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Increased Hippocampal Neurogenesis and p21 Expression in Depression: Dependent on Antidepressants, Sex, Age, and Antipsychotic Exposure

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The mammalian hippocampus continues to generate new neurons throughout life. The function of adult-generated neurons remains controversial, but adult neurogenesis in the hippocampus is related to depression. Studies show that neurogenesis in the hippocampus is regulated by antidepressants in both humans and rodents, but no studies have examined the effects of age, sex, or antipsychotic exposure on the relationship between depression, antidepressant exposure, and hippocampal neurogenesis in humans. Hippocampal sections were obtained from the Stanley Medical Research Institute and were immunohistochemically labeled for the immature neuron marker doublecortin and the cell cycle arrest marker p21. We compared the number of cells in the granule cell layer and subgranular zone that expressed these proteins in brains from control subjects ($n=12$), patients with major depressive disorder (MDD) without psychotic symptoms ($n=12$), and patients with MDD and psychotic symptoms ($n=12$). We show here that the density of doublecortin/NeuN expression was increased in MDD patients compared with controls and MDD patients with psychosis, with the effect greater in women. Further, we show that older depressed patients without psychosis had higher levels of p21/NeuN expression and that depressed individuals prescribed antidepressants had higher levels of p21/NeuN expression, but only in older women. We show for the first time that changes in neurogenesis due to prescribed antidepressants or depression are dependent on age, sex, and the presence of antipsychotics or psychotic symptoms.

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INTRODUCTION

Major depressive disorder (MDD) is two times more common in women than in men (Gutierrez-Lobos *et al*, 2002). Exposure to stress is a precipitating factor for MDD (Bale, 2006; Beck, 2008; Schule, 2007). Depressed patients show abnormal hypothalamic–pituitary–adrenal (HPA) function such as hypersecretion of cortisol (Stetler and Miller, 2011) and abnormal HPA negative feedback inhibition (Parker *et al*, 2003b; Schule, 2007). Normalizing HPA function is one of the major targets of recent therapies (Ising *et al*, 2007; Schule, 2007).

Depressed patients have smaller hippocampi (Campbell *et al*, 2004; Sheline *et al*, 1999; 2003) that is related to duration of illness and seen primarily in older adults (>65 years) (McKinnon *et al*, 2009). Further, antidepressant use protects against reductions in hippocampal volume principally in women (Lorenzetti *et al*, 2009; Sheline *et al*, 2003). The smaller hippocampal volume may be due to a number of factors including reduced neurogenesis. Intriguingly, both age and sex influence hippocampal neurogenesis in rodents (Galea, 2008; Kuhn *et al*, 1996) and hippocampal volume in depressed patients.

Adult neurogenesis in the hippocampus exists in most mammalian species including humans (Eriksson *et al*, 1998) and occurs via proliferation of progenitor cells and migration, differentiation, and survival of the resulting daughter cells (van Praag *et al*, 2002). Chronic stress and high glucocorticoids, both precipitating factors for MDD, reduce levels of cell proliferation and cell survival in the hippocampus (Brummelte and Galea, 2010; Gould *et al*, 1997). Conversely, antidepressant treatments (including MAOIs, SSRIs, tricyclics) increase cell proliferation in the adult rodent hippocampus (Green and Galea, 2008; Lagace *et al*, 2007; Malberg *et al*, 2000). These complementary observations generated the neurogenic hypothesis of depression, which suggests that the underlying etiology of MDD lies in a diminished rate of hippocampal neurogenesis

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(Drew and Hen, 2007) and that antidepressants exert their therapeutic effects by restoring neurogenesis to normal levels (Perera *et al*, 2011). However, simultaneously administering antidepressants while blocking or inhibiting neurogenesis improves many but not all depressive symptoms, indicating that adult neurogenesis in the hippocampus does not contribute to all the therapeutic effects of antidepressants (David *et al*, 2009).

Cell cycle progression is controlled by various factors including the cyclin/cdk complexes, which are inhibited by the cdk inhibitor p21 (Waga *et al*, 1994). p21 is decreased (increasing cell proliferation) in response to antidepressant treatment, and the knockout of p21 attenuates the therapeutic effects of antidepressants in mice (Pechnick *et al*, 2008, 2011). These findings suggest an intricate relationship between depression, antidepressant efficacy, neurogenesis, and p21.

Two studies showed enhanced hippocampal cell proliferation with antidepressant treatment in patients with MDD (Boldrini *et al*, 2009; Lucassen *et al*, 2010); one study did not (Reif *et al*, 2006). However, the latter study did not consider sex, age, or duration of illness as contributing factors. Furthermore, these studies focused on cell proliferation (Boldrini *et al*, 2009; Lucassen *et al*, 2010; Reif *et al*, 2006) and that the survival of new neurons is also important. In this study, we considered the effect of age, sex, and duration of illness on the density of immature neurons and p21 expression in the hippocampus.

