LETTERS

Wnt signalling regulates adult hippocampal neurogenesis

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The generation of new neurons from neural stem cells is restricted to two regions of the adult mammalian central nervous system: the subventricular zone of the lateral ventricle, and the subgranular zone of the hippocampal dentate gyrus[1]. In both regions, signals provided by the microenvironment regulate the maintenance, proliferation and neuronal fate commitment of the local stem cell population[1]. The identity of these signals is largely unknown. Here we show that adult hippocampal stem/progenitor cells (AHPs) express receptors and signalling components for Wnt proteins, which are key regulators of neural stem cell behaviour in embryonic development[2]. We also show that the Wnt/β-catenin pathway is active and that Wnt3 is expressed in the hippocampal neurogenic niche. Overexpression of Wnt3 is sufficient to increase neurogenesis from AHPs *in vitro* and *in vivo*. By contrast, blockade of Wnt signalling reduces neurogenesis from AHPs *in vitro* and abolishes neurogenesis almost completely *in vivo*. Our data show that Wnt signalling is a principal regulator of adult hippocampal neurogenesis and provide evidence that Wnt proteins have a role in adult hippocampal function.

Using *in situ* hybridization, we found that Wnt3 is expressed in close proximity to the subgranular zone (SGZ), the region in which AHPs proliferate and differentiate into dentate granule neurons[1] (Fig. 1a). Analysis of adult Wnt/β-catenin reporter mice (BATGAL)[3] showed that this pathway is active in the SGZ and the dentate granule cell layer (Fig. 1b). We have previously shown that factors derived from the local astrocyte population participate in the regulation of proliferation and neuronal differentiation in hippocampal neurogenesis[4]. Notably, we found that several Wnt family members, including Wnt3, are expressed in adult hippocampal astrocytes (Fig. 1c and data not shown) and that AHPs express receptors for Wnts and crucial Wnt/β-catenin signalling pathway components[5] (Fig. 1d and data not shown). On the basis of these expression patterns, we considered that astrocyte-derived Wnts and Wnt signalling might be involved in the regulation of hippocampal neurogenesis.

We first tested this hypothesis in a co-culture system of AHPs and adult hippocampal astrocytes that models the interaction of these cell populations in the hippocampal neurogenic niche[4]. AHPs expressing green fluorescent protein (GFP) were co-cultured for 4 d with hippocampal astrocytes in the presence or absence of a Wnt inhibitor, secreted Frizzled-related protein 2 and 3 (sFRP2/3)[6]. Neuronal differentiation was evaluated every 24 h by quantifying the percentage of AHPs expressing the immature neuronal marker doublecortin[7] (DCX; Fig. 1e). We also determined the percentage of AHPs that expressed the mature neuronal marker MAP2ab[4] after 4 d (Fig. 1f). At all time points, the percentage of AHPs that differentiated into neurons was significantly decreased in the presence of sFRP2/3 (Fig. 1e, f), indicating that Wnts participate in the induction of neuronal differentiation of AHPs by factors derived from hippocampal astrocytes[4].

The interaction of Wnts with their receptors can trigger several signalling pathways, including β-catenin-dependent ('canonical') and β-catenin-independent pathways[5,8]. The Wnt/β-catenin signalling reporter Super8XTOPFLASH[9] was electroporated into AHPs that were subsequently cultured in the presence or absence of a hippocampal astrocyte feeder layer. Co-culture with hippocampal astrocytes resulted in a fourfold increase in luciferase reporter activity (Fig. 1g), suggesting that Wnts derived from hippocampal astrocytes stimulate Wnt/β-catenin signalling in AHPs. Next, AHPs were electroporated with the Wnt/β-catenin signalling reporter TOPGAL[10] and cultured on hippocampal astrocytes for 4 d. Expression of the β-galactosidase reporter was found almost exclusively in cells (>95% of the β-gal$^+$ cells; $n = 300$) that were positive for DCX (data not shown) or MAP2ab (Fig. 1h), but was not observed in the small fraction of AHPs that differentiated into glial fibrillary acidic protein (GFAP)-positive astrocytes[4].

Given this apparent association between Wnt signalling and neuronal lineage commitment, we tested the requirement for intact Wnt/β-catenin signalling in AHPs during neuronal differentiation induced by hippocampal astrocytes. Activation of the canonical Wnt pathway stabilizes β-catenin, which subsequently forms a transcriptionally active complex with members of the TCF/LEF transcription factor family[5]. Mutant forms of TCF/LEF that lack the ability to bind β-catenin act as dominant-negative regulators of Wnt/β-catenin signalling. AHPs were electroporated with a dominant-negative Lef1 (dnLef1) expression construct or empty vector (see Methods) and plated onto a hippocampal astrocyte feeder layer. After 4 d of co-culture, differentiation into DCX-positive neurons was reduced by 50% in dnLef1-expressing AHPs as compared with controls ($P < 0.05$, Mann–Whitney rank sum test; Fig. 1i). Taken together, these results indicate that astrocyte-derived Wnts and intact Wnt/β-catenin signalling in AHPs are substantial contributors to the neuronal differentiation of AHPs induced by hippocampal astrocytes.

