Behavioral/Systems/Cognitive

α_2-Adrenoceptor Blockade Accelerates the Neurogenic, Neurotrophic, and Behavioral Effects of Chronic Antidepressant Treatment

Sudhirkumar U. Yanpallewar,[1]* Kimberly Fernandes,[1]* Swananda V. Marathe,[1]* Krishna C. Vadodaria,[1] Dhanisha Jhaveri,[2] Karen Rommelfanger,[3] Uma Ladiwala,[1] Shanker Jha,[1] Verena Muthig,[4] Lutz Hein,[4] Perry Bartlett,[2] David Weinshenker,[3] and Vidita A. Vaidya[1]

[1]Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400005, India, [2]Queensland Brain Institute, University of Queensland, Brisbane, Queensland 4072, Australia, [3]Department of Human Genetics, Emory University, Atlanta, Georgia 30322, and [4]Department of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, 79098 Freiburg, Germany

Slow-onset adaptive changes that arise from sustained antidepressant treatment, such as enhanced adult hippocampal neurogenesis and increased trophic factor expression, play a key role in the behavioral effects of antidepressants. α_2-Adrenoceptors contribute to the modulation of mood and are potential targets for the development of faster acting antidepressants. We investigated the influence of α_2-adrenoceptors on adult hippocampal neurogenesis. Our results indicate that α_2-adrenoceptor agonists, clonidine and guanabenz, decrease adult hippocampal neurogenesis through a selective effect on the proliferation, but not the survival or differentiation, of progenitors. These effects persist in dopamine β-hydroxylase knock-out ($Dbh^{-/-}$) mice lacking norepinephrine, supporting a role for α_2-heteroceptors on progenitor cells, rather than α_2-autoreceptors on noradrenergic neurons that inhibit norepinephrine release. Adult hippocampal progenitors *in vitro* express all the α_2-adrenoceptor subtypes, and decreased neurosphere frequency and BrdU incorporation indicate direct effects of α_2-adrenoceptor stimulation on progenitors. Furthermore, coadministration of the α_2-adrenoceptor antagonist yohimbine with the antidepressant imipramine significantly accelerates effects on hippocampal progenitor proliferation, the morphological maturation of newborn neurons, and the increase in expression of brain derived neurotrophic factor and vascular endothelial growth factor implicated in the neurogenic and behavioral effects of antidepressants. Finally, short-duration (7 d) yohimbine and imipramine treatment results in robust behavioral responses in the novelty suppressed feeding test, which normally requires 3 weeks of treatment with classical antidepressants. Our results demonstrate that α_2-adrenoceptors, expressed by progenitor cells, decrease adult hippocampal neurogenesis, while their blockade speeds up antidepressant action, highlighting their importance as targets for faster acting antidepressants.

Introduction

Depression is a prevalent psychiatric disorder, and current antidepressant medications share the major drawback of a delayed onset of therapeutic action (Thompson, 2002). While the mechanisms underlying this time lag are poorly understood, slow-onset adaptive changes in corticolimbic brain regions like the hippocampus are hypothesized to contribute to the delayed beneficial effects of antidepressants (Sahay and Hen, 2007; Krishnan and Nestler, 2008). Hippocampal plasticity induced in response to chronic antidepressant treatment occurs both at the cellular level, through an increase in adult hippocampal neurogenesis (Malberg et al., 2000), and at the molecular level, via increased expression of trophic factors like brain derived neurotrophic factor (BDNF) (Nibuya et al., 1995) and vascular endothelial growth factor (VEGF) (Warner-Schmidt and Duman, 2007). The neurogenic changes following chronic antidepressant treatment involve both increased progenitor proliferation and morphological maturation of newborn neurons (Wang et al., 2008). These molecular and cellular adaptations arise only after 2–3 weeks of sustained antidepressant administration, and are demonstrated to play an important role in the behavioral effects of antidepressants (Shirayama et al., 2002; Santarelli et al., 2003; Airan et al., 2007; Warner-Schmidt and Duman, 2007). Treatments that would hasten the onset of these adaptive changes are of particular interest, as they may serve to identify putative rapid action antidepressants.

A slow recovery is not an inherent feature of depression, as electroconvulsive seizure therapy and combination drug treatments are reported to exhibit faster clinical effects (Daly et al., 2001; Blier, 2003). Among the targets for adjunct drug therapy is

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*S.U.Y., K.F., and S.V.M. contributed equally to this work.

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Correspondence should be addressed to Dr. Vidita A. Vaidya, Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400005, India. E-mail: vvaidya@tifr.res.in.

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the α_2-adrenoceptor (Blier, 2003). Clinical evidence indicates that coadministration of the α_2-adrenoceptor antagonist yohimbine with an antidepressant hastens improvement in mood, and antidepressants that exhibit preferential α_2-adrenoceptor antagonism like mirtazapine can exhibit faster therapeutic effects (Quitkin et al., 2001; Carpenter et al., 2002; Sanacora et al., 2004). Furthermore, chronic antidepressant treatments have been demonstrated to desensitize and/or downregulate the α_2-adrenoceptor, an effect speculated to play an important role in determining the speed of antidepressant action (Esteban et al., 1999; Andrade and Sudha, 2000). Several studies indicate enhanced α_2-adrenoceptor mRNA, binding density or function in animal models of depression, and in postmortem studies of major depression (Fulford et al., 1994; Callado et al., 1998; García-Vallejo et al., 1998; García-Sevilla et al., 1999; Ribas et al., 2001). While preclinical and clinical studies implicate α_2-adrenoceptors in both the pathogenesis and treatment of depression, thus far little is known about how α_2-adrenoceptors regulate key adaptive plastic processes such as adult hippocampal neurogenesis that are thought to play an important role in antidepressant efficacy.

The present study was performed to assess the influence of α_2-adrenoceptors on adult hippocampal neurogenesis using *in vivo* and *in vitro* approaches. Furthermore, we addressed whether adjunct α_2-adrenoceptor antagonist treatment with an antidepressant accelerates the onset of adaptive changes such as increased hippocampal neurogenesis and enhanced trophic factor expression, and whether this translates into faster behavioral effects of antidepressant treatment in animal models. Our results underscore the importance of the α_2-adrenoceptor as a target for the development of faster acting antidepressants.

Materials and Methods

Animals. Adult male Wistar rats (250–300 g, 2 months of age) bred in the Tata Institute of Fundamental Research (TIFR) animal colony were used in the present study. Animals were group housed and maintained on a 12 h light/dark cycle with access to food and water *ad libitum*. For dispersed hippocampal progenitor culture studies, adult Wistar rats were used, and for the neurosphere assays, Wistar rat pups (7 d) were used. For the Dopamine β-hydroxylase (*Dbh*) knock-out experiments, adult $Dbh^{+/-}$ and $Dbh^{-/-}$ mice, maintained on a mixed 129/SvEv and C57BL/6J background and generated as previously described (Thomas et al., 1995; Thomas and Palmiter, 1998) were used. Pregnant $Dbh^{+/-}$ mice were given the adrenergic receptor agonists, isoproterenol and phenylephrine (20 μg/ml each) + vitamin C (2 mg/ml) from embryonic day 9.5 (E9.5)–E14.5, and L-3,4-dihydroxyphenylserine (DOPS; 2 mg/ml + vitamin C 2 mg/ml) from E14.5–birth in their drinking water to rescue the embryonic lethality associated with the $Dbh^{-/-}$ mutation. $Dbh^{+/-}$ mice were used as controls because they have normal norepinephrine/epinephrine levels and are phenotypically indistinguishable from $Dbh^{+/+}$ mice (Thomas et al., 1995; Thomas and Palmiter, 1998; Szot et al., 1999). Since no sex differences in hippocampal neurogenesis were observed, female $Dbh^{+/-}$ and $Dbh^{-/-}$ mice were used in this study. For the α_{2A}- and α_{2C}-adrenoceptor knock-out studies, adult male mutant mice generated as previously described (Altman et al., 1999; Hein et al., 1999) were used. The α_{2AC} knock-out mice were generated through crossing the single-knock-out strains (Hein et al., 1999). For all mouse studies, animals 3–6 months of age were used and genotypes were confirmed using PCR. All animal procedures were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the TIFR Institutional Animal Ethics committee, Animal Ethics Committee of the University of Queensland, the Emory University Animal Care and Use Committee, or the University of Freiburg, and appropriate government authorities.

Drug treatments and BrdU labeling paradigms. To study the influence of α_2-adrenoceptor stimulation, the α_2-adrenoceptor agonists, clonidine (1 mg/kg; Sigma), or guanabenz (2 mg/kg; Sigma) were used. To address the effects of α_2-adrenoceptor blockade, the α_2-adrenoceptor antagonists, yohimbine (2 mg/kg; Sigma) or RX811059 (2-ethoxy-idazoxan, 2.5 mg/kg; Sigma) were used. For the antidepressant studies, animals received treatment with the tricyclic antidepressant imipramine (20 mg/kg, Sigma). The vehicle used in this study was 0.9% saline, with the exception of yohimbine, for which it was 10% DMSO. The choice of drug doses was based on previous studies (Dursun and Handley, 1993; García-Vallejo et al., 1998; Bortolozzi and Artigas, 2003; Gobert et al., 2003; Sairanen et al., 2005; Nair et al., 2006) and all drugs were administered via intraperitoneal injection.

To study the influence of α_2-adrenoceptor stimulation or blockade on the proliferation of adult hippocampal progenitors, animals received two different treatment regimes (acute and chronic). In the acute treatment regime, rats received a single injection of the α_2-adrenoceptor drugs (clonidine, guanabenz, yohimbine, or RX811059) or vehicle, followed 45 min later by a single injection of the mitotic marker 5-bromo-2′-deoxyuridine (BrdU, 200 mg/kg; Sigma) and were killed 2 h later (n = 6/group; each experiment had its individual vehicle group). In the chronic treatment regime, rats received treatment with the α_2-adrenoceptor drugs (clonidine, guanabenz, or yohimbine) or vehicle once daily for 7 d. On the last day of treatment, 2 h after drug or vehicle administration, animals received a single injection of BrdU (200 mg/kg) and were killed 24 h later (n = 5–6/group; each experiment had its individual vehicle group). To address whether the effects of α_2-adrenoceptor stimulation on hippocampal progenitor proliferation could be prevented by prior α_2-adrenoceptor blockade, animals were divided into four experimental groups (vehicle/vehicle, vehicle/clonidine, yohimbine/vehicle and yohimbine/clonidine, n = 10/group; yohimbine/vehicle group, n = 6). The two injections were separated by 30 min. BrdU was administered 45 min after the last injection and the animals were killed 2 h later.

Dbh mutant mice were used to address whether the effects of α_2-adrenoceptor agonists on adult hippocampal progenitor proliferation involve stimulation of inhibitory α_2-autoreceptors or α_2-heteroceptors on target neurons. Because $Dbh^{-/-}$ mice lack endogenous norepinephrine, any effect of α_2-adrenoceptor agonists in these mice must be mediated by α_2-heteroceptors. $Dbh^{+/-}$ and $Dbh^{-/-}$ mice received treatment with guanabenz (2 mg/kg) or vehicle once daily for 7 d. On the last day of treatment, 2 h after drug or vehicle administration, animals received a single injection of BrdU (200 mg/kg) and were killed 24 h later (n = 5–6/group). To address the role of individual α_2-adrenoceptor subtypes on adult hippocampal progenitor proliferation, α2A, α2C and α2AC knock-out and wild-type control mice received a single administration of BrdU (100 mg/kg) and were killed 2 h later (n = 4–5/group).

