

Chronic treatment with AMPA receptor potentiator Org 26576 increases neuronal cell proliferation and survival in adult rodent hippocampus

Xiaowei W. Su · Xiao-Yuan Li · Mounira Banasr ·
Ja Wook Koo · Mohammed Shahid · Brian Henry ·
Ronald S. Duman

Received: 20 April 2009 / Accepted: 16 June 2009 / Published online: 15 July 2009
© Springer-Verlag 2009

Abstract

Rationale Currently available antidepressants upregulate hippocampal neurogenesis and prefrontal gliogenesis after chronic administration, which could block or reverse the effects of stress. Allosteric α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor potentiators (ARPs), which have novel targets compared to current antidepressants, have been shown to have antidepressant properties in neurogenic and behavioral models.

Objectives This study analyzed the effect of the ARP Org 26576 on the proliferation, survival, and differentiation of neurons and glia in the hippocampus and prefrontal cortex of adult rats.

Materials and methods Male Sprague-Dawley rats received acute (single day) or chronic (21 day) twice-daily intraperitoneal injections of Org 26576 (1–10 mg/kg). Bromodeoxyuridine (BrdU) immunohistochemistry was conducted 24 h or 28 days after the last drug injection for the analysis of cell proliferation or survival, respectively. Confocal

immunofluorescence analysis was used to determine the phenotype of surviving cells.

Results Acute administration of Org 26576 did not increase neuronal cell proliferation. However, chronic administration of Org 26576 increased progenitor cell proliferation in dentate gyrus (~40%) and in prefrontal cortex (~35%) at the 10-mg/kg dosage. Cells born in response to chronic Org 26576 in dentate gyrus exhibited increased rates of survival (~30%) with the majority of surviving cells expressing a neuronal phenotype.

Conclusion Findings suggest that Org 26576 may have antidepressant properties, which may be attributed, in part, to upregulation of hippocampal neurogenesis and prefrontal cell proliferation.

Keywords Antidepressant · Gliogenesis · Prefrontal cortex

Introduction

Major depressive disorder (MDD) is a debilitating, potentially fatal illness with greater than 15% lifetime prevalence among the US population (Kessler et al. 2003). Depression currently ranks among the top ten causes of disability among the world population (Lopez et al. 2006) and is projected to rank among the top three causes of disability by 2020–2030 (Mathers and Loncar 2006). Currently available treatments have limited efficacy, with only 65% of people with depression showing some improvement after any of several antidepressant medications and only after many weeks or months of treatment (Nestler et al. 2002). Furthermore, research suggests that presently available chemical antidepressants, which all act upon the monoamine neurotransmitters, serve to modulate other neuro-

X. W. Su · X.-Y. Li · M. Banasr · R. S. Duman (✉)

Division of Molecular Psychiatry,
Abraham Ribicoff Research Facilities,
Connecticut Mental Health Center,
Yale University School of Medicine,
New Haven, CT 06508, USA
e-mail: ronald.duman@yale.edu

J. W. Koo

Department of Psychiatry, Mount Sinai School of Medicine,
New York City, NY 10029, USA

M. Shahid · B. Henry

Department of Pharmacology, Schering-Plough,
Newhouse,
Lanarkshire, UK

biological systems which may play a more primary role in depression (Heninger et al. 1996).

Recent research aimed at elucidating these systems has led to a neurotrophic hypothesis of depression (Duman and Monteggia 2006). This posits that stress induces atrophy and loss of neurons in certain brain regions which may be involved in the neurobiology of depression and that antidepressant treatment can reverse or block these effects (Duman and Monteggia 2006). Various classes of antidepressants increase levels of hippocampal neurogenesis, which is required for the behavioral effects of antidepressants in certain animal models of the disorder (Santarelli et al. 2003; see Warner-Schmidt and Duman 2007). Furthermore, loss of glia in the prefrontal cortex is implicated in depression (Banasr and Duman 2008), and gliogenesis in the same region may play a role in treatment response (Banasr and Duman 2007; Kodama et al. 2004).

The limited effectiveness as well as tolerability issues of current antidepressant medications argues for the development of new molecular approaches targeting novel mechanisms in ongoing drug discovery for the treatment of depression. A novel class of antidepressants is the positive allosteric modulator of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtype of glutamate receptors, referred to as AMPA receptor potentiators (ARPs). ARP administration has been reported to increase cell proliferation in limbic brain structures implicated in depression (Bai et al. 2003), to be effective in the forced-swim test and tail suspension test, two behavioral models of antidepressant efficacy (Bai et al. 2001), and to increase levels of brain-derived neurotrophic factor (BDNF) expression in neuronal culture (Legutko et al. 2001) in entorhinal/hippocampal slice cultures (Lauterborn et al. 2000) and in rat hippocampus in vivo (Mackowiak et al. 2002). Furthermore, fluoxetine, a classical antidepressant, has been found to alter AMPA receptor phosphorylation in a manner expected to increase AMPA receptor signaling (Bleakman et al. 2007). The precise mechanisms by which ARPs exert their effects in neurogenic and behavioral models of depression remain unclear; however, they are a promising class of compounds for novel antidepressant discovery (Mathew et al. 2008; Zarate and Manji 2008). The latter notion is partially supported by recent evidence suggesting that the antidepressant properties of ketamine may at least in part be mediated by AMPA receptor activation (Maeng et al. 2008).