We sought to determine whether Wnt signalling is sufficient to enhance neurogenesis from AHPs. To this end we focused on the Wnt family member Wnt3, which stimulates Wnt/β-catenin signalling in AHPs (Supplementary Fig. 2). AHPs were transduced with a retroviral vector expressing Wnt3 under the control of the tetracycline-suppressible *tet* operator[11]. Wnt3-expressing AHPs in which

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transgene expression was suppressed by the addition of doxycycline (100 ng ml^{-1}) did not differ from wild-type AHPs in generating neurons, astrocytes and oligodendrocytes under differentiating conditions (data not shown). On removal of doxycycline, however, the presence of Wnt3 stimulated a roughly fivefold increase in the percentage of cells that differentiated into neurons as compared with wild-type AHPs (Fig. 2a, b) or Wnt3-expressing AHPs cultured in the presence of doxycycline (data not shown). By contrast, differentiation into oligodendrocytes or astrocytes was not significantly increased (Supplementary Fig. 3), indicating that Wnt3 specifically enhanced the generation of neurons.

Increased neurogenesis in the presence of Wnt3 could be caused by promotion of cell survival, increased proliferation of neuronally committed progenitors or enhanced neuronal cell fate instruction. We determined apoptotic cell death by a TdT-mediated dUTP nick end labelling (TUNEL) assay every 24 h for 4 d in Wnt3-expressing and wild-type AHP cultures under differentiating conditions. No significant difference in the percentage of cells undergoing apoptotic cell death was observed between the experimental groups, indicating that Wnt3 did not affect survival under these conditions (data not shown). We then evaluated the effects of Wnt3 on neuroblast proliferation. Parallel cultures of Wnt3-expressing and wild-type AHPs were cultured under differentiating conditions and fixed at 24-h intervals over 4 d after a 2-h pulse of 10 μM bromodeoxyuridine (BrdU). As expected, the percentage of DCX-positive cells, which comprises proliferating neuroblasts and immature postmitotic neurons[7], was significantly increased in Wnt3-expressing AHPs as compared with controls at all time points (Supplementary Fig. 4). In the DCX-positive population, the neuroblast fraction was identified by coexpression of the proliferation marker Ki67. The ratio of