To determine the influence of α_2-adrenoceptor stimulation or blockade on the survival and differentiation of adult hippocampal progenitors, drug-naive rats first received BrdU (200 mg/kg) once daily for three consecutive days. Five days following the last BrdU treatment, a time point at which BrdU-positive cells are likely to be postmitotic (Cameron and McKay, 2001), rats received treatment with the α_2-adrenoceptor agonist clonidine, the α_2-adrenoceptor antagonist yohimbine and respective vehicle treatment once daily for 21 d and were killed 24 h after the final drug treatment (n = 4–6/group). To assess effects of α_2-adrenoceptor stimulation on hippocampal progenitor maturation at earlier time-points after BrdU labeling, drug-naive animals first received BrdU (100 mg/kg) twice daily for two consecutive days followed by a single BrdU injection on the third day. Treatment with the α_2-adrenoceptor agonist, guanabenz, commenced the day after the final BrdU injection and continued for seven consecutive days. Animals were killed 24 h after the final vehicle/guanabenz treatment (n = 4/group).

To address the influence of α_2-adrenoceptor blockade on the behavioral and neurogenic effects of the antidepressant imipramine, rats were divided into four treatment groups: vehicle/vehicle, yohimbine/vehicle, vehicle/imipramine, yohimbine/imipramine (n = 10/group). The two injections were spaced apart by 30 min. All four groups received the above described drug treatment regime for 7 or 21 d. Two hours after the last drug treatment animals were tested for their behavior in the novelty suppressed feeding (NSF) test. The NSF test is reported to be responsive to chronic administration of classical antidepressants for 2–3

weeks (Bodnoff et al., 1988; Santarelli et al., 2003). Following the NSF test, animals were injected with BrdU (200 mg/kg, i.p.) and were killed 24 h later to address effects on progenitor proliferation using BrdU immunohistochemistry and immature neuron numbers using DCX immunohistochemistry. Furthermore, a group of animals that received the above treatment regime for 14 d was also used to address effects on endogenous markers of hippocampal neurogenesis ($n = 6$/group). An additional group of animals ($n = 6$/group) from the 7 d drug treatment regime were killed 28 d after the final BrdU treatment to study differentiation of BrdU-labeled hippocampal progenitors into neurons or glia. To address whether the 7 d combined drug treatment regime influences hippocampal progenitor maturation, drug-naive animals first received BrdU (100 mg/kg) twice daily for two consecutive days followed by a single BrdU injection on the third day. Animals were divided into the four treatment groups as described above, received the 7 d drug treatment paradigm with yohimbine and imipramine, and were killed 24 h after the final treatment ($n = 5$/group).

To address the effects of a short-duration (7 d) combined treatment with yohimbine and imipramine on gene expression in the hippocampus, animals received vehicle or yohimbine injections followed 30 min later by vehicle or imipramine treatment as described above, and were killed 2 h after the last injection ($n = 4$/group). To assess the influence of a short-duration (7 d) treatment with yohimbine and imipramine on norepinephrine levels in the hippocampus, animals received vehicle or yohimbine injections followed 30 min later by vehicle or imipramine treatment as described above ($n = 6$/group).

Dispersed adult hippocampal progenitor cultures. Dissociated adult hippocampal progenitor cell cultures were generated from adult male Wistar rats (2 months) based on a previously described method (Palmer et al., 1999), and cells were cultured in DMEM/F-12 with N2 supplement (Invitrogen) and 40 ng/ml FGF-2 (R&D Systems) on poly-ornithine- (Sigma) and laminin- (Sigma) coated tissue culture plates. Hippocampal progenitors were plated in 16-well poly-ornithine- and laminin-coated Labtek chamber slides (Nunc) at a density of 5×10^5 cells/well. To address the influence of α_2-adrenoceptor stimulation or blockade on hippocampal progenitor proliferation, cells were exposed for 24 h to growth medium with or without clonidine (10, 50, and 100 μM) or yohimbine (10, 50, and 100 μM). Cells were then exposed to a pulse of BrdU (40 μM, Sigma) for 2 h and were fixed by exposure to 4% paraformaldehyde (PFA) for 15 min before further processing. *In vitro* drug doses were selected based on prior literature (Ortego and Coca-Prados, 1998; Han et al., 2007). To examine the expression of α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptor subtypes in hippocampal progenitors, total RNA was isolated from the dispersed hippocampal progenitor cultures (Oligotex Direct RNA kit, Qiagen) and PCR was performed on reverse transcribed cDNA using specific forward and reverse primers. The primer sequences (DNA Technology) for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors and β-actin were based on prior literature (Faber et al., 2001; Mori et al., 2002).

Neurosphere assay. Seven-day-old Wistar pups were killed under isoflurane-induced anesthesia and hippocampi were dissected out into HEPES-buffered Eagle's medium. Hippocampal tissue was minced, treated with 0.1% trypsin-EDTA for 5 min at 37°C, and digestion was stopped by adding trypsin inhibitor (0.014% w/v, Sigma). Tissue was triturated to obtain a single cell suspension, and cells were plated at 1000 cells/well in a 96-well plate and cultured in NeuroCult NSC basal medium with proliferation supplements (StemCell Technologies), 2% bovine serum albumin (Invitrogen), 2 μg/ml heparin (Sigma), epidermal growth factor (20 ng/ml, BD Biosciences), and basic fibroblast growth factor (10 ng/ml, Roche). Cells were treated with clonidine (100 μM), guanabenz (10 μM), or yohimbine (10 μM) for the duration of the experiment. *In vitro* drug doses were selected based on prior literature (Ortego and Coca-Prados, 1998; Dunbar et al., 2006; Han et al., 2007). In each experiment, 20 wells were plated per treatment group and the experiment was repeated three times. The number of neurospheres (>100 μm) obtained per well was counted after 10 d in culture.

Immunohistochemistry and immunofluorescence. Animals were killed through transcardial perfusion with 4% PFA, and serial coronal sections (50 μm) through the rostrocaudal extent of the hippocampus were generated on a vibratome. Sections were processed for BrdU, proliferating cell nuclear antigen (PCNA) or doublecortin (DCX) immunohistochemistry as described previously (Kulkarni et al., 2002, Jha et al., 2006). In brief, BrdU immunohistochemistry involved DNA denaturation and acid hydrolysis followed by overnight incubation with anti-BrdU antibody (mouse anti-BrdU, 1:500, Boehringer Mannheim; rat anti-BrdU, 1:500, Accurate Biochemicals) before treatment with secondary antibody (biotinylated anti-mouse IgG or anti-rat IgG, 1:500, Vector Laboratories or Cy3-conjugated anti-mouse IgG, 1:1000, Invitrogen). For PCNA immunohistochemistry, sections were subjected to antigen retrieval in 0.1 M PB at 80°C for half an hour before blocking and overnight incubation with mouse anti-PCNA (1:250, Millipore Bioscience Research Reagents) followed by incubation with biotinylated horse anti-mouse secondary antibody (1:250, Vector) for 2 h. For DCX immunohistochemistry, sections were incubated with goat anti-DCX antibody (1:250, Santa Cruz Biotechnology) overnight followed by incubation with a biotinylated rabbit anti-goat secondary antibody (1:500, Vector) for 2 h. For NeuroD immunohistochemistry, sections were incubated with goat anti-NeuroD antibody (1:250, Santa Cruz Biotechnology) overnight followed by incubation with a biotinylated rabbit anti-goat secondary antibody (1:500, Vector) for 2 h. Signal amplification was performed using a Vectastain Elite Avidin-Biotin system (Vector) and was visualized using diaminobenzidine (Sigma) as a substrate.

For double label immunofluorescence, sections subjected to DNA denaturation and acid hydrolysis were exposed to the following combinations of antibodies: (1) mouse anti-BrdU (1:500, Roche) and goat anti-calretinin (1:250; Millipore Bioscience Research Reagents), (2) mouse anti-BrdU (1:500, Roche) and goat anti-DCX (1:250; Santa Cruz Biotechnology) overnight at room temperature. Sections were incubated with a mixture of secondary antibodies: biotinylated anti-mouse IgG (1:250, Vector) and rhodamine-conjugated anti-goat IgG (1:250, Invitrogen) before incubation with fluorescein-conjugated streptavidin (1:500, GE Healthcare) for 1 h.

For triple label immunofluorescence, sections subjected to DNA denaturation and acid hydrolysis were exposed to a mixture of antibodies: rat anti-BrdU (1:200, Accurate Biochemicals), mouse anti-neuronal nuclei (NeuN; 1:1000; Millipore Bioscience Research Reagents) and rabbit anti-glial fibrillary acidic protein (GFAP; 1:500; Sigma) overnight at room temperature. Sections were incubated with a mixture of secondary antibodies: biotinylated anti-rat IgG (1:500, Millipore Bioscience Research Reagents), rhodamine-conjugated anti-mouse IgG (1:500, Millipore Bioscience Research Reagents), and Cy-5-conjugated anti-rabbit IgG (1:500, Millipore Bioscience Research Reagents) for 2 h, followed by incubation with fluorescein-conjugated streptavidin (1:500, GE Healthcare) for 1 h.

Cell counting analysis. All cell counting analysis was performed on coded sections by an experimenter blind to the study code. Quantitation of BrdU-positive cell numbers in tissue sections was carried out using a previously described modified, unbiased stereology protocol (Malberg et al., 2000) on a Zeiss Axioskop microscope. Sections spanned the rostrocaudal extent of the hippocampus and every fifth hippocampal section was processed for BrdU quantitation (10 sections/animal). BrdU-positive cells within dentate gyrus (DG) were counted as being in the subgranular zone (SGZ)/granule cell layer (GCL) when they were directly touching the SGZ or within it. The total number of BrdU-positive cells per SGZ/GCL was estimated by multiplying the total number of BrdU-positive cells counted by the section periodicity (5) and results are expressed as the total number of BrdU-positive cells per SGZ/GCL. Quantitation of PCNA-positive cells followed the same approach described above for BrdU-quantitation. To determine DCX- or NeuroD-positive cell number in the hippocampus, the number of DCX- or NeuroD-positive cells in the DG were quantitated (four to six sections/animal) and results were expressed as the number of DCX- or NeuroD-positive cells per section. Furthermore, we also examined the morphological status of DCX-positive cells by categorizing them as (1) DCX-positive cells without tertiary dendrites or (2) DCX-positive cells with complex tertiary arbors (Wang et al., 2008). Quantitation of both DCX-positive cell number and morphological category was performed using a Zeiss Axioskop at a magnification of 400×.

To address effects on the maturation of hippocampal progenitors, the percentage of BrdU-positive cells that colocalized with the markers calretinin or DCX was determined using confocal microscopy. A minimum of 60 (BrdU/calretinin) or 30 (BrdU/DCX) BrdU-positive cells from each animal (4 sections, 500 μm apart) were analyzed using *z*-plane confocal sectioning with 1 μm steps on a Biorad MRC1024 to confirm colocalization of BrdU with calretinin or DCX. To study progenitor differentiation into NeuN-positive neurons or GFAP-positive glia, the percentage of BrdU-positive cells that colocalized with NeuN or GFAP was assessed. A minimum of 30 BrdU-positive cells from each animal (8 sections, 250 μm apart) was analyzed using *z*-plane sectioning with 0.5 μm steps on a Biorad MRC1024 confocal microscope to confirm colocalization with either NeuN or GFAP.