Older age is associated with hypercortisolemia, small hippocampal volume, and reduced SSRI efficacy in MDD patients (McKinnon *et al*, 2009; Parker *et al*, 2003a; Stetler and Miller, 2011; Thase *et al*, 2005). Furthermore, sex differences exist in the neural manifestations of depression (Lorenzetti *et al*, 2009) and HPA negative feedback normalization with antidepressant treatment (Binder *et al*, 2009). Thus, it is important to establish whether there are sex and age differences on the effects of antidepressants on neurogenesis in depression. In addition, patients treated for depression often display comorbid psychotic symptoms (Blazer *et al*, 1994) and as such are often prescribed antipsychotic drugs in addition to antidepressants. First- and second-generation antipsychotics may differentially modulate neurogenesis (Newton and Duman, 2007), and altered neurogenesis in the hippocampus has been reported in schizophrenia (Reif *et al*, 2006). Thus, the presence of psychotic symptoms or antipsychotics could impact the effects of antidepressants on neurogenesis in humans. Therefore, we examined post-mortem hippocampal tissue from patients with MDD, with and without psychotic symptoms, to assess immature neuron survival using doublecortin (DCX) and the expression of a cell cycle inhibitor, p21. We hypothesized that the density of immature neurons would be increased and p21 expression would be suppressed in MDD patients prescribed antidepressants, which may be attenuated in psychotic patients prescribed antipsychotics. Further, given age and sex differences in antidepressant efficacy (Thase *et al*, 2005) and hippocampal volume in depression (Lorenzetti *et al*, 2009; McKinnon *et al*, 2009), we expected age and sex differences would exist, with younger depressed patients showing more DCX and less p21 expression with antidepressants compared with older patients and that this relationship would be stronger in women.

SUBJECTS AND METHODS

Subjects and Demographics

Sections of human hippocampal tissue were received from the Stanley Brain Research Laboratory, which is a part of the Stanley Medical Research Institute (Chevy Chase, MD). Tissue was collected from a total of 36 subjects (depression collection) in three groups ($n = 12$ per group): subjects with MDD without psychosis, subjects with MDD and psychosis, and age-matched controls. pH was measured on homogenized brain tissue from another brain region by the Stanley Medical Research Institute. Age, sex ratio, cause of death, age of disease onset, and duration of illness are shown in Table 1.

Histology

The hippocampal tissue was received as 14-μm unfixed cryosections mounted on glass slides. The sections were stored at $-80\,^{\circ}C$ until processing. One series of sections

Table 1 Demographic Variables for Each Group

	Control	Depressed	Depressed with psychosis	*p*-Value
Number of males /12	8	7	6	0.71
Average age (years)	46.8 ± 3.49	42.83 ± 2.92	41.5 ± 3.47	0.50
Age of onset (years)	—	30.58 ± 12.16	29.17 ± 12.03	0.77
Suicide[a]	0/12	8/12	9/12	0.012–0.024
Post-mortem interval	25.25 ± 3.07	23.58 ± 1.93	33.09 ± 3.31	0.06 (0.14 outlier removed)
pH	6.63 ± 0.05	6.71 ± 0.04	6.59 ± 0.04	0.16
Brain weight(g)	1444.83 ± 33.70	1467.17 ± 42.55	1470.42 ± 46.89	0.71
Duration of illness (years)	—	12.26 ± 2.45	12.33 ± 2.02	0.95
Lifetime alcohol	2.08 ± 0.60	1.7 ± 0.56	2.63 ± 0.65	0.50
Lifetime drug	0.75 ± 0.39	1.0 ± 0.44	1.27 ± 0.54	0.85
Smoking (time of death)	0.57 ± 0.20	0.2 ± 0.2	0.67 ± 0.21	0.12

[a]Significantly different from controls. Only cause of death by suicide was significantly more common in the psychiatric compared with control groups. g, grams.

was labeled for DCX, one for p21, and a third was labeled for NeuN. There were six sections per subject.

Doublecortin and p21 Immunohistochemistry

Slides were thawed for 45 min and then fixed for 15 min in 4% formaldehyde (for DCX staining) or 45 min in Histo-Choice fixative (for p21 staining) at room temperature. The tissue was rinsed three times in 0.1 M Tris-buffered saline (TBS) and then incubated in 0.6% hydrogen peroxide for 20 min. The sections were again rinsed in TBS and then blocked for 30 min in 5% normal horse serum and 0.3% Triton X in TBS. Sections were incubated for 48 h in 2.5% normal horse serum, 0.3% Triton X, and a 1 : 250 dilution of goat anti-DCX polyclonal antibody (200 μg/ml, SC-8066, Santa Cruz Biotechnology) or 1 : 200 dilution of goat anti-p21 polyclonal antibody (200 μg/ml, SC-397-G, Santa Cruz Biotechnology). The slides were rinsed in TBS and then incubated with a solution of ImmPRESS anti-goat polymer reagent (Vector Labs) for 18 h at 4 °C. Labeling was visualized with NovaRed (Vector Labs), and sections were lightly counterstained with methyl green and dehydrated and coverslipped with Permount.

NeuN Immunohistochemistry

A final series of tissue was labeled for the mature neuronal marker NeuN. The sections were first thawed for 45 min and then fixed for 15 min in 4% formaldehyde at room temperature. The sections were then incubated at 4 °C for 24 h in a solution containing 0.3% Triton X, 3% normal donkey serum, and 1 : 500 dilution of mouse anti-NeuN monoclonal antibody (1.0 mg/ml, MAB377 clone A60, Millipore). The sections were rinsed in TBS and then incubated in a 1 : 500 dilution of donkey anti-mouse conjugated to Alexa 546 (Molecular Probes) for 18 h at 4 °C. The sections were then rinsed and counterstained in a solution containing 0.003 mg/ml DAPI (D-1306, Molecular Probes) for 30 min at room temperature. The sections were coverslipped with PVA-DABCO to preserve the fluorescent labeling.