The focus of the present study is to evaluate the effectiveness of the ARP Org 26576 (Erdemli et al. 2007; Jordan et al. 2005) on cell proliferation in limbic brain regions of rat brain. Because this compound was not designed to directly affect brain levels of the monoamine neurotransmitters, it represents a novel class that may have improved antidepressant efficacy or may be effective in treating a subset of patients who do not respond to traditional antidepressant treatment. Furthermore,

their novel pharmacologic properties suggest that ARPs may be effective in augmenting the action of existing antidepressants which act through monoamine neurotransmitter systems (Li et al. 2003). A growing body of evidence suggests that there is a correlation between hippocampal neurogenesis as well as prefrontal cortex gliogenesis and antidepressant treatment. The present study was conducted to determine whether the ARP Org 26576 would increase hippocampal cell proliferation, survival, and differentiation to neuronal phenotypes and whether it would also increase prefrontal cortex cell proliferation and survival.

Materials and methods

Animals Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) with initial weights of 230–250 g were used for experiments. Animals were housed, three per cage, under standard laboratory conditions (12-h light/dark cycle) and constant temperature (25°C) with ad libitum access to food and water. All procedures were in accordance with the National Institutes of Health guidelines for animal research and approved by the Yale Animal Care and Use Committee.

Drug administration protocols Dose selection for Org 26576 was based on maintaining consistency with doses showing in vivo activity (Erdemli et al. 2007; Jordan et al. 2005). For acute drug administration, rats were administered Org 26576 at 8:00 a.m. and 4:00 p.m. by intraperitoneal (IP) injection at 1, 3, or 10 mg/kg or given vehicle (Fig. 1a). Drug was dissolved in 5% mulgofen (a detergent which improves solubility) in 0.9% NaCl. For chronic drug administration, Org 26576 (10 mg/kg) or vehicle (5% mulgofen in 0.9% NaCl) was administered twice a day at 8:00 a.m. and 8:00 p.m. for 21 days (Fig. 1b). For the survival and differentiation study, Org 26576 (10 mg/kg) or vehicle was administered in exactly the same way for 21 days, after which rats were allowed to survive for 28 days (Fig. 1c). Animal weights were measured every 3 days in each experiment. There were no differences in body weight among the treatment groups.

Bromodeoxyuridine administration For proliferation studies, rats received two administrations of 150 mg/kg bromodeoxyuridine (BrdU; Sigma, St. Louis, MO, USA) by IP injection 30 and 150 min after the last drug injection, respectively. Twenty-four hours after the last BrdU injection, rats were anesthetized by 400 mg/kg chloral hydrate and transcardially perfused with 100 ml of 0.1 mol/l cold phosphate-buffered saline (PBS), followed by 200 ml of 4% cold paraformaldehyde plus PBS. For the survival and differentiation study, rats received 150 mg/kg BrdU once daily on each of the last 2 days of the 21-day drug administration and were then allowed to

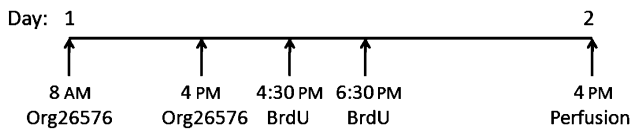
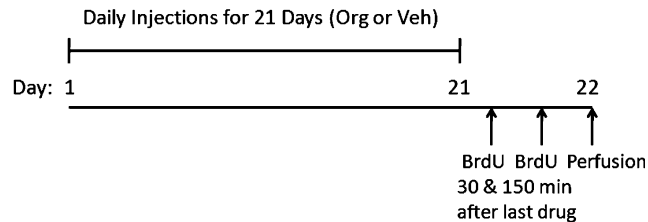
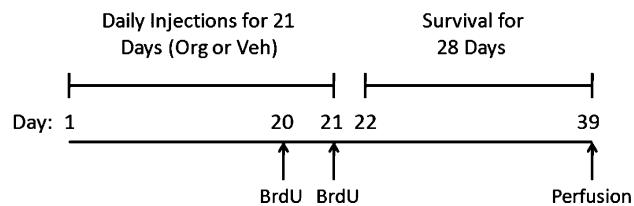
a. Acute Administration Study**b. Chronic Administration Study****c. Survival & Phenotype Study**

Fig. 1 Time schedule for drug and bromodeoxyuridine (BrdU) administration. **a** Acute Org 26576 administration paradigm. Animals received two injections, followed by BrdU, and perfusion 24 h later. **b** Chronic Org 26576 paradigm. Animals received two injections daily for 21 days, followed by BrdU and perfusion. **c** Survival and differentiation study paradigm. Animals received drug injections for 21 days with BrdU on days 20 and 21 and were allowed to survive for 28 days after the last BrdU injection

survive for 28 days after the last BrdU injection. Animals were then anesthetized and perfused exactly as described above.