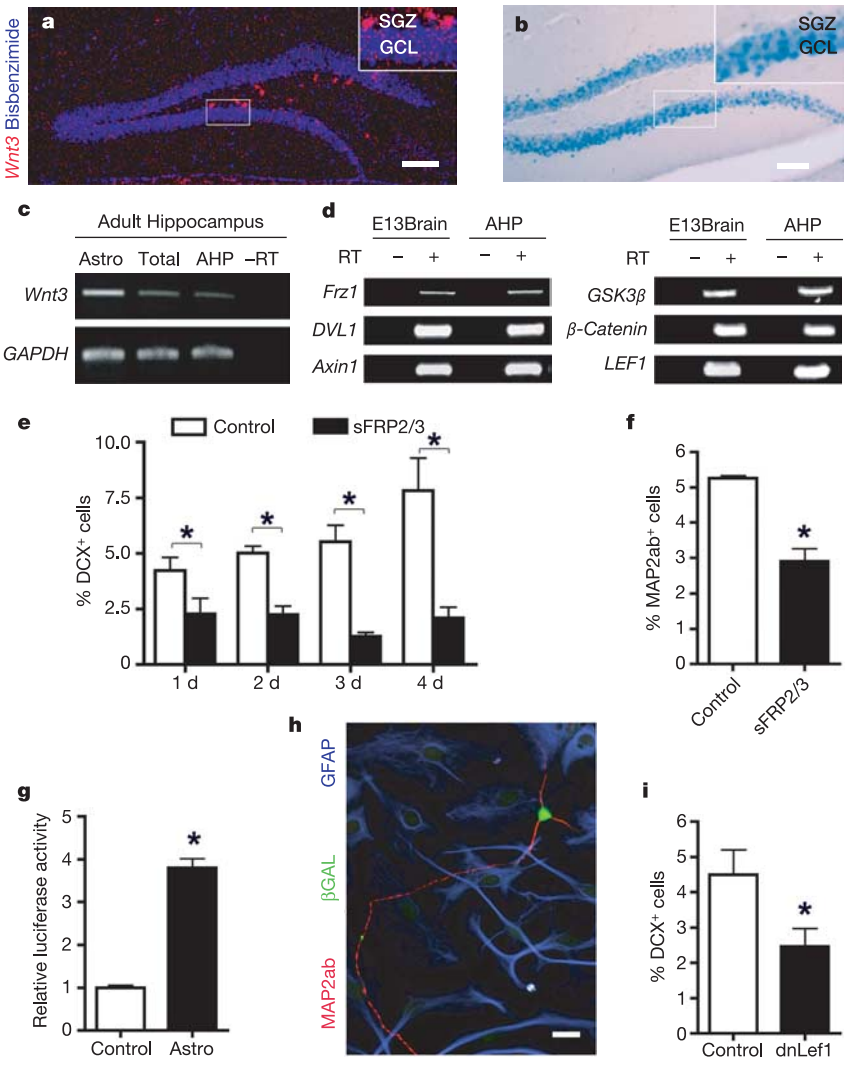

Figure 1 | Wnt signalling is involved in hippocampal-astrocyte-induced neurogenesis from AHPs. **a**, Wnt3 is expressed in close proximity to the SGZ of the adult mouse; *Wnt3* mRNA is red, the bisbenzimide counterstain is blue. Scale bar, 80 μm. GCL, granule cell layer. **b**, β-Galactosidase staining (blue) in BATGAL mice shows activity of the Wnt/β-catenin signalling pathway in the SGZ and granule cell layer of the dentate gyrus. **c**, RT–PCR confirms *Wnt3* expression in the adult hippocampus (Total) and adult hippocampal astrocytes (Astro). Less *Wnt3* mRNA is expressed in AHPs and is not sufficient to stimulate Wnt/β-catenin signalling significantly (Supplementary Fig. 1). *GAPDH* mRNA was measured as an internal control. **d**, RT–PCR analysis of Wnt signalling components in AHPs. *Frz1*, *Frizzled1*; *DVL1*, *Dishevelled1*. cDNA from embryonic day 13 brain was analysed as a positive control. **e**, **f**, Decrease in the percentage of neurons generated from AHPs in co-cultures of AHPs and hippocampal astrocytes caused by the addition of sFRP2/3. **g**, Hippocampal astrocytes stimulate β-catenin signalling in AHPs. As a control, AHPs were cultured in the absence of hippocampal astrocytes. **h**, AHPs expressing MAP2ab (red) show activation of the canonical Wnt signalling pathway (TOPGAL reporter; green) in co-cultures with hippocampal astrocytes (GFAP; blue). Scale bar, 20 μm. **i**, Blocking canonical Wnt signalling by expression of dnLef1 in AHPs reduces their neuronal differentiation in hippocampal astrocyte co-cultures. Asterisks indicate a statistical difference between experimental groups ($P < 0.05$, Mann–Whitney rank sum test [**e**, **f**, **i**] or Student's *t*-test ([**g**]). Error bars represent the s.e.m.

BrdU-positive neuroblasts ($BrdU^+DCX^+Ki67^+$) per total number of neuroblasts (DCX^+Ki67^+) was 2.5-fold higher in Wnt3-exposed AHPs on day 2 and remained significantly higher on day 3 (Fig. 2c, d). Despite this enhanced neuroblast proliferation, no change in overall proliferation was observed, as determined by the percentage of BrdU-positive cells among the total population (Fig. 2d), which was most probably due to the relatively small number of neuroblasts (<2% on day 2). The absence of detectable changes in bulk proliferation, however, indicated that Wnt3 does not have strong proliferative effects on cells other than neuroblasts under the current experimental conditions. The specificity of the proliferative effect was further substantiated by our finding that oligodendrocyte precursors did not show increased proliferation in the presence of Wnt3 (Supplementary Fig. 3).

Next, we determined whether Wnt3 also influences the neuronal fate choice of AHPs. To distinguish the proliferative effects of Wnt3 from potential instructive effects, differentiation was studied under growth-arresting conditions. Cells were cultured in differentiating conditions in the continuous presence of the DNA polymerase inhibitor aphidicolin[12] (10 μg ml^{-1}) or the ribonucleotide reductase inhibitor hydroxyurea[13] (1 μM). For the first 18 h, Wnt3-expressing AHPs were cultured in the presence of doxycycline to inhibit expression of Wnt3 before the onset of growth arrest. Doxycyline was then removed to allow expression of Wnt3 and the cells were cultured for an additional 40 h. During this period, BrdU was added to label cells that continued to proliferate despite the treatment with aphidicolin or hydroxyurea. Less than 5% of cells incorporated BrdU, indicating that most cells (~95%) were growth-arrested during the final 40 h of the assay (data not shown). In the growth-arrested population, we observed a 5–10-fold increase in the percentage of DCX-positive neurons generated from Wnt3-expressing AHPs as compared with controls (Fig. 2e, f). These results show that, in addition to stimulating neuroblast proliferation, Wnt3 instructs AHPs to adopt a neuronal fate.