Quantification

All quantification was performed using an Olympus BX-51 light/epifluorescent microscope. Cell counts were performed using a ×100 oil immersion lens (total magnification, ×1000). DCX- and p21-expressing cells (Figure 1) were counted throughout the granule cell layer (GCL) as well as the subgranular zone (defined as the 20-μm zone on the interior edge of the GCL) in each section analyzed. NeuN and DAPI cell densities were calculated by randomly placing a grid with 50/50 μm divisions over the section and calculating the number of nuclei present within the grid at five locations in each GCL.

The area of the GCL in each section was measured by capturing images using a ×4 lens and tracing around the GCL using the software package NIH ImageJ. Cell densities were then calculated by dividing the total number of cells counted by the measured area or the total number of NeuN cells for each subject within the region of interest.

Data Analyses

ANOVAs were conducted on DCX-, p21-, DCX/NeuN-, p21/NeuN-, NeuN-, and DAPI-expressing cells, with group (control, depressed without psychotic symptoms, and depressed with psychotic symptoms) and sex as the between-subjects variables and with age as the covariate. To further address the issue of age, we also used age split as a between-subjects dichotomous variable to examine younger *vs* older adults (<50 *vs* >50 years) as has been reported previously (Thase *et al*, 2005). Post hoc tests utilized LSD comparisons. Pearson product–moment correlations were conducted between DCX-, DCX/NeuN-, p21-, p21/NeuN-, NeuN-, and DAPI-expressing cells, and demographic and clinical variables included age, brain pH, postmortem interval (PMI), age of onset of illness, and duration of illness. Student's *t*-tests were conducted on psychiatric groups for age of onset of illness and duration of illness. Significance levels were set at $p = 0.05$.

RESULTS

Demographics

Demographic variables are listed in Table 1. There were no significant differences between groups in age (all *p*-values are >0.45) or sex ($p = 0.7$). Given sex differences in hippocampal neurogenesis and antidepressant efficacy, sex was used as a between-subjects variable. Furthermore, because age interacted with neurogenesis variables, age was used as covariate in all subsequent analyses. We also created a dichotomous variable for age (age split) in which subjects were divided into two age groups of younger or older than 50 years. The age 50 years was chosen as the average age of menopause for women is 51.7 years (Do *et al*, 1998). Furthermore, neurogenesis levels are altered with ovarian hormone levels in both younger and older rodents (Barha and Galea, 2011; Barha *et al*, 2009; Barker and Galea, 2008) and with aging (Kuhn *et al*, 1996). Table 2 shows the sample sizes for men and women within the different psychiatric groups between the two age groups. Furthermore, the number of patients prescribed antidepressants, antipsychotics, and/or mood stabilizers is listed in Table 2.

Because pH can affect immunohistochemistry, we examined pH levels across group and sex, with age as a covariate. There were no significant main or interaction effects of pH for group or age (all *p*-values are >0.14). PMI had a strong trend to be significantly different between groups ($p = 0.06$), with depressed patients with psychosis tending to have a longer PMI than any other group. However, when one outlier was removed, the significance level fell to 0.14. Covariate effects are not mentioned further unless they had a significant effect in the analyses.

Increasing age was associated with greater levels of the cell cycle arrest marker p21 but only in depressed women. There was a positive correlation between p21-expressing cells and age in the total cohort of depressed patients ($r = 0.52$, $p = 0.009$) but only a trend in controls ($r = 0.54$, $p = 0.08$). This positive correlation was evident in depressed females ($r = 0.70$, $p = 0.016$) but not in depressed