Immunohistochemistry After perfusion, all brains were postfixed overnight in 4% paraformaldehyde in PBS at 4°C and cryoprotected in 30% sucrose at 4°C. Serial sections of the brains (40- μ m thickness) were cut on a freezing microtome, and sections were stored in a glycerol-based cryoprotectant. Free-floating sections were subjected to deoxyribonucleic acid denaturation by incubation for 2 h in 50% formamide/2 \times standard saline citrate (SSC) at 65°C, followed by a 2 \times SSC rinse. Sections were then incubated for 30 min in 2 N HCl, PBS, and then 10 min in 0.1 mol/l boric acid, pH 8.5. Sections were incubated for 30 min in 3% hydrogen peroxide to eliminate endogenous peroxidases. Sections were incubated with blocking buffer (3% normal horse serum in 0.3% Triton X-100, PBS) for 30 min. Then, sections were incubated with mouse anti-BrdU (1:100; Becton Dickinson, San Jose, CA, USA) with blocking buffer overnight at 4°C. Sections were then incubated for 1 h with biotinylated horse antimouse antibody (1:200; Vector Laboratories, Burlingame, CA, USA) with blocking buffer, followed by amplification with an avidin–biotin complex

(Vector Laboratories), and cells were visualized with diaminobenzidine (Vector Laboratories) with a light microscope (Zeiss Axioskop, Carl Zeiss, Jena, Germany).

For double labeling, brain sections were incubated for 30 min with 2 N HCl and PBS, followed by blocking buffer (3% normal goat serum, 0.3% Triton X-100, PBS). Sections were then incubated for 2 days at 4°C with rat anti-BrdU (1:100; Accurate; Harlan Olac, Bicester, UK) and mouse antineuronal nuclei (NeuN; 1:500; Chemicon, Billerica, MA, USA). The secondary antibodies were Alexa Fluor 488 goat antimouse (1:200) and Alexa Fluor 546 goat antirat (1:200; Molecular Probes, Carlsbad, CA, USA) and were applied for 1 h and visualized with confocal Z-plane sectioning by a Zeiss LSM 510 (Carl Zeiss).

Quantification of BrdU labeling An experimenter blinded to the identity of the sections counted BrdU-labeled cells. Stereo Investigator software (MBF Bioscience, Williston, VT, USA) was used for all counting procedures. To distinguish single cells within clusters, all counts were performed at $\times 400$ under a light microscope (Zeiss Axioskop, Carl Zeiss). For cell counting in the dentate gyrus, every tenth section throughout the whole hippocampus was processed for BrdU immunohistochemistry. A cell was counted as being in the subgranular zone (SGZ) of the dentate gyrus if it was touching or in the subgranular zone, which was defined as the cell layer located between the granule cell layer and the hilus. For prelimbic cortex, every tenth section was collected and immunostained through 4.20- to 2.20-mm bregma with reference to the Paxinos and Watson (1998) Rat Atlas, resulting in five sections for each brain examined. For cell counting in the prelimbic cortex, a 1 \times 1-mm square scale was used. All positive cells within the square scale were counted under $\times 400$ magnification.

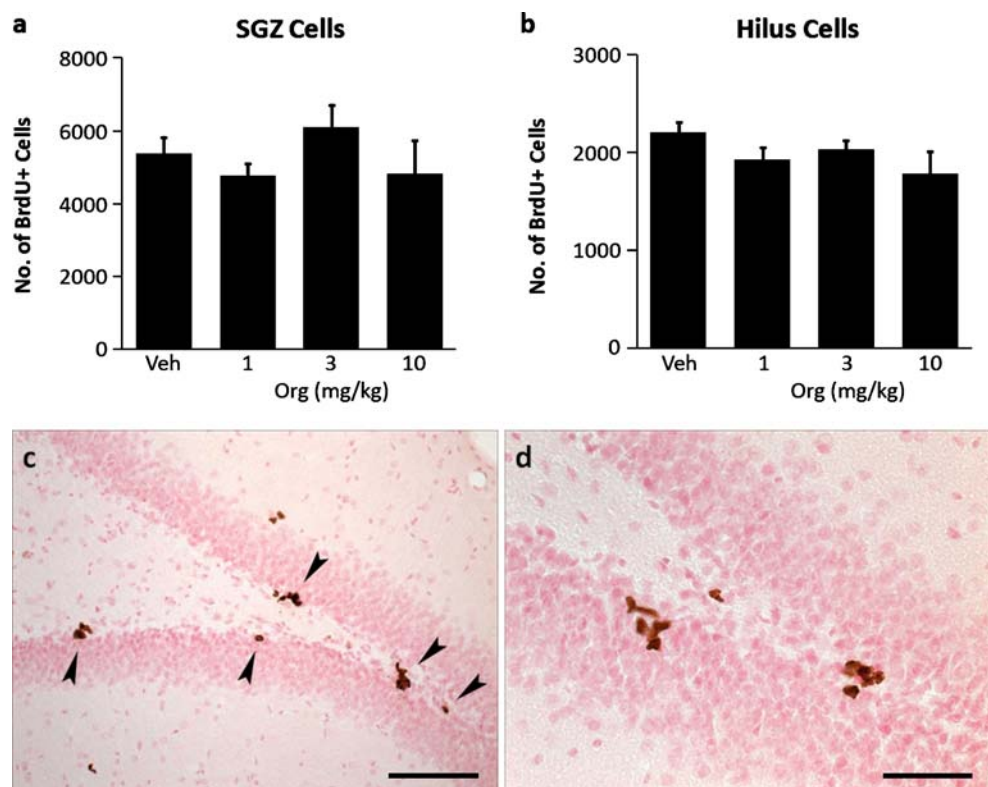
For double labeling, slices were analyzed on a confocal microscope (Zeiss Axiovert LSM510, Carl Zeiss). At least 50 dentate gyrus BrdU-positive cells per animal were analyzed with Z-plane sectioning (1- μ m steps) to confirm the colocalization of both BrdU and NeuN.