Quantitation of the numbers of BrdU-positive cells in adult rat hippocampal progenitor cultures was done using an epifluorescent Zeiss microscope by an experimenter blind to the treatment conditions. The percentage of DAPI-positive cells per field that were labeled with BrdU was determined. The number of BrdU-positive cells for the proliferation experiment was counted in >600 cells/well (three random, nonoverlapping individual fields per well) and three wells were scored per treatment condition.

In situ *hybridization and autoradiogram quantitation. In situ* hybridization was carried out as previously described (Nair et al., 2007). In brief, 14 μm cryostat cut coronal sections were fixed, acetylated and dehydrated before storage at −70°C. Rat BDNF, VEGF, fibroblast growth factor-2 (FGF-2), VGF (not an acronym), activity regulated cytoskeletal protein (Arc), and cAMP response element binding protein (CREB) cRNA probes were generated from either transcription competent plasmids or PCR generated templates. Plasmids for BDNF, FGF-2, VGF, Arc, and CREB were kindly provided by Dr. Julie Lauterborn (University of California, Irvine, Irvine, CA), Dr. Sondra Bland (University of Colorado at Boulder, Boulder, CO), Dr. Shubha Tole (Tata Institute of Fundamental Research, Mumbai, India), Dr. Oswald Steward (John Hopkins University, Baltimore, MD), and Dr. Ronald Duman (Yale University, New Haven, CT) respectively. The VEGF template for VEGF exon 3 was PCR generated using a forward primer with a T3 site and a reverse primer with a T7 site (forward primer: AATTAACCCTCACTAAAGGGTCCAATTGAGACCCTGGTG; reverse primer: TAATACGACTCACTATAGGGCCATAGTGACGTTGCTCTCCG). cRNA probes were transcribed using ^{35}S-labeled UTP (GE Healthcare), and slides were incubated with ^{35}S-UTP labeled riboprobes at a concentration of 2×10^6 cpm/150 μl in hybridization buffer. After hybridization, slides were subjected to RNase A (20 μg/ml) treatment at 45°C for 30 min, followed by stringent washes in decreasing concentrations of SSC. Slides were air dried and exposed to Hyperfilm β-max (GE Healthcare) for 1–2 weeks. Autoradiograms were analyzed using the Macintosh-based Scion Image software (Scion). To correct for nonlinearity, ^{14}C standards were used for calibration. The dentate gyrus granule cell region was analyzed and an equivalent area was outlined for each sample. Optical density measurements from both sides of 3–4 individual sections from each animal were analyzed, from which the mean value was calculated. Sense riboprobes or a ribonuclease A pretreatment wash (40 μg/ml at 37°C for 30 min) did not yield any significant hybridization (data not shown) confirming the specificity of signal.

Novelty suppressed feeding test. To address the influence of α_2-adrenoceptor blockade on the behavioral effects of the antidepressant imipramine, animals were subjected to the NSF paradigm, which has been previously reported to demonstrate differences in behavior following chronic, but not acute, antidepressant treatment (Bodnoff et al., 1988; Santarelli et al., 2003). Rats in the four different treatment groups, vehicle/vehicle, yohimbine/vehicle, vehicle/imipramine, and yohimbine/imipramine were tested on day 7 and day 21 of treatment in two distinct experiments. Food was withdrawn 48 h before the test with water available *ad libitum*. The test was performed in a black rectangular box of dimensions (52 × 48 × 32 cm) kept in a dark room. Food pellets were placed in the center of the box on a small elevated platform that was brightly illuminated. At the start of the test, rats were placed in a dark corner of the box facing the wall and were observed for a period lasting 5 min. The latency to feed was determined and animals that did not approach the food pellet were given a score of 300 s equaling the entire duration of the test. At the end of the test, animals were transferred to their home cages with food available, and their food consumption was measured for 30 min.

Biochemical detection of norepinephrine. To assess the levels of norepinephrine in the hippocampus following combined treatment with yohimbine and imipramine for 7 d, high performance liquid chromatography (HPLC) was used. Twenty-four hours after the final treatment, hippocampi were rapidly dissected out and frozen in liquid nitrogen. Homogenates were generated through sonication of hippocampi in 0.1 M perchloric acid followed by centrifugation at 15,000 rpm for 10 min at 4°C. Supernatants were processed for determination of norepinephrine. Levels of norepinephrine were determined with a fluorometric detection method for HPLC analysis as described previously (Lakshmana and Raju, 1997) using purified norepinephrine (Sigma) as a standard.

Statistical analysis. Results were subjected to statistical analysis using the statistical software Prism (Graphpad). Experiments with two groups were analyzed for differences using the unpaired Student's *t* test, with significance determined at $p < 0.05$. Experiments with three groups or more were subjected to statistical analyses using one-way or two-way ANOVA with significance determined at *p* values <0.05, followed by the Bonferroni *post hoc* test for group comparisons. The two-way ANOVA was used to assess interaction effects between two factors, namely: (1) α_2-adrenoceptor antagonist, yohimbine and α_2-adrenoceptor agonist, clonidine, (2) genotype (*Dbh*) and α_2-adrenoceptor agonist, guanabenz, and (3) the antidepressant, imipramine and the α_2-adrenoceptor antagonist, yohimbine.

Results

α_2-Adrenoceptor stimulation decreases adult hippocampal progenitor proliferation *in vivo*

Acute (single injection) (Fig. 1) or chronic (once daily injections for 7 d) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material) treatments with the α_2-adrenoceptor agonists, guanabenz or clonidine, resulted in a significant decline in the proliferation of adult hippocampal progenitors, assessed using the mitotic marker BrdU. BrdU-positive cells were predominantly localized to the SGZ (Fig. 1*B*) lining the border of the GCL and the hilus. Analysis of the numbers of BrdU-positive cells in the SGZ, using unbiased stereological approaches, revealed a significant decline of 34% and 38% in BrdU-positive cell number after acute guanabenz (Fig. 1*C*) or acute clonidine (Fig. 1*C*) treatment respectively. Chronic treatments with guanabenz or clonidine (supplemental Fig. 1, available at www.jneurosci.org as supplemental material) resulted in a 45% and 32% decline in the numbers of BrdU-positive cells in the SGZ respectively. To corroborate the effects of α_2-adrenoceptor stimulation on adult hippocampal progenitor proliferation observed with BrdU, we also examined the effect of acute clonidine treatment on an endogenous marker of cell division, PCNA. Acute clonidine treatment resulted in a significant decline (35%) in the number of PCNA-positive cells within the SGZ (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

Next, we addressed whether pretreatment with the α_2-adrenoceptor antagonist, yohimbine, would block the effects of clonidine on hippocampal progenitor proliferation. Pretreatment with yohimbine completely blocked the clonidine-induced decline in BrdU-positive cell number observed within the SGZ (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Two-way ANOVA revealed a significant yohimbine × clonidine interaction ($F_{(1,26)} = 11.59$, $p = 0.0022$). Furthermore, to assess whether α_2-adrenoceptor blockade itself influences adult hippocampal progenitor proliferation, animals received acute treatments with the α_2-adrenoceptor antagonists, yohimbine or RX811059. Acute treatment with either of the α_2-

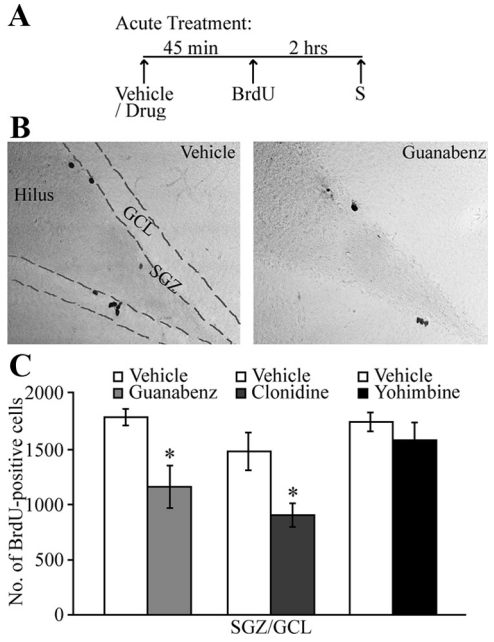

Figure 1. α_2-Adrenoceptor stimulation decreases the proliferation of adult hippocampal progenitors. Rats received acute treatment with the α_2-adrenoceptor agonists guanabenz or clonidine, or the α_2-adrenoceptor antagonist yohimbine as described in Materials and Methods. ***A***, Shown is a schematic representation of the experimental design to assess the influence of acute treatment with α_2-adrenoceptor agonists and antagonist on adult hippocampal progenitor proliferation (S-time point for kill). ***C***, Quantitative stereological analysis revealed a significant decrease in the number of BrdU-positive cells in the SGZ/GCL following acute treatment with the α_2-adrenoceptor agonists guanabenz and clonidine. ***B***, Shown are representative photomicrographs of BrdU-positive cells from vehicle- and acute guanabenz-treated groups. BrdU-positive cells were observed in the SGZ at the border of the GCL and the hilus. Acute treatment with the α2-adrenoceptor antagonist yohimbine did not alter the number of BrdU-positive cells in the SGZ/GCL (***C***). The results are expressed as the mean ± SEM number of BrdU-positive cells in the SGZ/GCL ($n = 5–6$ per group). *$p < 0.05$ compared with vehicle-treated controls (Student's *t* test).

adrenoceptor antagonists did not alter the number of BrdU-positive cells within the SGZ (Fig. 1*C*) (SGZ BrdU-positive cell number: Vehicle = 1215.83 ± 140.60, RX811059 = 1315 ± 155; $p > 0.05$, Results are the mean ± SEM, $p > 0.05$, Student's *t* test). In addition, chronic treatment with yohimbine also did not change the number of BrdU-positive cells in the SGZ (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

α_2-Adrenoceptor stimulation induced decline in adult hippocampal progenitor proliferation persists in *Dbh*$^{-/-}$ mice

α_2-Adrenoceptors are coupled to G_i protein, inhibit adenylyl cyclase, and exist both as presynaptic inhibitory autoreceptors that control norepinephrine release and as α_2-heteroceptors on target neurons receiving noradrenergic input. *Dbh*$^{-/-}$ mice were used to distinguish whether the effects of α_2-adrenoceptor stimulation on progenitor proliferation are mediated by α_2-autoreceptors or heteroceptors. Because *Dbh*$^{-/-}$ mice lack norepinephrine, α_2-adrenoceptor agonists cannot produce effects via autoreceptor modulation of norepinephrine release; their only activity is via α_2-heteroceptors (Szot et al., 2004). Chronic administration of the α_2-adrenoceptor agonist, guanabenz, to *Dbh*$^{-/-}$ mice resulted in a significant decline in the number of BrdU-positive cells in the SGZ (Fig. 2). The extent of this significant decline in hippocampal progenitor proliferation was similar to that