Finally, we tested whether Wnt signalling regulates neurogenesis *in vivo*. Adult BATGAL mice were injected with a single dose of BrdU and killed after 24 h. Wnt/β-catenin pathway activity was observed in BrdU-positive cells in the SGZ (Fig. 3a) and in DCX-expressing cells with morphological features of proliferating neuroblasts[7] (Fig. 3b). These *in vivo* results are consistent with our *in vitro* finding that Wnt/β-catenin signalling regulates neuronal fate commitment and neuroblast proliferation.

For *in vivo* loss- and gain-of-function studies, we used a self-inactivating lentiviral vector (LV)[14] expressing a bicistronic cassette encoding for a Wnt signalling inhibitor or stimulator, an internal ribosomal entry site (IRES) and GFP. To block Wnt signalling *in vivo*, we used a secreted mutant Wnt1 protein (dnWnt), which non-autonomously blocks Wnt signalling *in vivo*[15,16]. Luciferase assays showed that LV–dnWnt reduced Wnt3-initiated β-catenin signalling in AHPs (Supplementary Fig. 5). In addition, we determined that overexpression of dnWnt in hippocampal astrocytes did not increase cell death in co-cultures of AHPs and hippocampal astrocytes (Supplementary Fig. 6). LV–dnWnt or control LV expressing GFP (LV–GFP) lentiviruses were stereotactically injected into the dentate gyrus of young (8–9 weeks) adult rats ($n = 8$). After a 3-week period to allow transgene expression and to minimize confounding factors

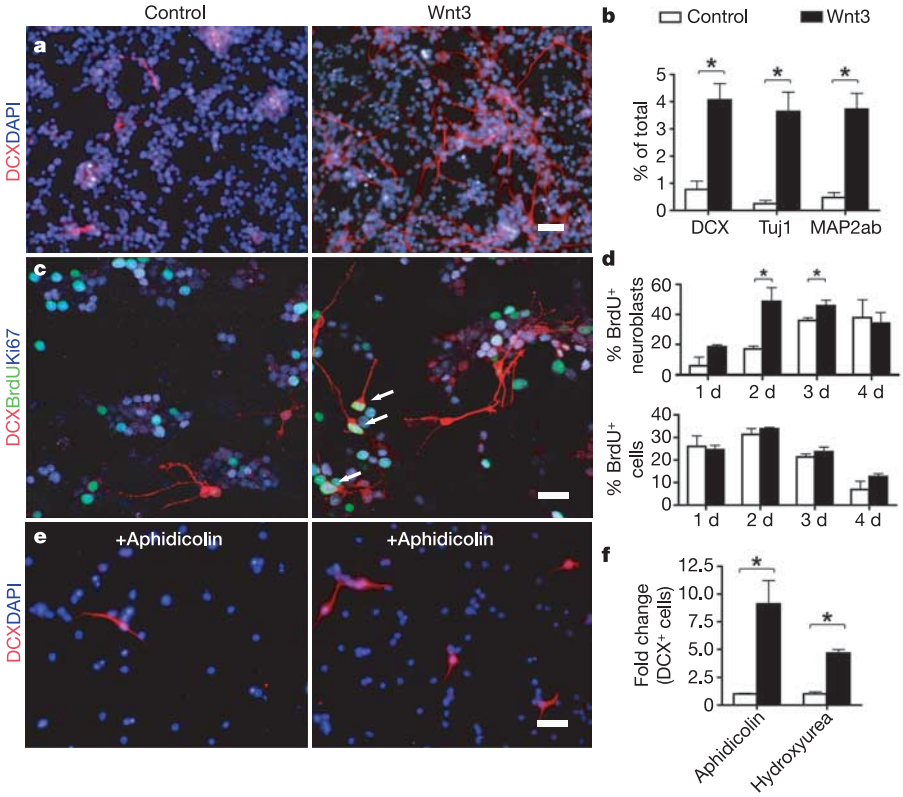

Figure 2 | Wnt3 is sufficient to increase neuronal production from AHP. **a**, Overexpression of Wnt3 in AHP leads to more cells expressing the early neuronal marker DCX (red). The DAPI counterstain is in blue. **b**, Quantitative analysis of three markers of neurogenesis after 4 d with or without Wnt3 overexpression. **c**, **d**, Analysis of proliferation by BrdU incorporation. Exposure to Wnt3 specifically increases cell division in the neuroblast population, whereas overall BrdU incorporation does not change significantly. Neuroblast proliferation was compared by determining the percentage of neuroblasts, namely DCX (red) and Ki67 (blue) double-positive cells, that had incorporated BrdU (green; arrows in **c**). **e**, **f**, Blocking cell division by aphidicolin (**e**, **f**) or hydroxyurea (**f**) shows that neuronal fate commitment (**e**; DCX, red) is increased 5–10-fold by Wnt3. The fraction of DCX-expressing cells in Wnt3-expressing AHPs is normalized to the DCX-positive fraction in control cells. Asterisks indicate a statistical difference between experimental groups ($P < 0.05$, Student's *t*-test). Error bars represent the s.e.m. Scale bars, 25 μm.

