocampus, 5-HT_{2A} stimulation enhances GABAergic neurotransmission [29], and that GABA plays a critical role in progenitor turnover and integration of newborn hippocampal progenitors [9,11], it is possible that GABAergic circuitry may play an important role in 5-HT_{2A}-receptor mediated progenitor proliferation.

Blockade of the 5-$HT_{2A/2C}$ receptor has an important significance in the treatment of depression. The 5-$HT_{2A/2C}$ receptor antagonist Mianserin is used as clinical antidepressant (reviewed in [18]), and 5-HT_{2A} receptor antisense treatment produces an antidepressant response in animal models of depression [30]. Further, patients suffering from depression have been reported to exhibit 5-HT_{2A} receptor hypersensitivity [23], and chronic antidepressant treatment is reported to decrease 5-HT_{2A} receptor binding [34]. Our results provide novel evidence that sustained administration of Ketanserin results in a significant increase in hippocampal progenitor proliferation. Interestingly, while the enhancement of hippocampal progenitor proliferation requires chronic administration by most pharmacological antidepressant treatments for at least 2–3 weeks [17], our results indicate an increase in hippocampal progenitor turnover within 1 week of treatment with a 5-$HT_{2A/2C}$ receptor antagonist. It has been previously suggested that 5-HT_{2A} antagonists when administered along with serotonin selective reuptake inhibitors exert augmentative and synergistic therapeutic effects [18]. Our results suggest that chronic administration of a 5-$HT_{2A/2C}$ antagonist also enhances hippocampal progenitor proliferation on a faster time-scale than that reported for pharmacological antidepressants [17]. Recent reports also indicate a faster regulation of adult hippocampal neurogenesis by selective 5-HT_4 agonists [16]. This raises the possibility that specific and selective perturbation of serotonergic receptors could result in faster effects on adult hippocampal neurogenesis, which may bear relevance to the use of combination therapy with antidepressants.

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