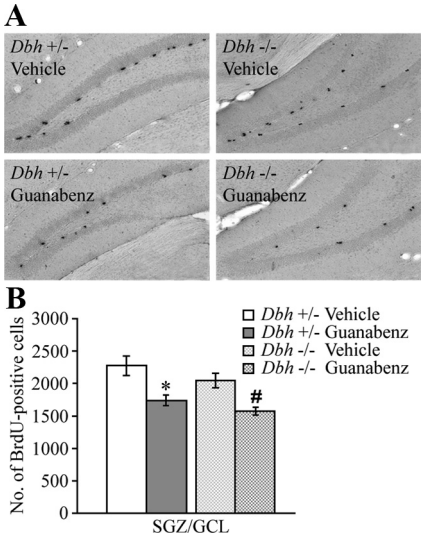

Figure 2. α_2-Adrenoceptor stimulation-induced decline in adult hippocampal progenitor proliferation persists in *Dbh*$^{-/-}$ mice. *Dbh*$^{+/-}$ and *Dbh*$^{-/-}$ mice received treatment with guanabenz or vehicle once daily for 7 d as described in Materials and Methods. ***A***, Shown are representative photomicrographs of BrdU-positive cells from vehicle- and guanabenz-treated *Dbh*$^{+/-}$ and *Dbh*$^{-/-}$ animals. ***B***, Quantitative analysis indicated a significant reduction in BrdU-positive cell number within the SGZ/GCL of both *Dbh*$^{+/-}$ and *Dbh*$^{-/-}$ mice following guanabenz treatment. The results are expressed as the mean ± SEM number of BrdU-positive cells in the SGZ/GCL ($n = 5–6$ per group). *$p < 0.05$ compared with *Dbh*$^{+/-}$ vehicle; #$p < 0.05$ compared with *Dbh*$^{-/-}$ vehicle (ANOVA and Bonferroni *post hoc* test).

observed in *Dbh*$^{+/-}$ controls treated with guanabenz. Two-way ANOVA indicated no genotype × guanabenz interaction ($F_{(1,13)} = 0.07$, $p = 0.80$). The persistence of the guanabenz-induced impairment of hippocampal progenitor proliferation in *Dbh*$^{-/-}$ mice supports a role for α_2-heteroceptors *in vivo*. However, it is not possible to rule out a role for other factors released by noradrenergic nerve terminals following α_2-autoreceptor stimulation that may modulate hippocampal progenitor turnover.

Dbh$^{-/-}$ mice did not exhibit a baseline decrease in hippocampal progenitor proliferation as compared to *Dbh*$^{+/-}$ controls (Fig. 2*B*). This suggests that genetic loss of norepinephrine through postnatal development and adulthood does not produce a significant alteration in the proliferation of adult hippocampal progenitors, unlike the decline observed with pharmacological norepinephrine depletion in adult animals (Kulkarni et al., 2002), suggesting possible compensatory mechanisms (Weinshenker et al., 2002; Schank et al., 2006).

α_2-Adrenoceptor stimulation decreases proliferation of adult hippocampal progenitors *in vitro*

Given that the effects of α_2-adrenoceptor agonists on hippocampal progenitor proliferation likely involve α_2-heteroceptors, we next sought to address whether adult hippocampal progenitors express α_2-adrenoceptor subtypes and whether α_2-adrenoceptor agonists modulate their proliferation *in vitro*. RT-PCR on nestin immunopositive, dispersed adult hippocampal progenitors in culture (Fig. 3*A*,*B*) demonstrated the mRNA expression of α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptor subtypes (Fig. 3*C*). To examine the effects of α_2-adrenoceptor stimulation on the proliferation of adult hippocampal progenitors in culture, progenitor cells were exposed to clonidine (10, 50, or 100 μM) for 24 h before a BrdU pulse for 2 h to determine the number of progenitors entering the S-phase of cell cycle. α_2-Adrenoceptor stimulation with clonidine

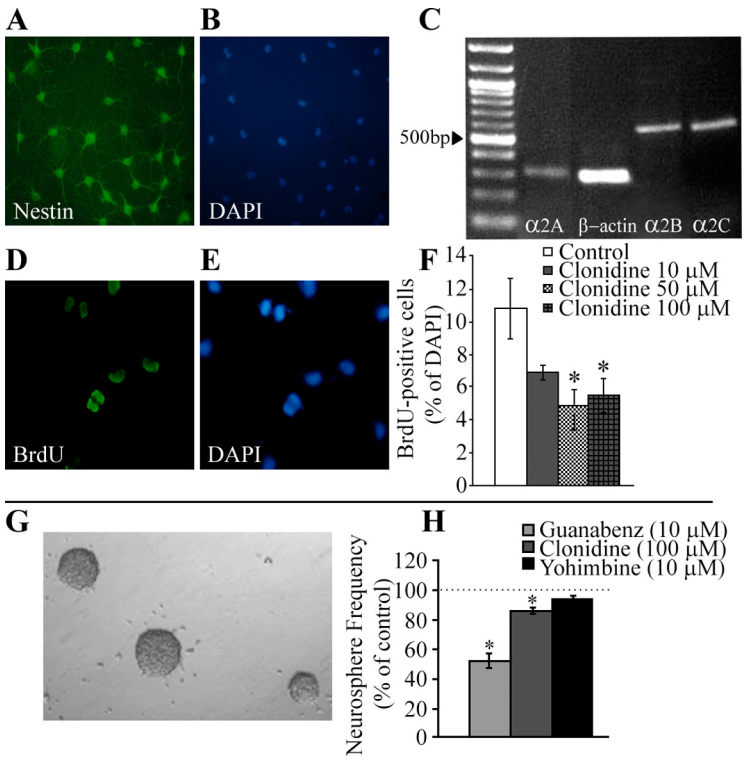

Figure 3. α_2-Adrenoceptor stimulation decreases the proliferation of adult hippocampal progenitors *in vitro* in two culture models, dispersed adult hippocampal progenitor cultures and the Neurosphere assay. ***A***, ***B***, Nestin-immunopositive (***A***, Nestin; ***B***, DAPI), dispersed adult hippocampal progenitors were derived as described in Materials and Methods and in the study by Palmer et al. (1999). ***C***, Reverse transcriptase-PCR results indicated that adult hippocampal progenitors express mRNA for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptor subtypes. ***F***, Treatment of dispersed hippocampal progenitors with clonidine (10, 50, or 100 μM) resulted in a significant decline in BrdU incorporation at the 50 and 100 μM doses. ***D***, ***E***, Shown is an epifluorescent image of BrdU-immunopositive progenitor cells (***D***, BrdU; ***E***, DAPI). α_2-Adrenoceptor stimulation also inhibited the proliferation of neural stem cells *in vitro* as observed using the neurosphere assay. The neurosphere assay was performed as described in Materials and Methods. ***H***, Treatment with the α_2-adrenoceptor agonists guanabenz (10 μM) or clonidine (100 μM) resulted in decreased neurosphere formation. Treatment with the α_2-adrenoceptor antagonist yohimbine (10 μM) did not alter neurosphere frequency. ***G***, Shown is a representative image of neurospheres. Results are expressed as mean ± SEM. BrdU-positive cells as a percentage of total DAPI-positive cells (***F***) or mean ± SEM. Neurosphere frequency is represented as a percentage of control (***H***) ($n = 3$/group). *$p < 0.05$ compared with control (ANOVA and Bonferroni *post hoc* test).

Table 1. Proliferation of adult hippocampal progenitors and the number of DCX-positive immature neurons are not altered in α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor subtype knock-out mice

	No. of BrdU-positive cells/DG	No. of DCX-positive cells/section
WT	651.00 ± 152.21	188.93 ± 6.497
$\alpha_{2A}^{-/-}$	451.71 ± 24.72	188.01 ± 30.69
$\alpha_{2C}^{-/-}$	615.60 ± 103.46	165.78 ± 12.85
$\alpha_{2AC}^{-/-}$	831.00 ± 133.18	163.10 ± 22.36

Wild-type, α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor subtype knock-out mice were injected with the mitotic marker BrdU as described in Materials and Methods. Quantitative analysis of BrdU-positive cells in the SGZ/GCL indicated no difference in the proliferation of adult hippocampal progenitors in α_2-adrenoceptor subtype knock-out mice compared with wild-type controls. The results are expressed as mean ± SEM BrdU-positive cells per SGZ/GCL (n = 5–6/group). Furthermore, the number of DCX-immunopositive immature neurons was also unchanged in α_2-adrenoceptor subtype knock-out mice compared with their wild-type controls. The results are expressed as mean ± SEM DCX-positive cells per section (n = 5–6/group).

led to a significant decrease in the numbers of BrdU-positive cells labeled *in vitro* (Fig. 3*D–F*). Similar to the *in vivo* results obtained with α_2-adrenoceptor antagonists, hippocampal progenitors *in vitro* treated with yohimbine (10, 50, or 100 μM) did not exhibit any change in proliferation (data not shown).

To further ascertain whether the α_2-adrenoceptor agonist induced decline in hippocampal progenitor proliferation is mediated through a direct effect on hippocampal progenitors, we also performed the neurosphere assay (Fig. 3*G*). Exposure of neurosphere cultures to clonidine or guanabenz resulted in a significant reduction in neurosphere formation (Fig. 3*H*). While treatment with guanabenz resulted in a robust and significant (44%) decline in neurosphere formation, the percentage decline induced by clonidine treatment, though significant, was relatively small (14%) (Fig. 3*H*). In contrast, treatment with yohimbine did not alter the numbers of neurospheres generated (Fig. 3*H*). Together, these results demonstrate that the direct stimulation of α_2-adrenoceptors on hippocampal progenitors inhibits proliferation.

Adult hippocampal progenitor proliferation is unaffected in α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor knock-out mice

To address whether turnover of adult hippocampal progenitors is altered in α_2-adrenoceptor subtype knock-out mice, we examined the number of BrdU-positive cells in $\alpha_{2A}^{-/-}$, $\alpha_{2C}^{-/-}$, and $\alpha_{2AC}^{-/-}$ mice. Total numbers of BrdU-positive cells in the SGZ of α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor knock-out mice were not different from those of wild-type controls (Table 1). Furthermore, we examined the numbers of cells expressing DCX, an endogenous marker of immature newborn neurons, and observed no difference in the numbers of DCX-positive cells in α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor knock-out mice compared with wild-type controls (Table 1).

α_2-Adrenoceptor stimulation or blockade does not influence the survival and differentiation of adult hippocampal progenitors

To examine the influence of α_2-adrenoceptor agonist or antagonist treatment on the survival and differentiation of adult hippocampal progenitors, drug-naive animals first received BrdU injections (Fig. 4*A*). Administration of either clonidine or yohimbine commenced 5 d after BrdU treatment and continued for 21 d (Fig. 4*A*). This particular BrdU labeling paradigm allows us to assess whether the postmitotic survival, or differentiation, of a BrdU-labeled cohort of hippocampal progenitors is influenced by α_2-adrenoceptor stimulation or blockade.