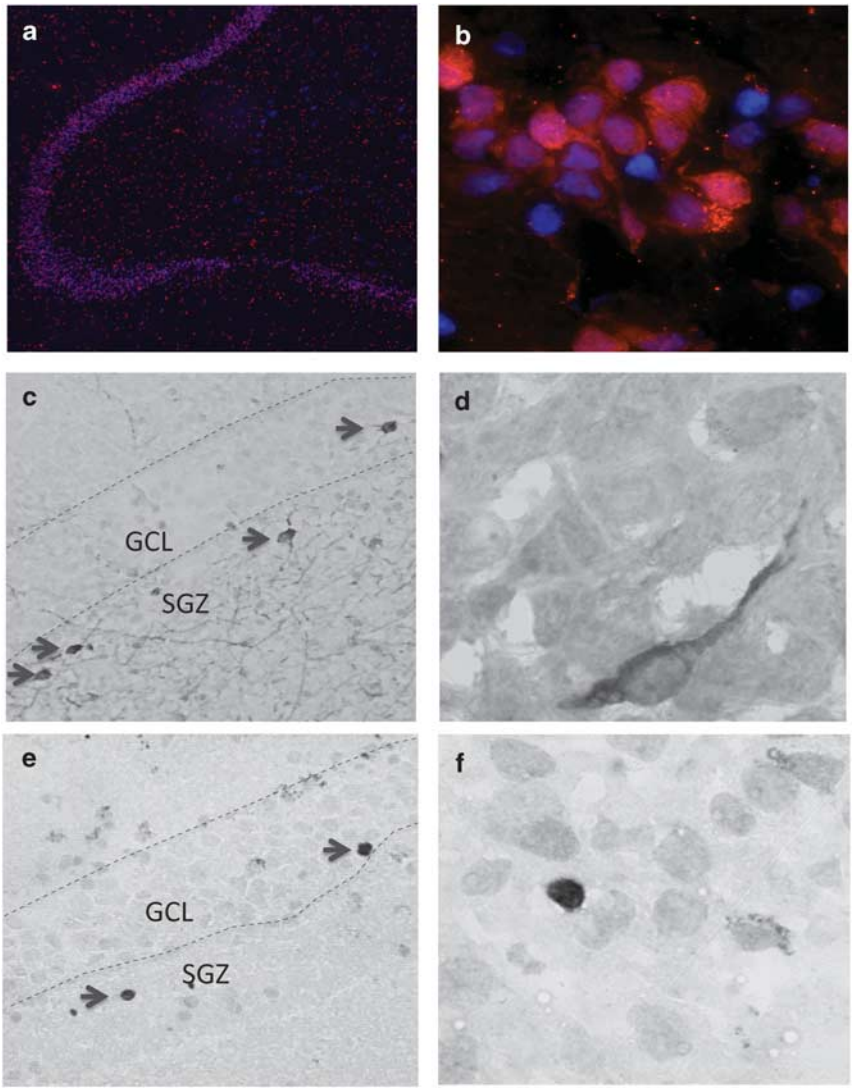

Figure 1 Photomicrographs of representative labeling of (a) low magnification stitched image of the granule cell layer (GCL) and (b) high magnification images of NeuN (red) counterstained with DAPI (blue). Panels (c) (× 400) and (d) (× 1000) show representative doublecortin labeling in the GCL and subgranular zone. Panels (e) (× 400) and (f) (× 1000) show the location of strongly labeled p21 cells at the border of the GCL and the subgranular zone.

males ($r = 0.42$, $p = 0.15$). When the MDD group was split according to the presence or absence of psychotic symptoms, the patients without psychotic symptoms had a significant positive correlation ($r = 0.61$, $p = 0.037$) but not the depressed patients with psychotic symptoms ($r = 0.52$, $p = 0.087$; Figure 2a). Unless indicated, correlations within each sex by group membership were not possible due to the low numbers in each group.

Higher age of onset of depression with psychosis is associated with more neurons in the dentate gyrus. Age of onset of disease was significantly positively correlated with the number of NeuN- and DAPI-expressing cells in the depressed patients with psychotic symptoms ($r = 0.61$, $p = 0.035$ and $r = 0.62$, $p = 0.032$, respectively), indicating that an older age of onset was associated with greater number of neurons in the dentate gyrus in this group (Figure 2b). This relationship was not observed in patients without psychosis, and there were no other significant correlations between the number of p21-, DCX-, NeuN-, or DAPI-expressing cells and age of onset.

Older depressed patients without psychosis have higher levels of p21 expression in the dentate gyrus of the hippocampus. There were no significant main or interaction effects on the number of DCX-, NeuN-, or DAPI-expressing cells on differences between groups (all p-values are >0.2). However, using age as the covariate and group, sex, and age split as the between-subjects variables, we conducted an ANOVA on the number of p21-expressing cells in the dentate gyrus. We found that there was a significant age split by group interaction ($F(2,23) = 3.55$, $p = 0.045$) as well as a main effect of group ($F(2,23) = 3.31$, $p = 0.05$) and a significant covariate effect of age ($F(1,23) = 4.52$, $p = 0.044$). Post hoc tests indicated that the older depressed patients without psychosis had higher levels of p21-expressing cells in the dentate gyrus than all other groups (all p-values are <0.035).

Table 2 Sample Sizes of Psychiatric Groups of Men and Women Prescribed Medications and of Age Split Groups

Drug	Group		
	Control	Depressed	Depressed/psychosis
Antidepressant	0	10 (5)	7 (4)
Antipsychotic	0	3 (1)	9 (5)
Mood stabilizer	0	4 (1)	2 (1)
Male	8 (4)	7 (5)	6 (6)
Age (years)			
<50	6 (1)	9 (4)	9 (5)
>50	6 (3)	3 (1)	3 (1)

The numbers of women within each group are in parentheses.
Antidepressants prescribed were SSRIs: fluoxetine (2), paroxetine (2), sertraline (2); SNRI: venlafaxine (3); TCA: desipramine (2); tetracyclic antidepressant: mirtazapine (1); monoamine oxidase inhibitor: tranylcypromine (1); atypical: bupropion (4), trazodone (3), unknown (2), nefazodone (2). Seven people were prescribed more than one medication.
Mood stabilizers prescribed were valproate (3), lithium (2), gabapentin (2).