Statistical analysis The differences among groups were analyzed by one-way analysis of variance followed by the Fischer's protected least significant difference or unpaired *t* test, respectively; data were presented as mean \pm SEM. The level of statistical significance was set at $p < 0.05$. Statistical analysis was conducted using StatView 5 software (SAS, Cary, NC, USA).

Results

Acute administration of Org 26576 does not significantly affect cell proliferation in the hippocampus The effect of

Fig. 2 Effects of acute Org 26576 on cell proliferation in SGZ and hilus in rats. **a** Acute administration does not increase cell proliferation in SGZ ($n=6$ per group). **b** Acute administration does not affect hilus cell proliferation. **c** Representative bright-field image showing BrdU+ cells (black arrowheads) in the SGZ under the 3-mg/kg dosage ($\times 200$ magnification; scale bar, 100 μm). **d** BrdU+ clustered proliferating cells in SGZ ($\times 400$ magnification; scale bar, 50 μm)



acute Org 26576 administration is shown in Fig. 2a, b. Representative sections of BrdU immunohistochemistry in the 3-mg/kg Org 26576 group for the dentate gyrus and hilus are shown in Fig. 2c, d. BrdU-positive cells are primarily found along the subgranular zone (Fig. 2c). BrdU-positive cells are often observed as clusters of three or more adjacent cells (Fig. 2d). None of the doses tested significantly increased the number of BrdU-positive cells in the dentate gyrus compared with the vehicle group (Fig. 2a). There was no significant effect of Org 26576 on BrdU-positive cells in the hilus (Fig. 2b).

Chronic administration of Org 26576 increases cell proliferation in the hippocampus Chronic Org 26576 significantly increases the number of BrdU-positive cells

in the subgranular zone versus vehicle (Org 26576 increase vs. vehicle=42%, two-sample $t_{22}=2.457$, $P=0.0224$; $n=12$ per group; Fig. 3a). Two experiments with groups of six animals each were conducted under identical protocols and the results were pooled. There was a robust increase in the number of cells within each BrdU-positive cluster, with a cluster defined as three or more adjacent cells (Org 26576 increase vs. vehicle=20% representing an average of one more cell per cluster, two-sample $t_{22}=3.984$, $P=0.0006$; Fig. 3b). Org 26576 did not significantly influence the number of BrdU-positive cells in hilus (Fig. 3c).

Survival and phenotype of cells after chronic Org 26576 The numbers of cells surviving after chronic Org 26576 was significantly increased as measured by numbers

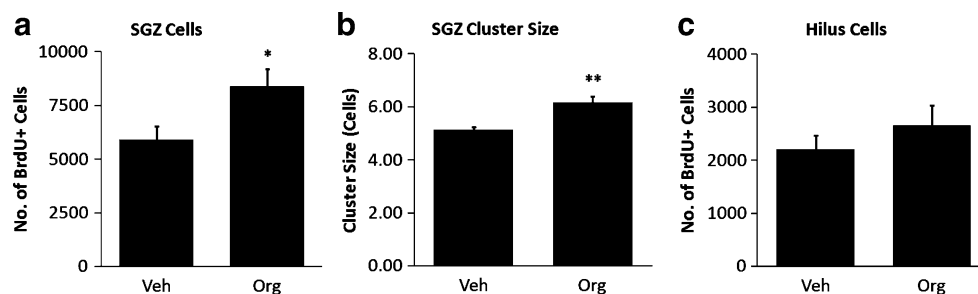


Fig. 3 Effects of chronic Org 26576 on cell proliferation in SGZ and hilus. **a** Chronic Org 26576 at the 10-mg/kg dosage increases cell proliferation in SGZ as measured by number of BrdU+ cells ($P<0.05$;

$n=12$ per group). **b** Chronic Org 26576 increases size of average proliferating clusters in SGZ ($P<0.005$). **c** Chronic Org 26576 does not significantly affect hilus cell proliferation

of BrdU-positive cells in the granular cell layer (GCL) sampled at the 28-day time point after the last drug administration (Org 26576 increase vs. vehicle=32%, two-sample $t_{10}=2.484$, $P=0.0323$; $n=6$ per group; Fig. 4a). A majority of surviving cells express the neuronal marker NeuN (vehicle, $93\pm 3\%$; Org 26576, $91\pm 2\%$; Fig. 4b). The percent of NeuN and BrdU double-labeled cells was not changed by Org 26576. Representative confocal images showing a BrdU-positive and NeuN-positive double-labeled cell are shown in Fig. 4c–e.