The survival of hippocampal progenitors in the SGZ/GCL was unaffected by clonidine or yohimbine treatment, as the numbers of BrdU-positive cells that persist in the SGZ/GCL was unchanged in both the clonidine- (Fig. 4*B*,*C*) and yohimbine- (Fig. 4*B*,*D*) treated groups compared with their respective vehicle-treated controls. We then determined whether the differentiation of BrdU-positive progenitors in the SGZ/GCL into a neuronal (NeuN-immunopositive, Fig. 4*G*) or glial (GFAP-immunopositive, Fig. 4*H*) fate is influenced by α_2-adrenoceptor stimulation or blockade. Confocal analysis with *z*-plane sectioning indicated no change in the percentage of BrdU-positive cells that acquired a neuronal or glial phenotype in vehicle- versus clonidine- (Fig. 4*E*) or yohimbine-treated groups (Fig. 4*F*). A majority of BrdU-

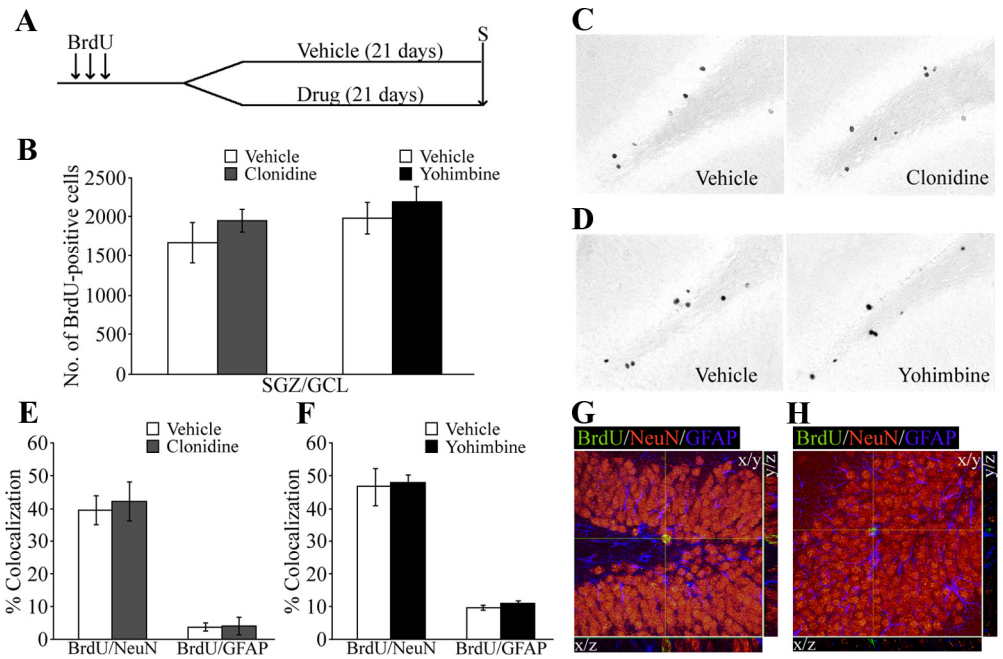

Figure 4. α_2-Adrenoceptor stimulation or blockade does not influence the survival and differentiation of adult hippocampal progenitors. Drug-naive rats first received BrdU injections followed by treatment with vehicle, clonidine or yohimbine for 21 d as described in Materials and Methods. ***A***, Shown is a schematic representation of the experimental design to assess the influence of α_2-adrenoceptor stimulation or blockade on adult hippocampal progenitor survival and differentiation (S-time point for kill). ***B***, Quantitative analysis revealed no difference in the number of BrdU-positive cells in the SGZ/GCL following treatment with the α_2-adrenoceptor agonist, clonidine or the antagonist, yohimbine. ***C***, ***D***, Shown are representative photomicrographs of BrdU-positive cells from clonidine- (***C***) and yohimbine- (***D***) treated groups along with representative images from respective vehicle-treated animals. The results are expressed as the mean ± SEM number of BrdU-positive cells in the SGZ/GCL ($n = 5–6$ per group). ***E***, ***F***, Quantitative analysis showed that clonidine (***E***) or yohimbine (***F***) treatment did not affect the percentage colocalization of BrdU-positive cells with either the neuronal marker NeuN or the glial marker GFAP, in the SGZ and GCL, compared with vehicle-treated controls. ***G***, ***H***, Shown are representative confocal *z*-stack images for colocalization of a BrdU-positive cell with NeuN (***G***) or GFAP (***H***). Results are expressed as the mean ± SEM percentage colocalization of BrdU-positive cells with NeuN or GFAP in the SGZ/GCL ($n = 4$/group).

positive cells acquired a neuronal phenotype with far fewer numbers taking on a glial fate. BrdU-positive cells that did not colocalize with either NeuN or GFAP may represent as yet undifferentiated progenitors.

We also examined whether α_2-adrenoceptor stimulation would influence earlier stages of maturation of hippocampal progenitors by addressing the percentage colocalization of BrdU with DCX. Using a BrdU labeling paradigm (supplemental Fig. 2, available at www.jneurosci.org as supplemental material) that did not involve a washout following BrdU administration to drug-naive animals, we carried out a short-duration (7 d) treatment with the α_2-adrenoceptor agonist, guanabenz. BrdU-DCX percentage colocalization was unaltered following guanabenz treatment (supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

α_2-Adrenoceptor blockade accelerates antidepressant–induced adult hippocampal neurogenesis

To assess whether α_2-adrenoceptor blockade accelerates the effects of antidepressant drugs on adult hippocampal progenitor proliferation, we examined the influence of combined treatment (7 or 21 d) with the α_2-adrenoceptor antagonist yohimbine and the tricyclic antidepressant imipramine on BrdU-positive cell number in the SGZ/GCL (Fig. 5*A*). At 21 d, a time-point when antidepressant treatments have been previously reported to enhance BrdU-positive cell number (Malberg et al., 2000, Santarelli et al., 2003), we observed a significant increase in the number of BrdU-positive cells in both the group that received only imipramine (Fig. 5*F*,*G*) and the group that received combined yohimbine and imipramine treatment (Fig. 5*F*,*G*). Two-way ANOVA analysis of the 21 d treatment experiment indicated no significant interaction of yohimbine × imipramine ($F_{(1,12)} = 1.22, p = 0.29$). In contrast, only the combined yohimbine- and imipramine-treated group showed a significant increase in the number of BrdU-positive cells in the SGZ at the 7 d time point (Fig. 5*B*,*C*). Two-way ANOVA revealed a significant yohimbine × imipramine interaction in the 7 d treatment experiment ($F_{(1,20)} = 20.99$, $p = 0.0002$). Imipramine treatment by itself for 7 d showed no significant effect on hippocampal progenitor proliferation (Fig. 5*C*). These results indicate that coadministration of yohimbine with imipramine shortens the duration of antidepressant treatment required to increase adult hippocampal progenitor proliferation.

We also examined the effects of the above combined treatments for 7 or 21 d on the number of DCX-positive immature neurons in the SGZ/GCL. At 21 d, both the imipramine and combined yohimbine and imipramine treatment groups showed a significant increase in the number of DCX-positive cells/section (Fig. 5*H*,*I*). Two-way ANOVA revealed no significant yohimbine × imipramine interaction at 21 d ($F_{(1,20)} = 0.69, p = 0.42$). At 7 d, we did not observe a change in DCX-positive cell numbers in any of the groups (Fig. 5*D*,*E*). Although a change in DCX-positive cell number was not seen at 7 d after combined treatment, 14 d of yohimbine and imipramine coadministration resulted in a significant increase in DCX-positive cell number (14 day combined yohimbine + imipramine experiment, number of DCX-positive cells/section: control = 153.60 ± 16.02, yohimbine = 154.43 ± 11.57, imipramine = 154.60 ± 10.63, yohimbine + imipramine = 225.20 ± 14.96). Two-way ANOVA analysis indicated a significant yohimbine × imipramine inter-

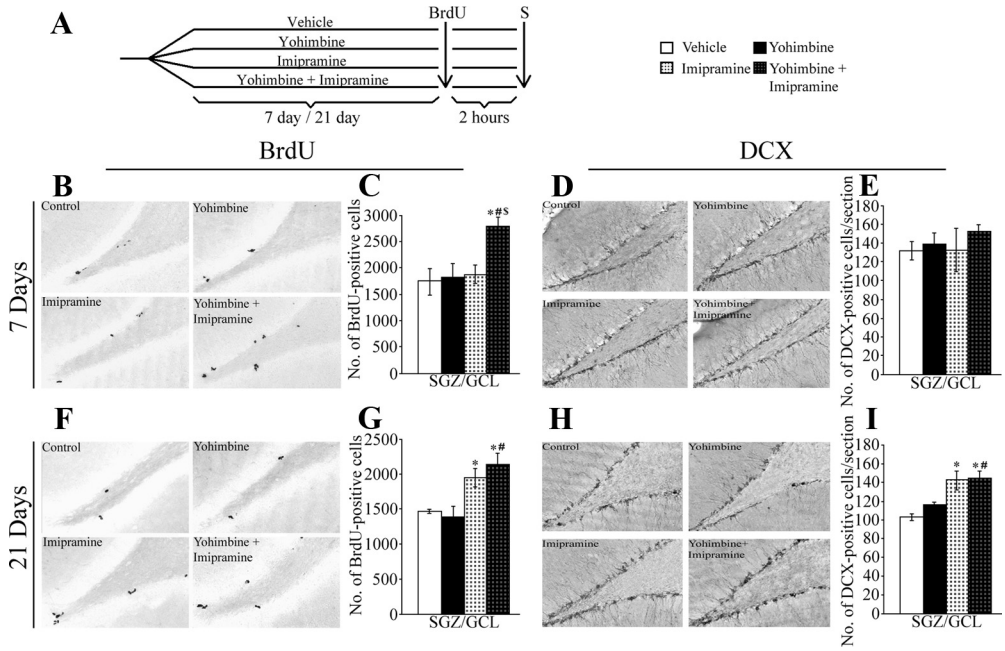

Figure 5. α_2-Adrenoceptor blockade results in faster effects of the antidepressant imipramine on adult hippocampal progenitor proliferation. Rats received combined treatment with the α_2-adrenoceptor antagonist, yohimbine, and the antidepressant imipramine for 7 or 21 d as described in Materials and Methods. ***A***, Shown is a schematic representation of the experimental design (S-time point for kill). Influence of 7 or 21 d combined yohimbine and imipramine treatments on adult hippocampal neurogenesis was assessed using the mitotic marker BrdU to determine effects on proliferation, and DCX to determine effects on immature neuron number. ***C***, Quantitative analysis revealed a significant increase in BrdU-positive cell number in the SGZ/GCL of animals that received short-duration 7 d combined treatments with yohimbine and imipramine. Imipramine treatment by itself for 7 d did not alter BrdU-positive cell number in the SGZ/GCL (***C***). ***G***, Twenty-one day treatment with imipramine, or with combined yohimbine and imipramine, resulted in a significant increase in BrdU-positive cell number in the SGZ/GCL. ***B***, ***F***, Shown are representative photomicrographs of BrdU-positive cells from control-, yohimbine-, imipramine-, and yohimbine + imipramine-treated groups from the 7 d (***B***) and 21 d (***F***) experiments. ***E***, ***I***, While in the 7 d experiment the number of DCX-positive cells per section was not altered in any of the groups (***E***), in the 21 d experiment imipramine, as well as combined yohimbine and imipramine, treated groups showed a significant increase in DCX-positive cells per section as compared to control (***I***). ***D***, ***H***, Shown are representative photomicrographs of DCX-positive cells from control-, yohimbine-, imipramine-, and yohimbine + imipramine-treated groups from the 7 d (***D***) and 21 d (***H***) experiments. The results are expressed as the mean ± SEM number of BrdU-positive cells per SGZ/GCL, or the mean ± SEM. Number of DCX-positive cells per section ($n = 10$/group). $^*p < 0.05$ compared with control, $^{\#}p < 0.05$ compared with yohimbine, $^{\$}p < 0.05$ compared with imipramine (ANOVA and Bonferroni *post hoc* test).

action effect ($F_{(1,19)} = 11.51$, $p = 0.0031$) in the 14 d combined treatment experiment. The 14 d imipramine only treatment group did not show any change in DCX-positive cell number (Bonferroni *post hoc* test, $p > 0.05$). These results suggest that coadministration of yohimbine hastens the increase in immature neuron generation observed with imipramine treatment.