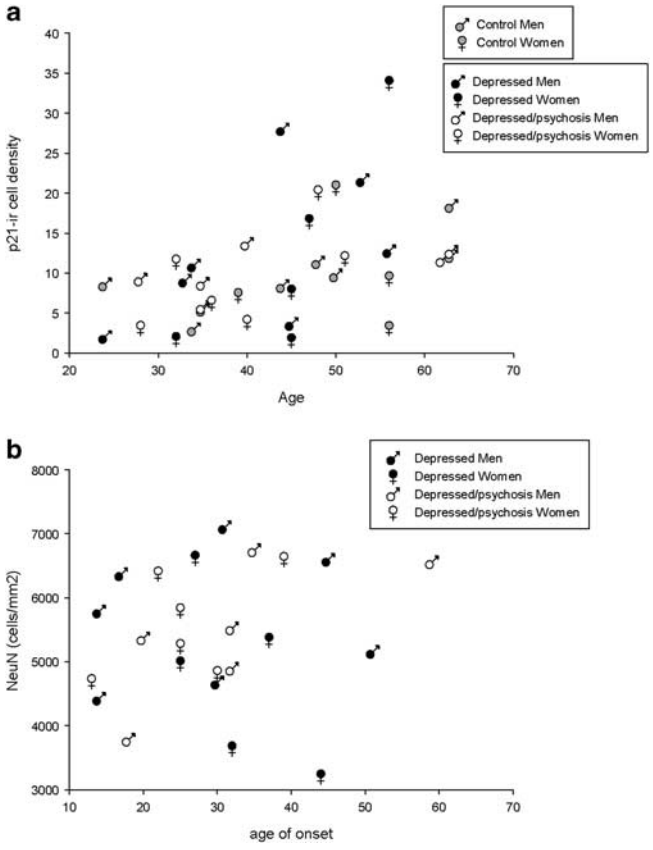

Figure 2 (a) p21 expression correlates with patient age. There was a significant positive correlation between p21 expression and age in depressed women ($r = 0.70$, $p = 0.016$) but not men ($r = 0.42$, $p = 0.15$). Age and p21 expression were not significantly correlated with each other in control or depressed patients with psychotic symptoms but were significantly positively correlated in depressed patients without psychotic symptoms ($r = 0.61$, $p = 0.037$). (b) NeuN density correlates with age of onset of depression. There was a significant positive correlation between NeuN density in the GCL of the hippocampus and age of onset of depression. However, this correlation was only significant in depressed patients with psychosis ($r = 0.61$, $p = 0.035$).

Older depressed patients without psychosis had higher density of p21/NeuN expression in the dentate gyrus of the hippocampus than all other groups. Due to correlations between p21 expression and age in the depressed groups and the positive correlation of NeuN-ir cells with age of onset of symptoms, we also examined the ratio of the counted p21- and DCX-expressing cells based on the number of NeuN-expressing cells that were counted. We calculated p21/NeuN as an index of the relative ratio of the p21 population compared with the mature granule cell population in the sections counted. There were significant two-way interactions of age split by group ($F(2,23) = 3.42$, $p = 0.0499$) and age split by sex ($F(1,23) = 4.41$, $p = 0.047$) and a main effect of group ($F(2,23) = 4.45$, $p = 0.023$) on the density of p21/NeuN-expressing cells. There were no other significant main or interaction effects. *Post hoc* tests demonstrated that the older depressed group without psychosis had the highest levels of p21/NeuN-expressing cells in the dentate gyrus compared with all other groups ($p < 0.034$; Figure 3a). In addition, older females had the highest levels of p21/NeuN-expressing cells compared with younger men ($p = 0.028$) and younger women ($p = 0.029$) but not compared with older men ($p = 0.34$; Figure 3b). This indicates that older women had a greater density of p21-expressing cells (in relation to the density of mature neurons) compared with younger men and women.

These effects may have been due to either prescribed antidepressants or antipsychotics; thus we ran further ANOVAs on the patients using antidepressants or antipsychotics as between-subjects variables in two separate ANOVAs. There was a significant three-way interaction between antidepressants, sex, and age split ($p < 0.049$). *Post hoc* tests indicated that older females prescribed antidepressants had the highest density of p21/NeuN-expressing cells compared with all other groups (all p-values are < 0.042; Figure 3c). The ANOVA on the density of p21/NeuN-expressing cells with antipsychotics revealed trends for interactions between age split and sex ($p = 0.052$) and antipsychotic by sex ($p = 0.086$) but no other effects (all p-values are > 0.1).

Depressed patients without psychosis had higher density of DCX/NeuN-expressing cells than depressed patients with psychosis. We also calculated DCX/NeuN as an index of the relative ratio of the immature neuron population compared with the mature granule cell population. The ANOVA on the density of DCX/NeuN expression revealed a significant effect of age covariate ($p = 0.026$) and a main effect of group ($F(2,28) = 3.44$, $p = 0.043$) but no significant effects of sex or interaction effects (all p-values are > 0.25). *Post hoc* tests revealed that depressed patients without psychosis had higher density of DCX/NeuN-expressing cells than depressed patients with psychotic symptoms ($p = 0.03$) and a trend for higher density than controls ($p = 0.08$; Figure 4a). Furthermore, due to previous work indicating antidepressant efficacy differences in men and women interacting with age (Thase *et al*, 2005) and because of our