Chronic administration of Org 26576 increases cell proliferation in prelimbic cortex Chronic Org 26576 significantly increased the number of BrdU-positive cells per square millimeter of prelimbic cortex in the cell proliferation experiment (Org 26576 increase vs. vehicle=35%, two-sample $t_9=3.425$, $P=0.0076$; vehicle, $n=5$; Org 26576, $n=6$; Fig. 5a). One animal in the group receiving vehicle exhibited poor BrdU staining and was excluded. Survival of cells born in prelimbic cortex as measured by numbers of BrdU-positive cells sampled at the 28-day time point after the last drug Org 26576 administration was not significantly changed (Fig. 5b).

A representative section showing BrdU-positive cells in prelimbic cortex in the proliferation study is shown in Fig. 5c. Significantly fewer BrdU-positive cells are found

in prelimbic cortex as compared to dentate gyrus. A schematic adapted from the Paxinos and Watson (1998) Rat Atlas diagramming the location of the 1×1 -mm counting frame used in quantifying prelimbic cortex is shown in Fig. 5d.

Discussion

The results of the present study extend previous work on the potential role of ARPs as novel antidepressants with two significant findings. First, the results demonstrate that chronic but not acute administration of Org 26576 increases neurogenesis in the adult hippocampus, as reflected by statistically significant increased levels of cell proliferation and survival, with the majority of surviving cells expressing a neuronal phenotype. Previous findings (Bai et al. 2003) analyzed the effect of another ARP, LY451646, on cell proliferation in the same brain region and demonstrated an increase but did not measure survival or differentiation to neuronal phenotypes. Second, the results demonstrate for the first time that chronic ARP administration (Org 26576) increases cell proliferation in prelimbic cortex. Reduced volume and decreased cell number in the prelimbic cortex have been reported in brain imaging and postmortem

Fig. 4 Effects of Org 26576 on cell survival and phenotype in GCL. **a** Chronic Org 26576 administration at the 10-mg/kg dosage increases survival of BrdU+ cells in GCL measured 28 days later ($P<0.05$, $n=6$ per group). **b** The majority of BrdU+ cells which survive in GCL express the neuronal marker NeuN. Org 26576 does not change the percentages of double-labeled cells. **c–e** Representative confocal image showing NeuN and BrdU colocalizations. NeuN: **c**, BrdU: **d**, merge: **e** (scale bar, 25 μm)