We next assessed whether short-duration (7 d) combined yohimbine and imipramine treatment influenced the morphological maturation of DCX-positive immature neurons. DCX-positive neurons were subdivided into (1) those without tertiary dendrites (Fig. 6*A*), and (2) those bearing tertiary dendrites (Fig. 6*B*), as described previously (Wang et al., 2008). Combined treatment with yohimbine and imipramine for 7 d significantly enhanced the number of DCX-positive cells bearing complex, tertiary dendrites (Fig. 6*C*). Individual yohimbine or imipramine treatment had no significant effect on the morphology of DCX-positive cells (Fig. 6*C*). Two-way ANOVA indicated a significant yohimbine × imipramine interaction ($F_{(1,15)} = 7.52$, $p = 0.0151$). This effect was seen although no changes in DCX-positive cell number were observed with 7 d combined treatment (Fig. 5*D*,*E*), suggesting a shift in distribution of DCX-positive cells to those bearing a more complex morphology. Previous studies indicate that chronic (28 d), but not subchronic (5 d), antidepressant treatment enhances the dendritic maturation of immature newborn neurons (Wang et al., 2008). Our results indicate that combined yohimbine and imipramine administration shifts the effects of antidepressant treatment on morphological maturation to an earlier onset.

We then addressed whether the expression of NeuroD, a neurogenic transcription factor required for the survival and maturation of hippocampal progenitors (Gao et al., 2009), is altered in the SGZ following short-duration yohimbine and imipramine treatment. The combined yohimbine- and imipramine-treated group showed a significant increase in the number of NeuroD-positive cells/section in the SGZ at the 7 d time point (Fig. 6*D*,*E*). Two-way ANOVA revealed a significant yohimbine × imipramine interaction ($F_{(1,15)} = 20.25$, $p = 0.0004$). Imipramine treatment by itself showed no significant effect on NeuroD-positive cell number (Fig. 6*E*). A previous study indicates that chronic (14 d), but not subchronic (5 d), antidepressant treatment enhances NeuroD-positive cell number in the SGZ/GCL (Larsen et al., 2007). Our results demonstrate that adjunct α_2-adrenoceptor blockade accelerates the effects of imipramine on NeuroD-positive progenitor number, and thus indicates that the increased numbers of hippocampal progenitors observed following combined treatment exhibit neuronal commitment.

To address whether short-duration yohimbine and imipramine treatment influences the developmental progression of newborn neurons we examined the percentage colocalization of BrdU-positive cells with calretinin, a transient marker of immature neurons (Brandt et al., 2003). Drug-naive animals were subjected to a BrdU labeling paradigm (supplemental Fig. 3, available at www.jneurosci.org as supplemental material), following which they received 7 d combined yohimbine and imipramine treatment. Confocal analysis indicated a significant increase in the percentage BrdU/calretinin colocalization in the

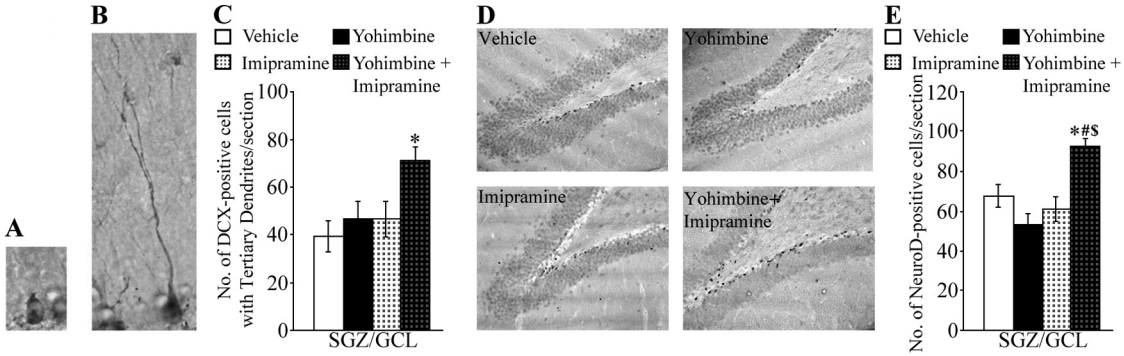

Figure 6. α_2-Adrenoceptor blockade accelerates the effect of the antidepressant imipramine on the morphological maturation of DCX-positive cells and the number of NeuroD-positive progenitors. Rats received combined treatment with the α_2-adrenoceptor antagonist, yohimbine and the antidepressant, imipramine for 7 d as described in Materials and Methods. The effect of combination treatment on the morphological maturation of newborn neurons was assessed by counting the number of DCX immunopositive cells bearing a complex dendritic morphology. ***A***, ***B***, Shown are representative photomicrographs of a DCX-positive cell without tertiary dendrites (***A***) and a cell with tertiary dendrites (***B***). ***C***, Quantitative analysis revealed a significant increase in the number of DCX-positive cells with tertiary dendrites/section in the SGZ/GCL of yohimbine + imipramine-treated animals compared with vehicle-treated controls. The results are expressed as the mean ± SEM number of DCX-positive cells with tertiary dendrites per section ($n = 4$–5/group). ***D***, Influence of 7 d combined yohimbine and imipramine treatment on the number of NeuroD-positive progenitors was assessed using NeuroD immunohistochemistry. Shown are representative photomicrographs of NeuroD-positive cells within the SGZ/GCL of vehicle-, yohimbine-, imipramine-, and yohimbine + imipramine-treated groups. ***E***, Quantitative analysis revealed a significant increase in the number of NeuroD-positive cells/section in the combined yohimbine and imipramine group, with no change observed following individual treatments with yohimbine or imipramine. The results are expressed as the mean ± SEM. Number of NeuroD-positive cells per section ($n = 5$/group). $^{*}p < 0.05$ compared with control, $^{\#}p < 0.05$ compared with yohimbine, $^{\$}p < 0.05$ compared with imipramine (ANOVA and Bonferroni *post hoc* test).

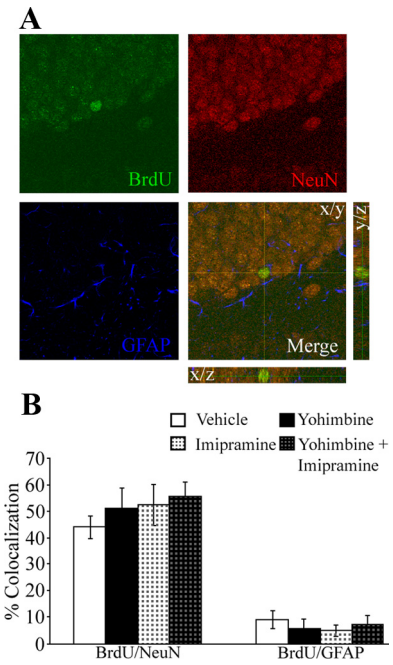

Figure 7. Combined α_2-adrenoceptor antagonist, yohimbine and antidepressant, imipramine treatment for 7 d results in an increase in adult hippocampal neurogenesis. Rats received BrdU at the end of the 7 d of combined treatment with yohimbine and imipramine, and were killed 28 d later as described in Materials and Methods. ***B***, Confocal *z*-stack analysis confirmed that BrdU-positive cells differentiate primarily into NeuN-immunopositive neurons. Quantitative analysis indicated no difference in the percentage colocalization of BrdU with NeuN or GFAP across the treatment groups. ***A***, Shown are confocal *z*-stack images of a BrdU-positive cell colocalizing with NeuN. The merged image demonstrates the colocalization of BrdU with NeuN. The results are expressed as the mean ± SEM percentage colocalization of BrdU-positive cells with NeuN or GFAP in the SGZ/GCL ($n = 4$/group).

combined yohimbine and imipramine treatment group, with no BrdU/calretinin colocalization observed in vehicle, yohimbine or imipramine treatment groups at this time point (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). Two-way ANOVA indicated a significant yohimbine × imipramine interaction ($F_{(1,16)} = 6.77$, $p = 0.0019$). Together, the influence of combined yohimbine and imipramine treatment on DCX morphology, NeuroD-positive cell number and BrdU/calretinin colocalization suggest that combination treatment influences the differentiation of newborn neurons within the adult hippocampal neurogenic niche.

To confirm whether the increase in BrdU-positive cell number that is observed with short-duration (7 d) combined yohimbine and imipramine treatment results in eventual differentiation into neurons or glia, we carried out triple immunolabeling with BrdU, NeuN and GFAP. Confocal analysis revealed that most of the BrdU-positive cells 4 weeks later had differentiated into neurons with few taking on a glial phenotype. The percentage distribution of BrdU cells that acquire a neuronal or glial fate was not altered across all the treatment groups at this time point (Fig. 7*A*,*B*).

Short-duration combined yohimbine and imipramine treatment enhances hippocampal gene expression of BDNF, VEGF, FGF-2, VGF, and Arc

As the next step we addressed whether short-duration (7 d) combined yohimbine and imipramine treatment is sufficient to increase the hippocampal expression of trophic factors like BDNF, VEGF, and FGF-2 that are known to be enhanced only following longer duration (2–3 weeks) antidepressant treatment (Nibuya et al., 1995, Dias et al., 2003; Warner-Schmidt and Duman, 2007; Bachis et al., 2008). We also examined the influence of this treatment on the gene expression of the neuropeptide VGF, the immediate early gene Arc and the transcription factor CREB, which are regulated by chronic antidepressant treatment and contribute to antidepressant efficacy (Nibuya et al., 1996; Pei et al., 2003; Thakker-Varia et al., 2007; Molteni et al., 2008). We found that combined yohimbine and imipramine administration for 7 d resulted in a robust and significant increase (176%) in BDNF mRNA in the dentate gyrus (DG) (Fig. 8*A*,*B*). Treatment with only yohimbine or imipramine for 7 d did not alter BDNF mRNA expression in the DG. Two-way ANOVA indicated a significant yohimbine × imipramine interaction ($F_{(1,16)} = 880.36$, $p < 0.0001$). Seven day combination treatment also significantly induced the mRNA expression of VEGF (79%) (Fig. 8*C*,*D*) and