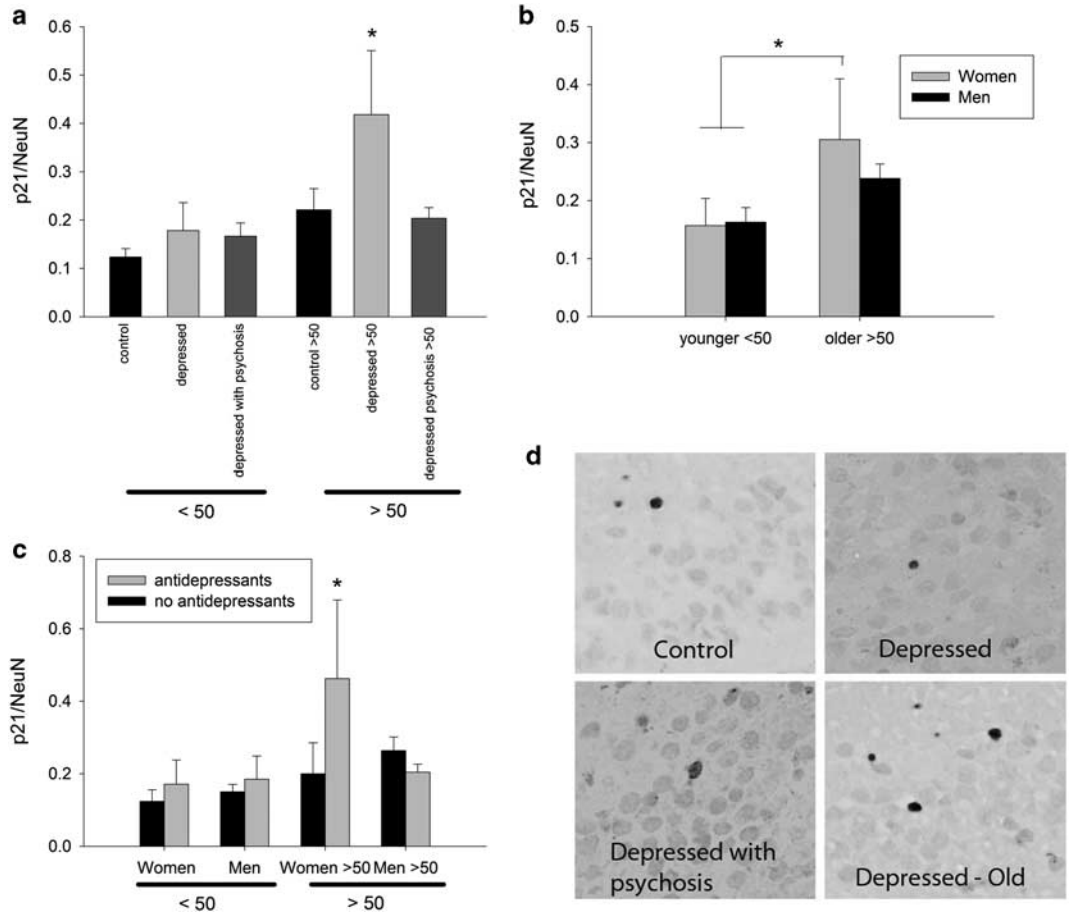

Figure 3 Increased density of p21/NeuN expression in older depressed patients. (a) There was a significantly greater density of p21/NeuN-expressing cells in the dentate gyrus of older depressed patients (>50 years) compared with controls. (b) Older women (>50 years) had significantly greater density of p21/NeuN-expressing cells than younger men and women. (c) Older women that had been prescribed antidepressants had significantly greater density of p21/NeuN-expressing cells than all other groups. Data shown is mean + SEM. (d) Photomicrographs show representative density of p21 expression in patients that were diagnosed as depressed (<50 or >50 years), depressed with psychosis, or controls. *$P \leqslant 0.05$.

findings of age effects on p21/NeuN expression, we expected that antidepressants may work differently on neurogenesis parameters in men *vs* women and older *vs* younger patients. Thus, we ran an *a priori* analysis to examine these effects on DCX/NeuN cells. We found that depressed women had a greater density of DCX/NeuN-expressing cells than control women ($p = 0.05$) and depressed women with psychotic symptoms ($p = 0.04$; Figure 4b). However, there were no significant differences between groups of men (depressed *vs* control men ($p = 0.97$) or depressed *vs* depressed with psychosis ($p = 0.49$)). This increase in the density of DCX/NeuN-expressing cells in depressed patients may be due to antidepressants, so we ran a comparison on the depressed groups that were prescribed antidepressants or not. Intriguingly, antidepressants were associated with different densities of DCX/NeuN expression depending on whether or not psychotic symptoms were present. The depressed patients without psychotic symptoms prescribed antidepressants had significantly higher density of DCX/NeuN expression than depressed patients with psychotic symptoms prescribed antidepressants, using sex and age as covariates ($p = 0.008$; Figure 4c). This was still significant when lifetime antipsychotic exposure was taken into account ($p = 0.011$). *A priori* we expected age differences in these effects, and when we examined age split by antidepressants in the psychiatric groups, we found that younger patients prescribed antidepressants (0.62 ± 0.08) had a greater density of DCX/NeuN expression than older patients prescribed antidepressants (0.36 ± 0.09; $p = 0.045$). Furthermore, when we examined whether prescribed antipsychotics affected DCX/NeuN density in depressed patients (with and without psychosis), we found that younger patients who were not prescribed antipsychotics had higher DCX/NeuN densities than all other groups (younger patients prescribed antipsychotics ($p < 0.005$) and older patients prescribed antipsychotics ($p < 0.01$) or not ($p < 0.001$); antipsychotic by age split interaction: $p < 0.037$; Figure 4d).

In addition, we ran subsequent analyses to address whether those patients prescribed antidepressants differed from controls for the density of DCX/NeuN-expressing cells, using sex and age as covariates. We found that although prescribed antidepressants increased DCX/NeuN ratio compared with controls, this failed to reach significance