caused by the surgery, rats were injected with 200 mg per kg (body-weight) of BrdU daily for 7 d to label proliferating AHPs and their progeny. Rats were perfused 24 h after the final BrdU injection. Quantification of TUNEL-positive cells in the transduced dentate gyrus areas did not show any significant difference between the experimental groups (Supplementary Fig. 6). To evaluate potential changes in neurogenesis, we determined the number of BrdU-positive cells and the percentage of BrdU-positive cells that expressed DCX in the targeted (GFP-expressing) areas. As compared with rats injected with LV–GFP, those injected with LV–dnWnt showed a marked reduction in the number of BrdU-positive cells ($11{,}170 \pm 1{,}122$ and $2{,}751 \pm 281.4$ per mm^3, respectively, $P < 0.0001$, Student's *t*-test; Fig. 3c) and in the percentage of BrdU-positive cells that differentiated into DCX-positive neurons ($57.62 \pm 4.65\%$ and $31.03 \pm 5.48\%$, respectively, $P < 0.005$, Student's *t*-test; Fig. 3d), resulting in roughly an eightfold reduction in neurogenesis in the targeted areas ($6{,}302 \pm 571.7$ versus 813.6 ± 118.7 new DCX-positive neurons per mm^3, respectively; Fig. 3e).

To examine the effects of increased Wnt signalling on hippocampal neurogenesis, LV expressing Wnt3 (LV–Wnt3) or control LV–GFP was injected into the dentate gyrus of mature (15-week-old) rats