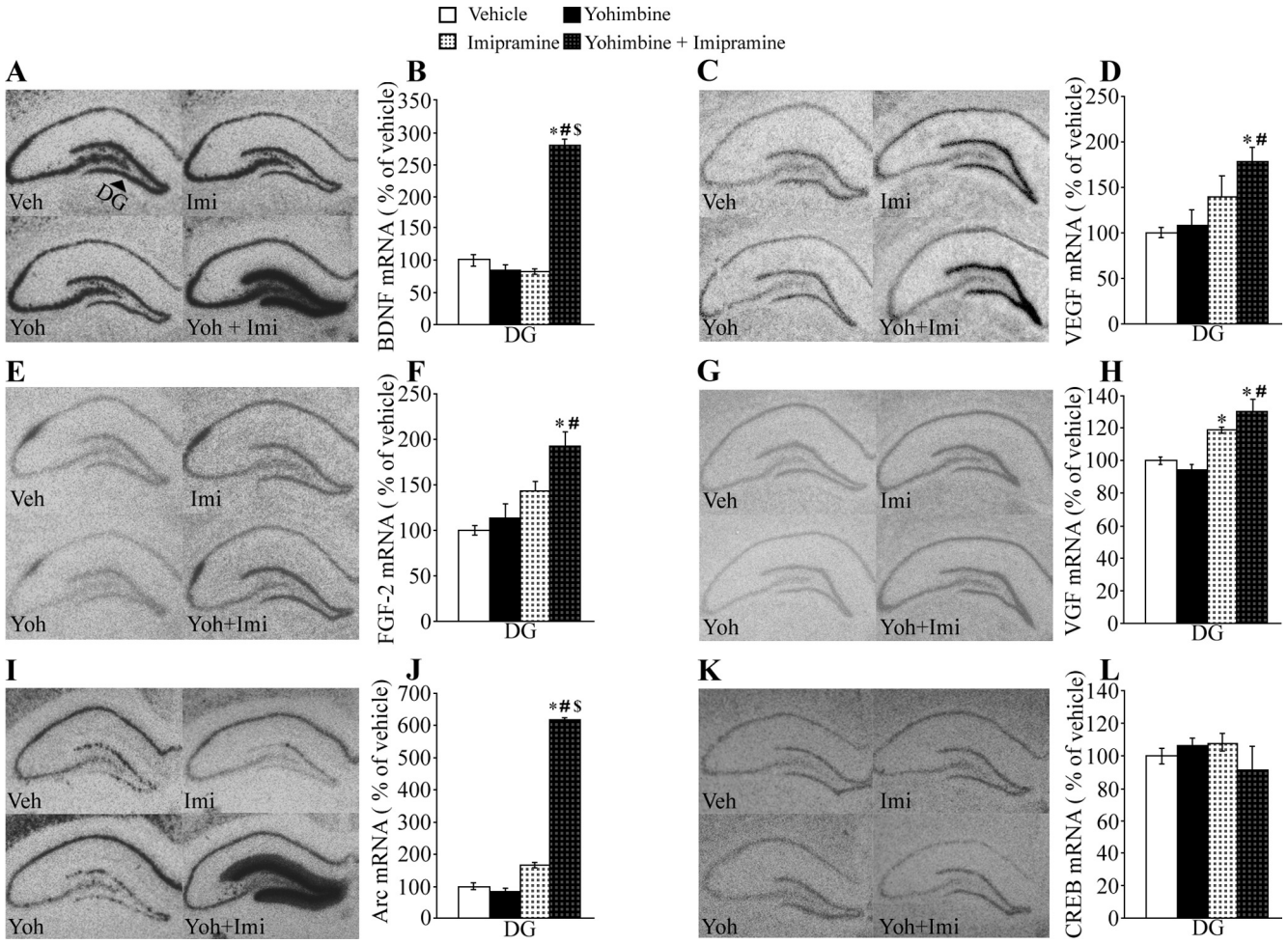

Figure 8. Short-duration combined yohimbine and imipramine treatment for 7 d upregulates the mRNA expression of plasticity associated genes like BDNF, VEGF, FGF-2, VGF, and Arc in the dentate gyrus. Rats received treatment with the α_2-adrenoceptor antagonist, yohimbine and the antidepressant, imipramine for 7 d before kill and processing for *in situ* hybridization as described in Materials and Methods. *In situ* hybridization for BDNF, VEGF, FGF-2, VGF, Arc, and CREB transcripts was performed using specific riboprobes and transcript levels in the hippocampal DG subfield were quantified using densitometric analysis. ***A***, ***C***, ***E***, ***G***, ***I***, ***K***, Representative autoradiograms of hippocampal sections from vehicle-, yohimbine-, imipramine-, and yohimbine + imipramine-treated animals are shown for BDNF (***A***), VEGF (***C***), FGF-2 (***E***), VGF (***G***), Arc (***I***) and CREB (***K***) mRNA. Arrowhead indicates the DG (***A***). Levels of mRNA in the DG hippocampal subfield are shown in the bar graphs next to the representative autoradiograms. ***B***, ***D***, ***F***, ***H***, ***J***, Levels of BDNF (***B***), VEGF (***D***), FGF-2 (***F***), VGF (***H***) and Arc (***J***) mRNA in the DG were significantly increased following 7 d combined yohimbine + imipramine treatment compared with vehicle-treated controls. ***K***, ***L***, CREB mRNA levels were unchanged following combination treatment with yohimbine and imipramine. Results are expressed as percentage of vehicle and are the mean ± SEM ($n = 4$/group). $^*p < 0.05$ compared with vehicle, $^{\#}p < 0.05$ compared with yohimbine, $^{\$}p < 0.05$ compared with imipramine (ANOVA and Bonferroni *post hoc* test).

FGF-2 (77%) (Fig. 8*E*,*F*) in the DG. Yohimbine or imipramine treatments by themselves for 7 d did not alter VEGF or FGF-2 mRNA expression in the DG significantly. However, the two-way ANOVA analysis of the VEGF and FGF-2 data indicated no significant yohimbine × imipramine interaction (VEGF: $F_{(1,13)} = 0.66$, $p = 0.43$; FGF-2: $F_{(1,12)} = 2.09$, $p = 0.17$).

Furthermore, the short-duration (7 d) combined treatment of yohimbine and imipramine significantly upregulated (30%) the mRNA expression of the neuropeptide VGF in the DG (Fig. 8*G*,*H*). Seven day of treatment with imipramine alone was also sufficient to induce a significant increase (19%) in VGF expression. Two-way ANOVA analysis of a yohimbine × imipramine interaction indicated a trend toward significance ($F_{(1,12)} = 3.89$, $p = 0.0721$). Interestingly, 7 d combined yohimbine and imipramine treatment also resulted in a highly robust increase (~5-fold) in the mRNA expression of the immediate early gene Arc within the DG (Fig. 8*I*,*J*). No change in Arc mRNA levels was observed with individual yohimbine or imipramine treatment for 7 d. Two-way ANOVA revealed a significant yohimbine × imipramine interaction ($F_{(1,16)} = 6894.33$, $p < 0.0001$). In contrast to the effects seen with trophic factors, the neuropeptide VGF and the immediate early gene Arc, combined yohimbine and imipramine treatment did not alter CREB mRNA expression in the DG (Fig. 8*K*,*L*).

To address whether short-duration (7 d) combined yohimbine and imipramine treatment changes hippocampal norepinephrine levels we carried out HPLC analysis. Hippocampal levels of norepinephrine in all treatment groups were found to be indistinguishable from vehicle-treated controls [level of NE (ng/g tissue weight) in the hippocampus: vehicle = 303.57 ± 23.35, yohimbine = 256.76 ± 10.99, imipramine = 254.22 ± 13.68, yohimbine + imipramine = 257.30 ± 16.54].

α_2-Adrenoceptor blockade accelerates the behavioral effects of the antidepressant imipramine in the novelty suppressed feeding test

Given the effects of short-duration (7 d) combined yohimbine and imipramine treatment on hippocampal neurogenesis and

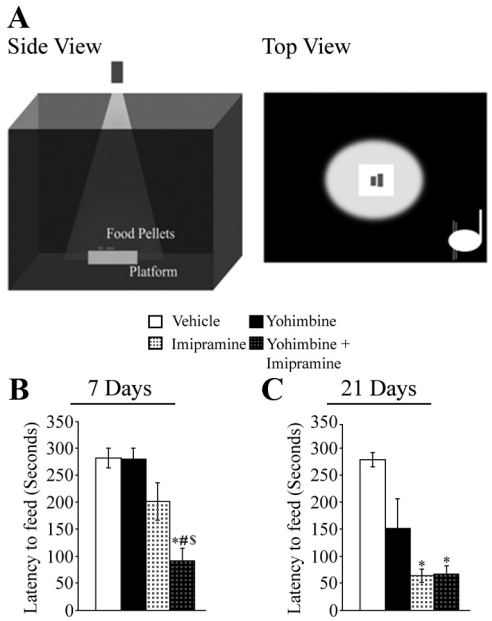

Figure 9. α_2-Adrenoceptor blockade hastens the effects of the antidepressant imipramine on behavior in the NSF test. Rats received combined treatment with the α_2-adrenoceptor antagonist, yohimbine and the antidepressant imipramine for 7 or 21 d, and behavior was assessed using the NSF test as described in Materials and Methods. Antidepressants require chronic (2–3 weeks) administration to exhibit behavioral effects on the NSF test. ***A***, Shown is a schematic illustration of the side, and top, views of an NSF arena in which food pellets are placed in the center on an illuminated platform and latency to feed is measured. ***C***, Administration of imipramine or combined yohimbine + imipramine treatment for 21 d resulted a significant reduction in the latency to feed in the NSF test. Twenty-one day yohimbine treatment did not result in a significant effect on the NSF test. ***B***, A reduced latency to feed was observed in the short-duration (7 d) experiment only in the combined yohimbine + imipramine treatment group. The results are expressed as the mean ± SEM latency to feed in seconds ($n = 10$/group). $^{*}p < 0.05$ compared with vehicle, $^{\#}p < 0.05$ compared with yohimbine, $^{\$}p < 0.05$ compared with imipramine (ANOVA and Bonferroni *post hoc* test).

gene expression of plasticity associated genes, we asked whether this short-duration combined treatment is also capable of inducing a behavioral response in the NSF test (Fig. 9*A*) that has been previously shown to be responsive to chronic (2–3 weeks), but not acute, antidepressant administration (Bodnoff et al., 1988; Santarelli et al., 2003). Following 21 d of treatment, we observed a significant reduction in the latency to feed in both the group that received just the antidepressant, imipramine, as well as the group that received the combination treatment with yohimbine and imipramine, with no significant effect of only yohimbine treatment (Fig. 9*C*). In contrast, we observed a significant reduction in the latency to feed only in the group that received a combination treatment with both yohimbine and imipramine in the short (7 d) duration experiment (Fig. 9*B*). Two-way ANOVA analysis of the 7 d experiment revealed a significant yohimbine × imipramine interaction ($F_{(1,36)} = 4.93$, $p = 0.0328$). To control for effects of drug treatments on appetite, we also assessed home cage food consumption immediately following the NSF test. Food consumption in the home-cage was found to be unaltered across all the treatment groups [home cage food consumption—7 d experiment (g/30 min): vehicle = 5.14 ± 0.34, yohimbine = 5.28 ± 0.25, imipramine = 4.43 ± 0.62, yohimbine + imipramine = 4.54 ± 0.80]. Together, these results suggest that a short-duration (7 d) combination treatment with yohimbine and imipramine reduces the time lag to observe a behavioral response in the NSF test.

Discussion

The novel findings of our study are that α_2-adrenoceptor stimulation decreases adult hippocampal neurogenesis, while simultaneous α_2-adrenoceptor blockade and antidepressant treatment hastens effects on hippocampal neurogenesis, plasticity-associated gene expression, and depression-related behavior. Our results highlight the importance of α_2-adrenoceptors as targets for rapid-action antidepressants.