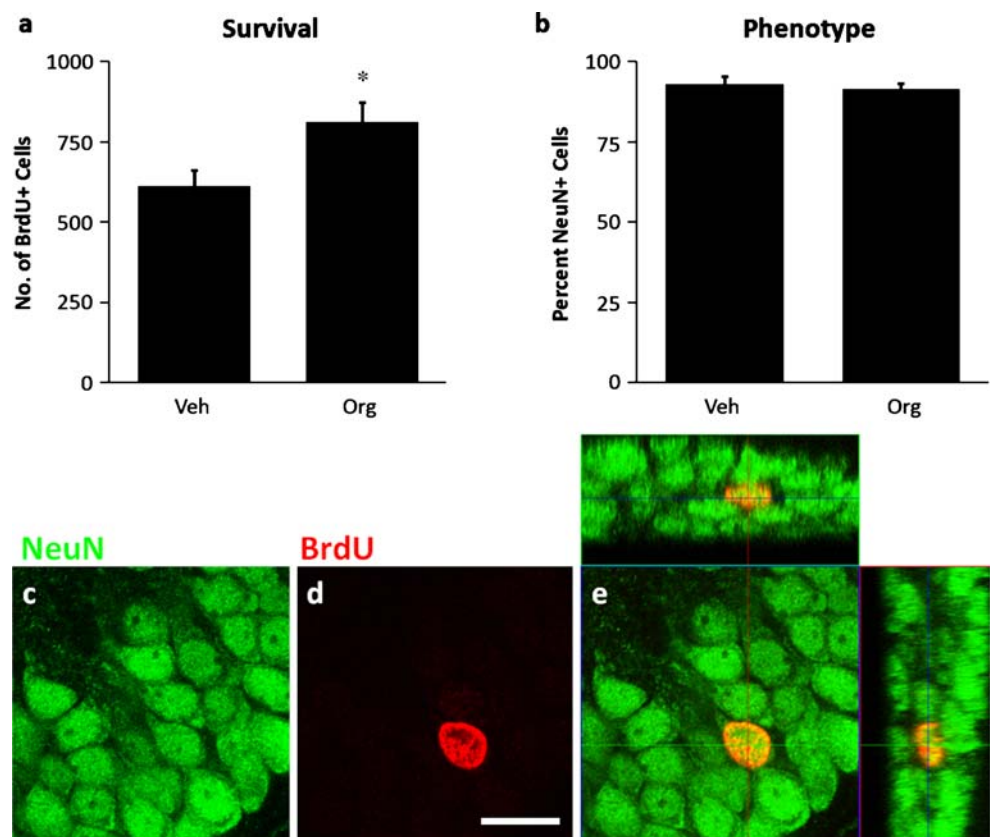
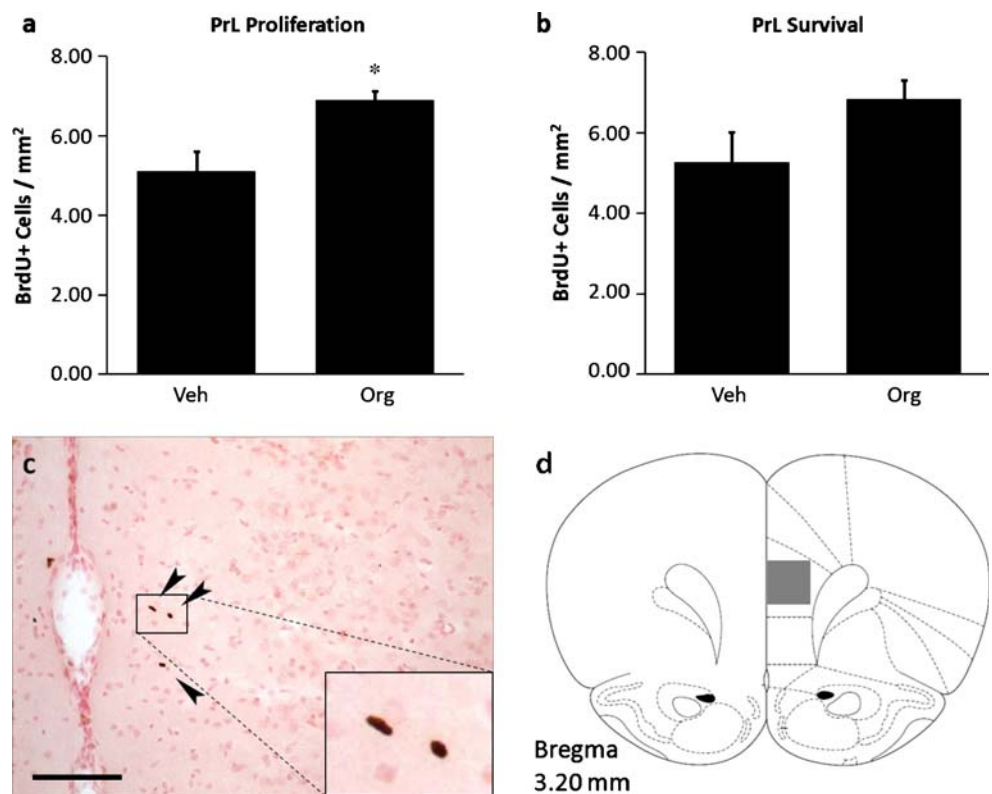


Fig. 5 Effects of chronic Org 26576 treatment on cell proliferation and survival in PFC. **a** Chronic Org 26576 administration at the 10-mg/kg dosage increases cell proliferation in prefrontal cortex ($P < 0.05$; vehicle, $n = 5$; Org 26576, $n = 6$). **b** Chronic Org 26576 does not significantly influence survival of BrdU+ cells in prefrontal cortex ($n = 6$ per group). **c** Representative bright-field image showing BrdU+ cells in prefrontal cortex ($\times 200$ magnification; scale bar, 100 μm ; inset shows detail of boxed region containing BrdU+ cells). **d** Schematic diagramming 1 \times 1-mm counting frame used to quantify BrdU+ cells in prefrontal cortex (shaded gray box)



studies of depressed patients (Cotter et al. 2002; Drevets et al. 1997), whereas gliogenesis in this region has been implicated in the actions of antidepressant treatment (Banasr et al. 2007). Previous animal research has shown newly dividing cells in prefrontal cortex adopt a glial phenotype (Banasr et al. 2007; Kodama et al. 2004) which, taken together with the present findings, further supports the antidepressant properties of Org 26576. The active dose of Org 26576 in the current study is consistent with the dose range used in other reports which examined the *in vivo* AMPA-receptor-mediated central effects of Org 26576 (Erdemli et al. 2007; Jordan et al. 2005), although these studies analyzed the effects of acute, rather than chronic, administration.

The upregulation of neurogenesis in the adult hippocampus in response to Org 26576 was observed after chronic (21 days) but not acute (two injections 8 h apart) administration, consistent with the time course for known chemical antidepressants (Malberg et al. 2000; Santarelli et al. 2003). All current chemical antidepressants require chronic treatment before a clinical antidepressant effect emerges. The findings that the neurogenic actions of Org 26576 are dependent on chronic administration are consistent with the time lag for the actions of antidepressant, and with a previous report of ARP induction of BrdU-positive cells in the hippocampus, although the latter study did report an acute effect on cell clusters (Bai et al. 2003).