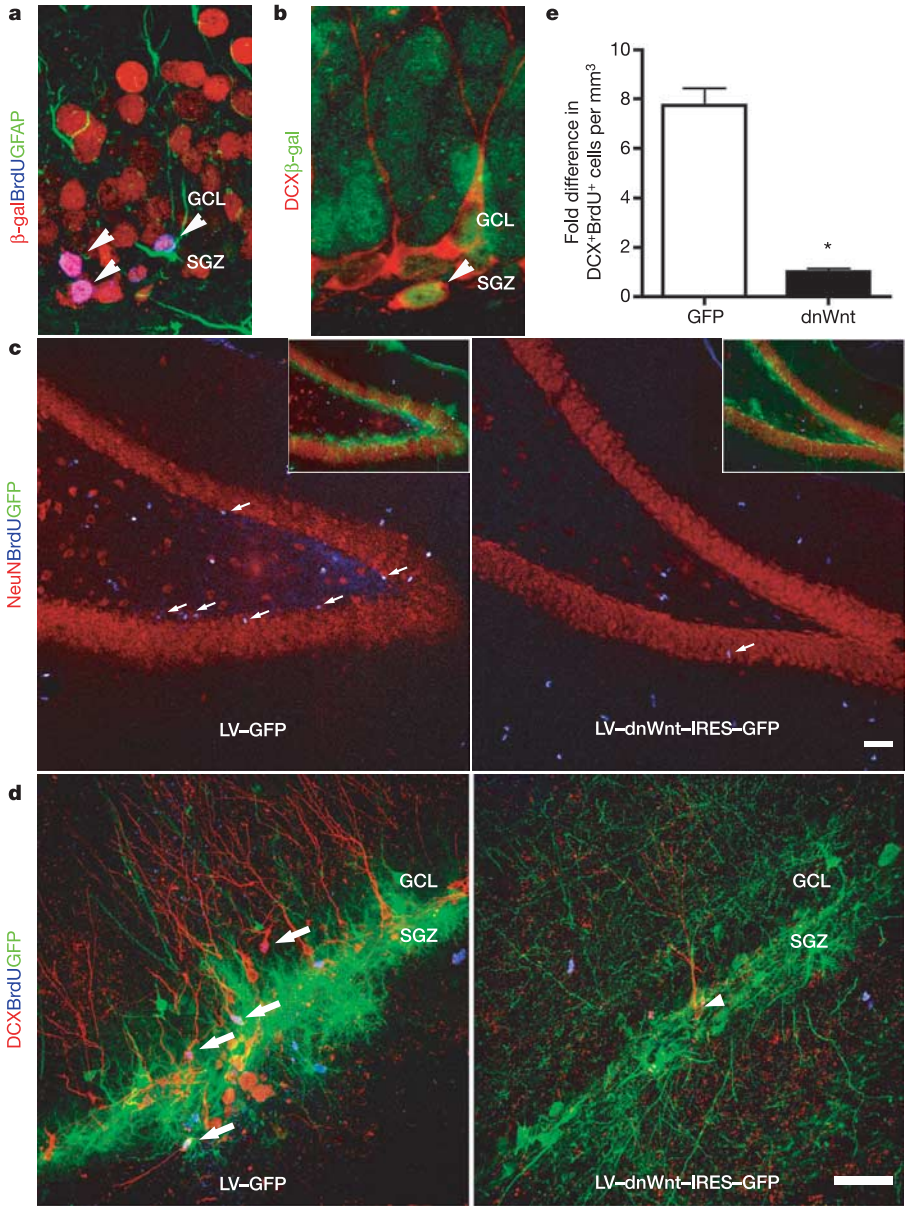

Figure 3 | Blocking Wnt signalling *in vivo* suppresses adult hippocampal neurogenesis. a, Wnt/β-catenin reporter activity (β-gal, red) in BrdU-positive progenitors (blue, arrowheads) in the SGZ of adult BATGAL mice. GFAP, green. **b**, Reporter activity (β-gal, green) in DCX-positive cells (red) with short processes oriented parallel to the dentate granule cell layer (arrowhead). This morphology is consistent with proliferating neuroblasts. **c**, Stereotactic injection of LV–GFP or LV–dnWnt (LV–dnWnt–IRES–GFP) into the adult dentate gyrus. Expression of dnWnt protein inhibits proliferation as determined by BrdU incorporation (blue, arrows) in the SGZ. Inset shows the viral infection, marked by expression of the GFP reporter (green). Mature dentate granule neurons are stained for NeuN (red). Scale bar, 50 μm. **d**, dnWnt inhibits expression of the neuroblast marker DCX (red, arrowhead). Newborn neurons double-labelled with DCX and BrdU (arrows) are almost eliminated as compared with controls. Scale bar, 70 μm. **e**. Differences in the number of newborn neurons. The rate of neurogenesis was calculated as the number of DCX and BrdU double-positive cells per volume of transduced dentate granule cell layer. The average number of new neurons per transduced volume in dnWnt-injected rats was used for normalization. Asterisks indicate a statistical difference between experimental groups ($P < 0.0001$, Student's *t*-test). Error bars represent the s.e.m.

($n = 6$). Injections of BrdU (100 mg per kg for seven consecutive days) were started 3 weeks after the viral injections, and rats were perfused 24 h after the final BrdU injection. In the transduced areas, rats injected with LV–Wnt3 showed a significantly higher percentage of BrdU-positive cells expressing DCX as compared with those injected with LV–GFP (70.29 ± 4.29% and 53.93 ± 6.76%, $P < 0.05$, Mann–Whitney rank sum test). These newborn immature neurons were often found in large clusters of more than eight DCX and BrdU double-positive cells in rats injected with LV–Wnt3 (Fig. 4a). We also observed a clear trend towards increased numbers of BrdU-positive cells in rats injected with LV–Wnt3 as compared with controls injected with LV–GFP (9,738.1 ± 2,460.1 and 6,211.1 ± 558.3 per mm^3, respectively). Together, these changes led to a doubling in neurogenesis in the targeted areas in rats injected with LV–Wnt3 (7,010.9 ± 1,963.7 versus 3,324.9 ± 543.0 new DCX-positive neurons per mm^3, $P < 0.05$, Mann–Whitney rank sum test; Fig. 4b), showing that increased Wnt signalling is sufficient to stimulate adult hippocampal neurogenesis.