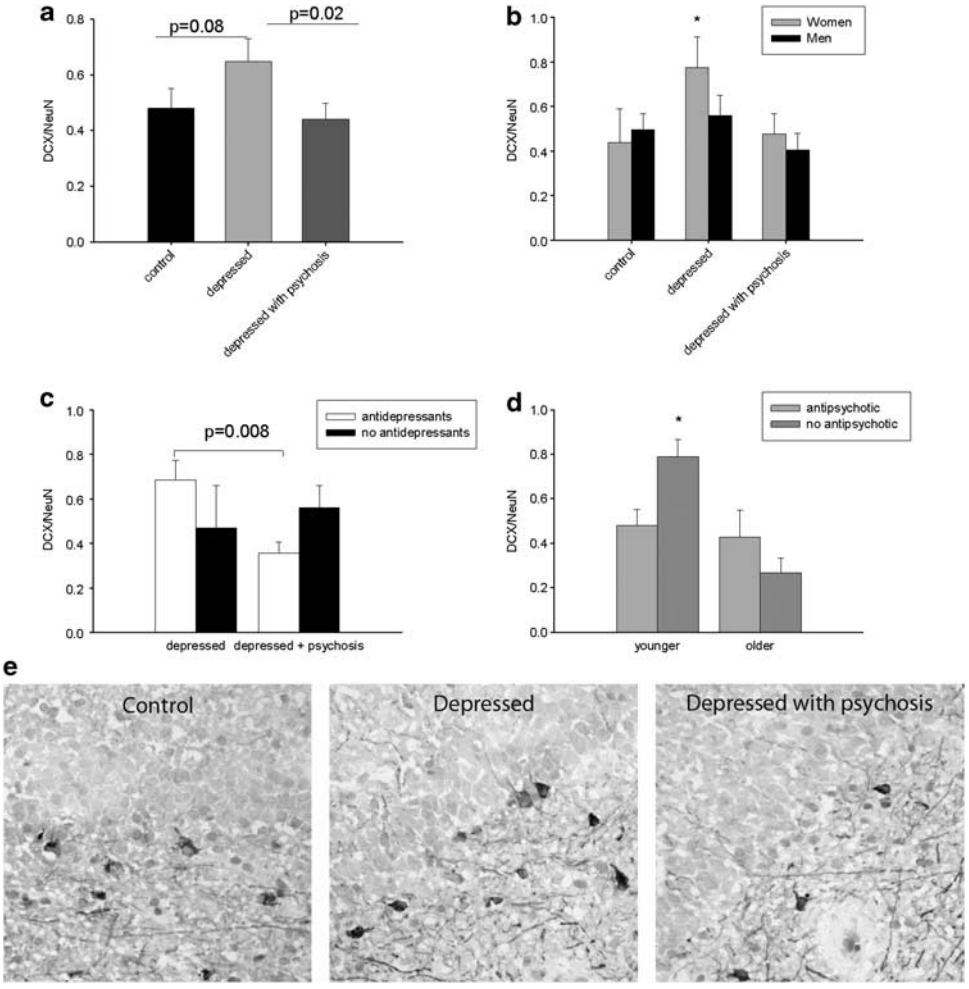

Figure 4 The density of doublecortin (DCX)/NeuN expression in the dentate gyrus is increased in depressed patients. (a) The density of DCX/NeuN expression was increased in depressed patients compared with depressed patients with psychosis ($p = 0.02$) and with a strong trend in controls ($p = 0.08$). (b) The density of DCX/NeuN expression was significantly greater in depressed women compared with all other groups. (c) Depressed patients prescribed antidepressants had greater density of DCX/NeuN expression compared with depressed patients with psychosis prescribed antidepressants (compare white bars, $p = 0.008$). (d) In younger (age <50 years), but not older (age >50 years) age groups, depressed patients that were not prescribed antipsychotics had significantly greater DCX/NeuN expression compared with all other groups. Data shown is mean + SEM. (e) Representative DCX expression is shown from depressed patients, depressed patients with psychosis, and controls. $^*P \leqslant 0.05$.

(controls: 0.48 ± 0.07; depressed patients prescribed antidepressants: 0.76 ± 0.10; main effect of antidepressants: $p = 0.10$).

Collectively these findings indicate that younger depressed patients taking antidepressants had a larger density of DCX-expressing cells relative to mature neurons than older patients, and this effect was not evident if patients were also prescribed antipsychotics.

Depressed patients that died by suicide have higher density of DCX/NeuN-expressing cells than depressed patients that died by other means. As expected, there were significantly more suicides among psychiatric groups compared with controls ($\chi^2 = 16.27$, $p = 0.0003$; $p = 0.020$ for depressed patients without psychosis, $p = 0.012$ for depressed patients with psychosis).

A priori we were interested in suicide as a variable of interest, and comparisons showed that depressed patients that had committed suicide had higher DCX/NeuN-expressing cell densities than depressed patients that had not committed suicide ($p = 0.04$). There were no significant differences between depressed patients with psychotic symptoms that had committed suicide and those who had died by other means ($p = 0.7$).

DISCUSSION

We found higher levels of cell cycle inhibition (p21 expression) in the dentate gyrus of older depressed patients (>50 years) and in patients who were prescribed antidepressants compared with controls. Intriguingly, we found that the presence of psychotic symptoms or prescribed antipsychotics was not associated with significant changes in p21 expression. In addition, older depressed women prescribed antidepressants had the highest density of p21/NeuN-expressing cells compared with all other groups.

We also found an increase in the density of new immature neurons compared with mature neurons in depressed patients compared with depressed patients with psychotic symptoms. Younger depressed patients and women had the highest density of DCX/NeuN-expressing cells. Furthermore, patients prescribed antipsychotics had the lowest density of DCX/NeuN-expressing cells, suggesting that antipsychotics may decrease production of new immature neurons. As expected, sex and age were significant factors in our analyses. In terms of age, the greater density of DCX/NeuN-expressing cells with prescribed antidepressants was mainly seen in the younger patients (<50 years) compared with the older patients, whereas p21 expression was higher in the older depressed group without psychosis. These findings are consistent with previous work illustrating an increase in cell proliferation with tricyclic antidepressants in younger adult depressed patients (mean age = 40 years (Boldrini *et al*, 2012)) and a lack of increase in cell proliferation with antidepressants in older depressed patients (mean age = 64 years (Lucassen *et al*, 2010)). Furthermore, sex differences were noted, with women showing more dramatic effects on the expression of immature neurons and the cell cycle arrest marker, p21, than men with either depression or prescribed antidepressants. Depressed women were more likely to have higher density of DCX/NeuN expression than depressed women with psychosis or controls, whereas the same relationship was not seen in men. Our study is the first to specifically examine sex and age differences in neurogenesis measures in human populations with psychiatric disease. These results are important in that we find the ability of antidepressants to significantly affect immature neurons and that cell cycle kinetics in the dentate gyrus is dependent on antipsychotic exposure, sex, and age.