Chronic Org 26576 significantly increased cell proliferation in the adult hippocampus as measured by the number

of BrdU-positive cells. This effect was seen in measures of total number of BrdU-positive cells and average cells per cluster in the SGZ. Clusters, which are defined as groups of more than three adjacent BrdU-positive cells, likely represent amplifying neural progenitors stimulated to undergo more than one round of mitotic division, as opposed to the proliferation of quiescent neural progenitors, which is induced by electroconvulsive therapy but not most chemical antidepressants (Segi-Nishida et al. 2008). However, as ARPs act by upregulation of AMPA-receptor-mediated signaling rather than modulation of monoamine neurotransmitters, further research is needed before it may be concluded that ARPs do not influence quiescent progenitors. We also found that the newborn cells survived when analyzed 28 days after the last Org 26576 administration and that these cells expressed a neuronal phenotype, demonstrating an overall increase in neurogenesis in response to ARP treatment.

There was no effect of Org 26576 on cell proliferation in the hilus of the hippocampus. Cells of the hilus primarily express a glial phenotype, and hilar gliogenesis has not been implicated in depression or antidepressant treatment. Results in the hilus serve as a negative control for the experimental methods utilized in the present study and provide further validation for results obtained.

Hippocampal neurogenesis is a correlate of antidepressant efficacy (Duman et al. 2001) and has been shown to be required for the behavioral effects of antidepressants in

certain animal models (Santarelli et al. 2003). However, research has suggested that this requirement may be species and strain dependent, with BALB/cJ mice showing behavioral responses to the SSRI fluoxetine in a neurogenesis-independent manner (Holick et al. 2008). This demonstrates that several mechanisms may underlie antidepressant-like effects, with neurogenesis not necessarily an essential correlate in all cases and not in the BALB/cJ background characterized. However, a body of research supports the link between hippocampal neurogenesis and antidepressant efficacy (Malberg et al. 2000; Sahay and Hen 2007). Taken together, the results in the hippocampus showing enhanced neurogenesis suggest that the ARP Org 26576 may have antidepressant properties under chronic administration.

Currently, the functional significance of new neurons generated in the adult hippocampus is not well-characterized; however, many possibilities exist. It has been shown that newly generated hippocampal neurons adopt the passive membrane properties of mature neurons, fire action potentials, and integrate into the existing network circuitry (van Praag et al. 2002). As such, new neurons may enhance hippocampal function involved with antidepressant action. It has been hypothesized that neurogenesis in the ventral hippocampus may be involved in the regulation of emotional responses (Sahay and Hen 2007). Adult neurogenesis may likewise serve to modulate hippocampal function or possibly regulate function of the hypothalamic–pituitary–adrenal axis. Finally, given the role of hippocampus in learning, neurogenesis in this region may influence plasticity and adaptation responses (Sahay and Hen 2007).

An increasing body of evidence suggests that there is a role for AMPA-receptor-mediated signaling in depression and the action of antidepressant treatments (Alt et al. 2006; Zarate and Manji 2008). ARPs hold promise in ongoing drug discovery because of their differential mechanisms of action compared to currently available antidepressant medications. ARPs are known to exert their positive allosteric effects on AMPA receptors by attenuating receptor deactivation and/or desensitization in the presence of agonist (Alt et al. 2006; Jin et al. 2005). Although it is not known which of these respective receptor processes Org 26576 preferentially targets, nonetheless, Org 26576 exerts a primary influence through a different pathway compared with existing chemical antidepressants (Erdemli et al. 2007), all of which influence monoamine neurotransmitter systems. As such, ARPs such as Org 26576 may represent a different means of treatment for depressive symptoms. Furthermore, Org 26576 represents a structurally distinct series derived from the first-generation ARP CX516 (Staubli et al. 1994) and displays 10–30 greater potency in potentiating AMPA-mediated electrophysiological responses in rat hippocampal cultured neurons (Jordan et al. 2005), arguing for its increased specificity. At present,

the half-life of Org 26576 in the brain is not known, although it is possible that the requirement of chronic rather than acute administration for its effects may be due in part to lag time needed to reach maximal brain concentration.

Results presented in the current as well as previous studies support the antidepressant-like effect of ARPs in neurogenic and gliogenic models of depression. The precise mechanisms by which ARPs exert these effects downstream of AMPA receptor signaling remain to be elucidated; however, a number of different pathways may be implicated. ARPs may exert an indirect effect on progenitor populations via a neuronal or glial intermediate. ARPs may act by increasing levels of the neurotrophin BDNF, a pathway that has been previously implicated (Lauterborn et al. 2000; Mackowiak et al. 2002). Or ARPs may influence other trophic factors implicated in antidepressant action, notably VEGF (Warner-Schmidt and Duman 2007). Results obtained in this study show that a downstream effect of chronic administration of the ARP Org 26576 is to increase neurogenesis in adult rat hippocampus and cell proliferation in prefrontal cortex. Combined with previous findings that implicate adult hippocampal neurogenesis and prefrontal gliogenesis in antidepressant action, this supports the conclusion that Org 26576 has antidepressant properties in a neurogenic model of depression.