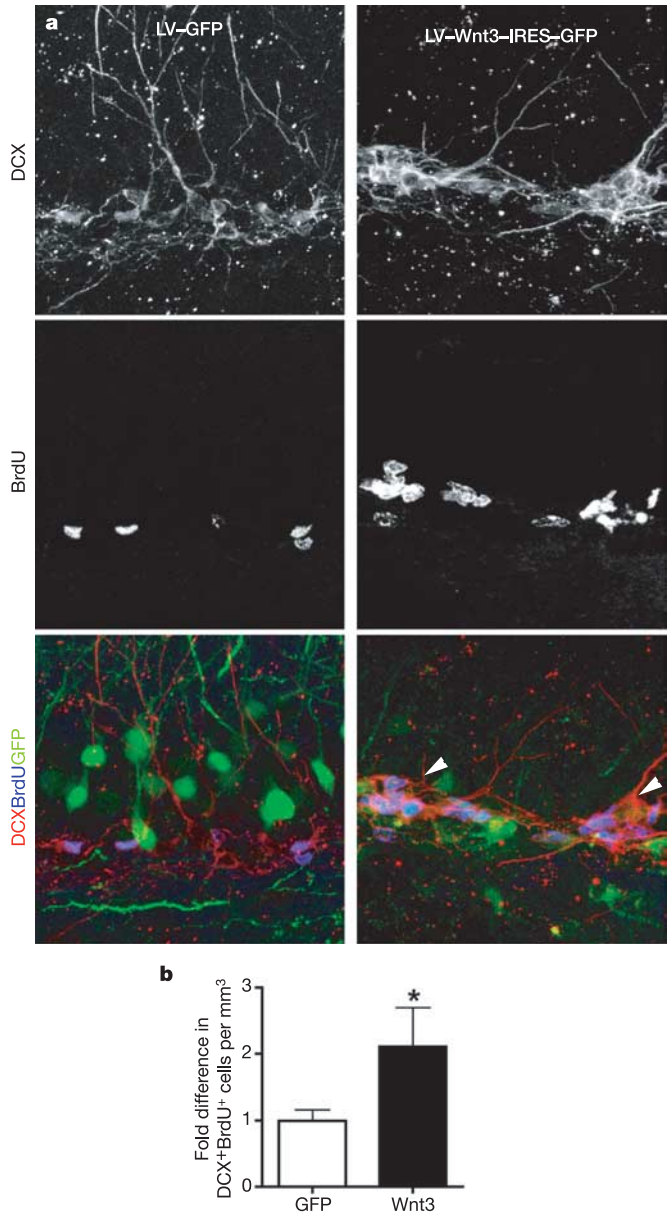

Figure 4 | Enhanced Wnt signalling *in vivo* increases adult hippocampal neurogenesis. a, LV–GFP or LV–Wnt3 was stereotactically injected into the adult rat dentate gyrus. Newborn neurons double labelled for DCX (red) and BrdU (blue) were found mostly in large clusters of about 8–10 cells (arrowheads) in the SGZ of rats injected with LV–Wnt3. Newborn neurons in rats injected with LV–GFP were predominantly found in smaller clusters of 2–4 cells. GCL, granule cell layer. Scale bar, 35 μm. **b**, Differences in the number of newborn neurons in control GFP and Wnt3-expressing hippocampi. The rate of neurogenesis in the experimental groups was calculated as the number of DCX and BrdU double-positive cells per volume of infected dentate granule cell layer. The average number of new neurons per infected volume in GFP-injected rats was used for normalization. Asterisk indicates a statistical difference between experimental groups ($P < 0.05$, Mann–Whitney rank sum test). Error bars represent the s.e.m.

Because genetic manipulation of the Wnt pathway often results in embryonic lethality, little is known about the role of Wnt signalling in the adult central nervous system. Our work identifies Wnt signalling as a regulatory pathway in adult hippocampal neurogenesis involved in the control of neuronal fate commitment and the proliferation of neuronally committed precursor cells. The extent to which the inhibition of Wnt signalling reduces adult hippocampal neurogenesis shows that Wnt signalling has a central role in these processes, although future experiments must address its relative contribution to proliferation and differentiation, as well as its potential involvement in later maturational events. There is evidence that precursor cell responses to Wnts can be modified by other signalling molecules[17–20]. We expect that such interactions are also involved in the regulation of AHPs given that adult hippocampal neurogenesis can be modulated, albeit to a far lesser extent, by *in vivo* inhibition of several other signalling pathways[21–23]. Finally, the discovery that hippocampal neurogenesis is almost extinguished by inhibition of Wnt signalling provides a powerful tool to study the as yet elusive role of adult neurogenesis and Wnt signalling in hippocampal function and plasticity.

METHODS

Cell culture. The isolation, characterization and culturing of AHPs used in this study have been described[24]. Fibroblast growth factor 2 was withdrawn to promote differentiation. The generation of AHPs expressing GFP has been described[4]. AHP cell lines expressing Wnt3 were generated by transduction with the pPIT–Wnt3 vector (Wnt3 cDNA was a gift from R. Nusse, Stanford University, CA). For proliferation studies, 10 μM BrdU (Sigma-Aldrich) was added for 2 h before fixation. To inhibit proliferation, we exposed AHPs to either 10 μg ml^{-1} of aphidicolin (Sigma) and 200 μM thymidine (Sigma) or 1 μM hydroxyurea (Sigma).

Primary astrocytes were isolated from rat hippocampus and cultured as described[4]. For co-cultures of AHPs and hippocampal astrocytes, AHPs were plated on a confluent astrocyte feeder layer at a density of 5×10^3 cells per cm^2 in serum-free conditions[4]. Recombinant sFRP2/3 protein (R&D Systems) was added at a concentration of 500 ng ml^{-1}.