Adult hippocampal neurogenesis encompasses progenitor proliferation, survival, and differentiation resulting in newborn neuron integration into hippocampal circuitry (Ming and Song, 2005). The hippocampal neurogenic niche receives dense noradrenergic innervation, expresses all the different classes of adrenoceptors, and hippocampal progenitor proliferation is stimulated by norepinephrine (Nicholas et al., 1996; Kulkarni et al., 2002) (D. Jhaveri and P. Bartlett, personal communication). Paradoxically, we found that α_2-adrenoceptor stimulation *in vivo* decreases hippocampal progenitor proliferation. α_2-Adrenoceptors exist both as heteroceptors on target neurons receiving noradrenergic innervation, and as α_2-autoreceptors that decrease norepinephrine release. Our *in vitro* and *in vivo* findings support a role for α_2-heteroceptors in mediating the effects on adult hippocampal progenitor turnover. First, we provide evidence that dispersed adult hippocampal progenitors in culture express the α_2-adrenoceptor subtypes, and exhibit decreased BrdU-incorporation following α_2-adrenoceptor stimulation. Second, α_2-adrenoceptor agonists significantly reduce neurosphere frequency in the neurosphere assay indicating a direct effect on the turnover of adult neural stem cells. Finally, our *in vivo* finding that this decreased proliferation persists in $Dbh^{-/-}$ mice indicates an effect on hippocampal progenitor proliferation via α_2-heteroceptors. While α_2-adrenoceptor stimulation selectively influences the proliferation, but not the survival or differentiation of hippocampal progenitors, α_2-adrenoceptor blockade does not influence hippocampal neurogenesis. Our results differ from a previous study (Rizk et al., 2006) that reported enhanced progenitor survival following long duration treatment with the α_2-adrenoceptor antagonist dexefaroxan. This discrepancy may be a consequence of differences in drug choice, dose, route of administration or treatment duration. We also did not observe any change in progenitor proliferation or immature neuron number in α_{2A}-, α_{2C}-, and α_{2AC}-adrenoceptor knock-out mice. Together, our results indicate that α_2-adrenoceptors exert a stimulation-induced, but not a basal tonic, inhibitory effect on adult hippocampal progenitor turnover.

Interestingly, α_2-adrenoceptors are expressed within proliferative germinal zones, and their stimulation decreases DNA synthesis in the developing forebrain (Lidow and Rakic, 1994; Kreider et al., 2004) suggesting possible common mechanisms in the developing and adult brain. Given that cAMP–CREB signaling enhances adult hippocampal neurogenesis (Nakagawa et al., 2002), α_2-adrenoceptor activation may decrease progenitor turnover through a G_i-mediated inhibition of adenylate cyclase. The mechanistic underpinnings of the effects of α_2-adrenoceptors on adult hippocampal progenitors warrant further investigation.

Hippocampal volumetric loss is observed in recurrent major depression, and decreased neurogenesis reported in animal models of depression (Campbell et al., 2004; Sahay and Hen, 2007). Enhanced α_2-adrenoceptor density and mRNA expression within limbic brain regions is observed in animal models of depression (Ribas et al., 2001; Flügge et al., 2003). Furthermore, increased hippocampal α_2-adrenoceptor binding (Meana et al.,

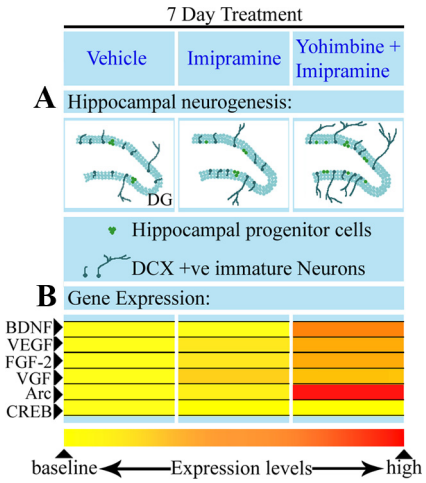

Figure 10. Short-duration (7 d) combined treatment with the α_2-adrenoceptor antagonist, yohimbine and the antidepressant, imipramine treatment hastens the effects of antidepressant treatment on adult hippocampal progenitor proliferation, morphological maturation of newborn neurons and the expression of plasticity-associated genes. ***A***, Shown in the illustration are the effects of combined yohimbine and imipramine treatment on adult hippocampal neurogenesis. The short-duration combined treatment results in an increased proliferation of adult hippocampal progenitors, whereas treatment with the antidepressant imipramine alone does not alter hippocampal progenitor proliferation. While the combination treatment for 7 d does not increase DCX-positive cell number, it significantly shifts the ratio of DCX-positive cells toward those bearing a more complex dendritic morphology indicating an effect of yohimbine + imipramine treatment on the morphological maturation of immature neurons. ***B***, Shown in the illustration are the effects of combined yohimbine and imipramine treatment on the mRNA expression of trophic factors like BDNF, VEGF, and FGF-2 and plasticity-associated transcripts like Arc, VGF, and CREB. The combination treatment significantly enhances the expression of BDNF, VEGF, FGF-2, VGF and Arc in the DG subfield of the hippocampus. Effects on mRNA expression levels are schematized based on the color scale (not to exact scale).

1992; González et al., 1994) and prefrontal α_{2A}-adrenoceptor immunoreactivity (García-Sevilla et al., 1999) have been observed in postmortem studies of major depression. While it remains uncertain whether the changes in α_2-adrenoceptor binding reflect alterations in terminal α_2-autoreceptors or α_2-heteroceptors in target fields, the mRNA expression changes indicate enhanced expression of α_2-heteroceptors in limbic neurocircuitry following chronic stress. Our results suggest a possible role for α_2-heteroceptors in the neurogenic decline observed in animal models of depression.

The "neurotrophic" and "neurogenic" hypotheses of depression posit a role for decreased trophic support and neurogenesis in the hippocampal damage observed in depression, and a contribution of antidepressant-induced trophic factor expression and hippocampal neurogenesis to their therapeutic action (Sahay and Hen, 2007; Schmidt and Duman, 2007). It is noteworthy that the neurotrophic and neurogenic changes exhibit a slow onset, and parallel the time course required for the behavioral benefits of sustained antidepressant administration (Sahay and Hen, 2007; Schmidt and Duman, 2007). One of our most interesting and novel findings is that α_2-adrenoceptor blockade accelerates the effects of chronic antidepressant treatment on hippocampal neurogenesis and trophic factor gene expression associated with faster behavioral effects (Fig. 10).

Combined 7 d yohimbine and imipramine (Y+I) treatment significantly improved behavioral outcome in the NSF test, which is normally sensitive only to longer periods (3 weeks) of antidepressant treatment (Santarelli et al., 2003). Y+I treatment also increased hippocampal progenitor proliferation and NeuroD-positive neuroblast number within 7 d, an effect not observed with imipramine treatment alone. Furthermore, a striking shift in the distribution of DCX-positive cells to those bearing complex, tertiary dendritic morphology was observed in the 7 d Y+I group. Enhanced BrdU/calretinin percentage colocalization observed at 7 d in the Y+I group suggests an influence on the developmental progression of immature neurons. Together, our results indicate that simultaneous α_2-adrenoceptor blockade hastens effects of antidepressant treatment on distinct aspects of adult hippocampal neurogenesis.

It is unlikely that increased progenitor proliferation contributes to the behavioral effects observed with 7 d Y+I treatment; rather our observation of effects on the morphological complexity of DCX-positive cells suggest that the integration of immature neurons into hippocampal neurocircuitry may be accelerated. It is tempting to speculate that this component of the neurogenic effects of 7 d Y+I treatment may contribute to the rapid rate of behavioral response. Future experiments are required to address whether 7 d Y+I treatment accelerates the differentiation, integration and recruitment of newborn neurons into hippocampal circuitry. Interestingly, 21 d Y+I treatment increased progenitor proliferation and DCX-positive cell number to a similar extent as imipramine treatment alone, suggesting that combined treatment may largely involve acceleration, rather than augmentation, of the neurogenic effects of imipramine.

Seven day Y+I treatment increased BDNF, VEGF, and FGF-2 mRNA expression in the DG subfield, an effect not observed with imipramine treatment. The combined treatment also enhanced VGF expression, an effect also observed with imipramine treatment. The effects of Y+I do not appear simply additive, and suggest the possible recruitment of distinct signaling mechanisms. The robust increase in the expression of the activity-dependent gene Arc (Bramham et al., 2008) in the DG subfield indicates enhanced neuronal activity following Y+I treatment. It is interesting to note that enhanced DG activity as measured by voltage-sensitive dye imaging has been previously strongly linked to antidepressant-induced neurogenic and behavioral responses (Airan et al., 2007). Neuronal activity is known to recruit latent stem/progenitor cells in the adult hippocampus, and enhance trophic factor expression (Gall and Lauterborn, 1992; Walker et al., 2008). Trophic factors could serve to link neuronal activity to structural plasticity in the hippocampal neurogenic niche. Our results also raise the strong possibility that non-cell autonomous effects via enhanced neuronal activity and trophic factor release may also contribute to the neurogenic effects of Y+I treatment. Furthermore, BDNF, FGF-2, VEGF, and VGF are implicated as key mediators of both the neurogenic and behavioral effects of antidepressants (Palmer et al., 1999; Warner-Schmidt et al., 2007; Bachis et al., 2008; Li et al., 2008; Thakker-Varia et al., 2007; Perez et al., 2009), and may play a critical role in mediating faster effects on neurogenesis and behavior with 7 d Y+I treatment.

Previous clinical literature has suggested the coadministration of α_2-adrenoceptor antagonists with antidepressants as a therapeutic strategy to hasten antidepressant responses (Blier, 2003). The mode of action for these faster effects has been unclear. Our results suggest that adjunct α_2-adrenoceptor antagonists accelerate the effects of antidepressant treatment on the slow-onset adaptive changes in neurogenesis and trophic factor expression that are thought to underlie the behavioral effects of antidepressants. Our findings support the view that structural remodeling and enhanced trophic support may be key events determining the latency in onset of antidepressant action. At present, we cannot distinguish whether blockade of α_2-autoreceptors or heteroceptors, or a combination of both, contribute to the faster effects of

Y+I treatment. It has been suggested that concomitant blockade of inhibitory presynaptic α_2-autoreceptors would increase noradrenergic neurotransmission, whereas in the absence of such a blockade a desensitization/downregulation of presynaptic α_2-adrenoceptors would have to precede an increase in noradrenergic neurotransmission, thus contributing to the delay in antidepressant action (Blier, 2003). It is possible that we observed faster effects with Y+I treatment due to the blockade of inhibitory α_2-autoreceptors, or heteroceptors on serotonergic neurons, thus enhancing noradrenergic and serotonergic neurotransmission. Our failure to observe a change in hippocampal norepinephrine levels following 7 d Y+I treatment does not rule out possible autoreceptor effects, particularly since we measured tissue norepinephrine levels, which are not always correlated with extracellular norepinephrine that is available for signaling. Future studies using genetic perturbation approaches will be required to dissect out the contribution of α_2-autoreceptors and heteroceptors to the faster effects of adjunct α_2-adrenoceptor antagonist and antidepressant treatment.

In conclusion, we demonstrate that α_2-adrenoceptor stimulation decreases adult hippocampal neurogenesis, while concomitant α_2-adrenoceptor blockade accelerates the neurogenic, neurotrophic, and behavioral effects of chronic antidepressant treatment. The delayed onset of action of antidepressants is a major drawback for both treatment and compliance. Our results highlight the importance of α_2-adrenoceptors in modulating the speed of antidepressant response, and motivate future research in the development of rapid-action antidepressants that target the α_2-adrenoceptor.

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