Acknowledgments Funding was provided through a grant from Schering-Plough.

References

- Alt A, Nisenbaum ES, Bleakman D, Witkin JM (2006) A role for AMPA receptors in mood disorders. *Biochem Pharmacol* 71:1273–1288
- Bai F, Li X, Clay M, Lindstrom T, Skolnick P (2001) Intra- and interstrain differences in models of “behavioral despair”. *Pharmacol Biochem Behav* 70:187–192
- Bai F, Bergeron M, Nelson DL (2003) Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. *Neuropharmacology* 44:1013–1021
- Banasr M, Duman RS (2007) Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. *CNS Neurol Disord Drug Targets* 6:311–320
- Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol Psychiatry* 64:863–870
- Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS (2007) Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biol Psychiatry* 62:496–504
- Bleakman D, Alt A, Witkin JM (2007) AMPA receptors in the therapeutic management of depression. *CNS Neurol Disord Drug Targets* 6:117–126
- Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall I (2002) Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Arch Gen Psychiatry* 58:545–553
- Drevets WC, Price JL, Simpson JR, Todd RF, Reich T, Vannier M (1997) Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386:824–827

- Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59:1116–1127
- Duman RS, Nakagawa S, Malberg J (2001) Regulation of adult neurogenesis by antidepressant treatment. *Neuropsychopharmacology* 25:836–844
- Erdemli G, Smith LH, Sammons M, Jeggo R, Shahid M (2007) Org 26576, a novel positive allosteric modulator, potentiates AMPA receptor responses in hippocampal neurons. *J Psychopharmacology* 21:A51
- Heninger GR, Delgado PL, Charney DS (1996) The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion studies in humans. *Pharmacopsychiatry* 29:2–11
- Holick KA, Lee DC, Hen R, Dulawa SC (2008) Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. *Neuropsychopharmacology* 33:406–417
- Jin R, Clark S, Weeks AM, Dudman JT, Gouaux E, Partin KM (2005) Mechanism of positive allosteric modulators acting on AMPA receptors. *J Neurosci* 25:9027–9036
- Jordan GR, McCulloch J, Shahid M, Hill DR, Henry B, Horsburgh K (2005) Regionally selective and dose-dependent effects of the ampakines Org 26576 and Org 24448 on local cerebral glucose utilisation in the mouse as assessed by ¹⁴C-2-deoxyglucose autoradiography. *Neuropharmacology* 49:254–264
- Kessler RC, Berglund P, Demler O, Jin R, Koretz R, Merikangas KR, Rush AJ, Walters EE, Wang PS (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *J Am Med Assoc* 289:3095–3105
- Kodama M, Fujioka T, Duman RS (2004) Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. *Biol Psychiatry* 56:570–580
- Lauterborn JC, Lynch G, Vanderklish P, Arai A, Gall CM (2000) Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J Neurosci* 20:8–21
- Legutko B, Li X, Skolnick P (2001) Regulation of BDNF expression in primary neuron culture by LY392098, a novel AMPA receptor potentiator. *Neuropharmacology* 40:1019–1027
- Li X, Witkin JM, Need AB, Skolnick P (2003) Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23:419–430
- Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ (2006) Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 367:1747–1757
- Mackowiak M, O'Neill MJ, Hicks CA, Bleakman D, Skolnick P (2002) An AMPA receptor potentiator modulates hippocampal expression of BDNF: an in vivo study. *Neuropharmacology* 43:1–10
- Maeng S, Zarate CA Jr, Du J, Schloesser RJ, McCammon J, Chen G, Manji HK (2008) Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 63:349–352
- Malberg J, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult hippocampus. *J Neurosci* 20:9104–9110
- Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002–2030. *PLoS Med* 3:2011–2030
- Mathew SJ, Manji HK, Charney DS (2008) Novel drugs and therapeutic targets for severe mood disorders. *Neuropsychopharmacology* 33:2080–2092
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002) Neurobiology of depression. *Neuron* 34:13–25
- Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edn. Academic, New York
- Sahay A, Hen R (2007) Adult hippocampal neurogenesis in depression. *Nature Neuroscience* 10:1110–1115
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub M, Lee J, Duman RS, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effect of antidepressants. *Science* 301:805–809
- Segi-Nishida E, Warner-Schmidt JL, Duman RS (2008) Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. *Proc Natl Acad Sci U S A* 105:11352–11357
- Staubli U, Perez Y, Xu FB, Rogers G, Ingvar M, Stone-Elander S, Lynch G (1994) Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Natl Acad Sci* 91:11158–11162
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. *Nature* 415:1030–1034
- Warner-Schmidt JL, Duman RS (2007) VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. *Proc Natl Acad Sci U S A* 104:4647–4652
- Zarate CA Jr, Manji HK (2008) The role of AMPA receptor modulation in the treatment of neuropsychiatric diseases. *Exp Neurol* 211:7–10