We found that antidepressants work differently to alter neurogenesis parameters in men *vs* women and in younger *vs* older adults. This is consistent with studies showing sex and age differences in antidepressant efficacy (Pae *et al*, 2009; Thase *et al*, 2005) and on hippocampal volume (McKinnon *et al*, 2009). Indeed, we found that the most potent effects of antidepressants on neurogenesis (ie, an increase in DCX expression and lower levels of p21 expression) were observed in younger depressed patients without psychotic symptoms and in younger depressed women. Antidepressant efficacy varies by age and sex, with the most effective outcomes occurring in younger women with SSRIs (Pae *et al*, 2009; Thase *et al*, 2005). According to the theoretical role of adult neurogenesis as a mechanism for antidepressant function, our current results fit well with these previous findings. In this study, antidepressants increased neurogenesis in younger depressed patients (<50 years) and cell cycle inhibition was elevated only in older depressed patients (>50 years). Together, this suggests that a possible mechanism for antidepressant action may in fact be due to increased neurogenesis and that the relative inefficacy of antidepressants in older depressed patients may be due to an inability to overcome an increase in cell cycle arrest.

The effects seen in this study may be understood in the context of disease and pharmacological interactions. As depression has been previously associated with decreased cell proliferation in the hippocampus of untreated elderly patients (mean age = 77 years (Lucassen *et al*, 2010)), it is quite likely that the observed increase in p21 expression in our sample of older depressed patients (>50 years) is a result of or a precipitating factor of the illness. The increase in the density of immature neuron expression in the younger depressed patients may be attributable to the effects of antidepressants prescribed to these patients. Antidepressants promote neurogenesis (Boldrini *et al*, 2009, 2012; Malberg *et al*, 2000), and some postulate that this is the mechanism of action of their clinical properties (Santarelli *et al*, 2003). The increase in p21 expression with age was seen only in older depressed patients more than 50 years of age. As such, it may be more difficult for antidepressants to reverse the cell cycle inhibition when depression becomes chronic or occurs later in life. This finding is also consistent with the meta-analysis that showed that smaller hippocampal volumes were not seen until 2 years after onset of illness and more so in middle-aged or older adults (McKinnon *et al*, 2009). The former idea is consistent with the finding that chronic depression, left untreated, becomes more difficult to treat later on (Bukh *et al*, 2011). Conversely, the latter idea may be consistent with reports indicating that late-onset depression is often associated with different etiological factors than early-onset depression (Brodaty *et al*, 2001; Bukh *et al*, 2011). Furthermore, it is important to note that the positive correlation between age and p21 expression was only seen in depressed women. Given that menopause is associated with lower levels of ovarian hormones and that the lack of antidepressant efficacy is more common in post- *vs* premenopausal women (Pae *et al*, 2009), it is possible that antidepressant-induced increases in neurogenesis may be tied to ovarian hormones as suggested in rats (Green and Galea, 2008). In addition, antidepressant efficacy varies with age and type of antidepressant, with TCAs or SNRIs more effective in older than in younger populations but the reverse seen with SSRIs (Kornstein *et al*, 2010; Parker *et al*, 2003a; Thase *et al*, 2005), and thus attention should be directed to the type of antidepressants prescribed in future studies. Together, these findings are compelling in that they suggest that the ability of antidepressants to influence hippocampal neurogenesis may be related to age and hormone status in women. However, our results need to be interpreted with caution as there was no independent confirmation of drug levels post mortem.

The effects of antipsychotic medications on neurogenesis may explain why we did not see similar results in depressed patients with and without psychotic symptoms. A variety of different psychiatric medications have diverse effects on cell proliferation and cell survival depending on dose and drug type (Kodama *et al*, 2004; Nandra and Agius, 2012; Wakade *et al*, 2002; Wang *et al*, 2004). In this study, we found that patients prescribed antipsychotics often had opposing effects on neurogenesis than patients prescribed antidepressants. Antipsychotics have variable affects on hippocampal neurogenesis in rodents with atypical increasing, but typical antipsychotics, such as haloperidol, not affecting neurogenesis in the hippocampus (Wang *et al*, 2004). Thus, in this study, the negative effects of these drugs on neurogenesis may have been balanced by the positive effects of the antidepressants on neurogenesis, resulting in no net difference in neurogenesis compared with controls.

In conclusion, our results show clear effects of prescribed antidepressants to upregulate the density of immature neurons in the dentate gyrus in adult depressed patients compared with depressed patients that also had psychosis. Furthermore, this effect was stronger in younger than in older patients and in women than in men. There are age and sex differences in antidepressant efficacy (Kornstein *et al*, 2010; Thase *et al*, 2005), and thus our findings parallel those indicating that antidepressant efficacy is stronger in younger depressed patients. Furthermore, the fact that the density of p21/NeuN-expressing cells was higher only in older women and prescribed antidepressants is also suggestive of age-related changes in cell cycle kinetics that may explain a potential mechanism of greater antidepressant failure with age. Intriguingly, we also show that the presence of psychotic symptoms and antipsychotic medication negates the positive effects of antidepressants on neurogenesis parameters in this study.

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