Plasmids. We used the following reporter plasmids: Super8xTOPFLASH[9] (with a TCF/LEF-binding motif) or Super8xFOPFLASH[9] (mutant motif; a gift from R. Moon, University of Washington, Seattle, WA), TOPGAL (a gift from E. Fuchs, Rockefeller University, NY), and Renilla-Luc under the control of the human elongation factor 1 promoter (internal control). For expression of Wnt3, we used the retroviral vector pPIT (a gift from D. Schaffer, University of California, Berkeley, CA). For expression of dnLef1 (a gift from C. Fryer, Salk Institute, La Jolla, CA), we used the retroviral vector PMY–IRES–GFP[25], which contains an IRES–GFP cassette that allows identification of transduced or electroporated cells.

Luciferase assays. AHPs were electroporated with reporter plasmids by a nucleofector device (Amaxa). R-Luc was co-electroporated as an internal control. Forty-eight hours after electroporation, luciferase activity in 10 μl of lysis supernantant was measured with the Dual-Luciferase Reporter Assay System (Promega) and an LB 9501 luminometer (Bechtold).

Immunostaining. Tissue and cultured cells were fixed with 4% paraformaldehyde and processed for immunostaining as described[26].

Cell counting. The percentage of antibody-labelled cells was determined by evaluating 1,000 cells in at least five randomly chosen fields of view. To evaluate the proliferation of neuroblasts, we analysed 100–200 DCX-positive cells for expression of Ki67 and incorporation of BrdU. Experiments were done in triplicate. Statistical analysis was done with the nonparametric Mann–Whitney rank sum test or Student's *t*-test.

***In situ* hybridization.** *In situ* hybridization was done according to standard protocols[27]. Slides were subsequently processed for emulsion autoradiography using Kodak NTB-2 emulsion (Eastman Kodak). Sense controls yielded only nonspecific background labelling (data not shown).

Lentiviral vectors. The control vectors were CSC.cPPT.hCMV.GFP.Wpre (loss-of-function studies) and pRRL.SIN.cPPT.hPGK.GFP (gain-of-function studies)[14]. To generate the dnWnt–IRES–GFP vector, we cloned the cDNA for dnWnt upstream of the IRES and GFP and inserted the bicistronic cassette in place of the GFP sequence in the CSC.cPPT.hCMV.GFP.Wpre vector. To construct the Wnt3–IRES–GFP vector, we cloned the cDNA of Wnt3 upstream of the IRES and GFP and inserted the bicistronic cassette in place of the GFP sequence in the pRRL.SIN.cPPT.hPGK.GFP.Wpre vector. Concentrated LV stocks, pseudotyped by the vesicular stomatitis viral envelope, were produced as described[14]. Expression titres, determined on HeLa cells by FACS analysis, were 5×10^9 to 1×10^{10} transducing units per ml with an HIV-1 p24 concentration of 200–400 $\mu g\,ml^{-1}$.

***In vivo* neurogenesis experiments.** All rat procedures were done in accordance with protocols approved by the animal care and use committee of The Salk Institute for Biological Studies. For loss-of-function studies, 1.5 μl of vector concentrate were stereotactically injected into the right hippocampal dentate gyrus (AP −4, ML ±2, DV −3.5 from Bregma, nose piece −3.3) of adult female Fisher 344 rats aged 8–9 weeks ($n = 8$ per group). For gain-of-function studies, 1.5 μl of vector concentrate were stereotactically injected into the right hippocampal dentate gyrus of adult female Fisher 344 rats aged 15 weeks ($n = 6$ per group).

Stereology. Areas infected by LVs were identified by expression of GFP. Experimental groups showed comparable viral spread, as determined by the presence of GFP-expressing cells along the anterior–posterior axis of the dentate gyrus (720–960 μm). We counted BrdU-positive cells in the dentate granule cell layer in a one-in-six series of sections (240-μm apart) throughout the rostrocaudal extent of the infected dentate granule cell layer. NeuN immunoreactivity was used to measure the granule cell layer volume. The granule cell area was traced by using a StereoInvestigator semiautomatic stereology system (MicroBrightfield) and a 10 × objective. The proliferation rate was expressed as BrdU cells per volume of dentate granule cell layer[26].

Phenotype analysis. Sections (40-μm thick) containing dentate gyrus areas infected by LVs were identified by expression of GFP. All BrdU-positive cells in the subgranular and granular layer of the hippocampal dentate gyrus were analysed by confocal microscopy for labelling for both BrdU and DCX. The differentiation rate was determined by dividing the number of BrdU-positive cells that stained for DCX by the number of BrdU-positive cells evaluated.

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