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ORIGINAL ARTICLE

The α7 nicotinic acetylcholine receptors regulate hippocampal adult-neurogenesis in a sexually dimorphic fashion

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Abstract

Disruption in cholinergic signaling has been linked to many environmental and/or pathological conditions known to modify adult neurogenesis. The α7 nAChRs are in the family of cys-loop receptor channels which have been shown to be neuroprotective in adult neurons and are thought to be critical for survival and integration of immature neurons. However, in developing neurons, poor calcium buffering may cause α7 nAChR activation to be neurotoxic. To investigate whether the α7 nAChR regulates neurogenesis in the hippocampus, we used a combination of mouse genetics and imaging to quantify neural stem cell (NSC) densities located in the dentate gyrus of adult mice. In addition, we considered whether the loss of α7 nAChRs had functional consequences on a spatial discrimination task that is thought to rely on pattern separation mechanisms. We found that the loss of α7 nAChRs resulted in increased neurogenesis in male mice only, while female mice showed increased cell divisions and intermediate progenitors but no change in neurogenesis. Knocking out the α7 nAChR from nestin$^+$ NSCs and their progeny showed signaling in these cells contributes to regulating neurogenesis. In addition, male, but not female, mice lacking α7 nAChRs performed significantly worse in the spatial discrimination task. This task was sexually dimorphic in wild-type mice, but not in the absence of α7 nAChRs. We conclude that α7 nAChRs regulate adult neurogenesis and impact spatial discrimination function in male, but not female mice, via a mechanism involving nestin$^+$ NSCs and their progeny.

Keywords α7 nicotinic acetylcholine receptors · Adult neurogenesis · Nestin · Pattern separation · Spatial discrimination · Neural stem cells · Sexually dimorphic

Introduction

Hippocampal adult-neurogenesis is a life-long process whereby neural stem cells (NSCs) located in the subgranular zone of the mammalian dentate gyrus divide, differentiate, mature and integrate into the local circuitry (Aimone et al. 2014; Altman and Das 1965; Bonaguidi et al. 2012; Eriksson et al. 1998; Goncalves et al. 2016; Spalding et al. 2013). NSCs are usually quiescent, while immature granule cells differ from sparsely activated (Chawla et al. 2005; Jung and McNaughton 1993) mature granule cells by responding to the normally inhibitory neurotransmitter GABA with excitation, and being prone to long-term potentiation (Esposito et al. 2005; Ge et al. 2007; Schmidt-Hieber et al. 2004). Despite some controversy (Sorrells et al. 2018), most data agree that adult neurogenesis occurs at a rate of 1.75% per year in humans (Spalding et al. 2013) and persists through aging (Boldrini et al. 2018), and can be modified by environmental factors such as exercise (van Praag et al. 1999) and social isolation (Holmes 2016). NSCs can be induced to differentiate through excitation (Deisseroth et al. 2004), but activated neurons may differentiate into astrocytes, leading to decreases in the NSC pool and a loss of neurogenic potential (Encinas et al. 2011). Pathogenic conditions that impact adult neurogenesis (Hollands et al. 2016; Jessberger and Parent 2015; Kang et al. 2016) are also linked to the disruption in cholinergic innervation. The dentate gyrus receives input from the basal forebrain through GABAergic and cholinergic projection neurons (Bao et al. 2017; Cooper-Kuhn et al. 2004), and the infusion of fibroblasts releasing

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acetylcholine (ACh) into the hippocampus reverses cognitive decline (Dickinson-Anson et al. 2003). Anti-cholinesterases, which inhibit the breakdown of acetylcholine, increase adult neurogenesis (Kotani et al. 2006) and promote survival of immature neurons through the α7 subtype of nicotinic ACh receptors (nAChRs) (Kita et al. 2014). By contrast, constitutive activation of the α7 nAChR results in increased apoptosis in the CNS of neonatal mice (Orr-Urtreger et al. 2000).

The α7 nAChR, located pre- and post-synaptically, contributes to cognition, attention, learning and memory (Hasselmo 2006; Levin 2012) through fast signal transduction and a higher level of Ca^{2+} flux than other nAChRs. Once thought to be strictly homomeric, they are now known to also form α7β2 heteromeric receptors (Khiroug et al. 2002; Wu et al. 2016) and couple to heterotrimeric G proteins (King et al. 2017). They are located on a diverse array of cells in the hippocampus (Albuquerque et al. 1997; Rubboli et al. 1994), and their loss, inhibition, or duplication contributes to multiple neuropsychiatric and neurodegenerative disorders (Gillentine et al. 2017; Graham et al. 2002). In particular, the α7 nAChR is implicated in diseases such as epilepsy, autism, schizophrenia (Adams et al. 2012), and Alzheimer's disease (Dineley et al. 2015). These disorders also show altered adult neurogenesis in the subgranular zone of the dentate gyrus (Hollands et al. 2016; Jessberger and Parent 2015; Kang et al. 2016). The α7 nAChR may also be neuroprotective (Hernandez et al. 2010) in adult neurons; activation of this receptor guides the normal maturation and integration of immature neurons (Campbell et al. 2010), thereby promoting their survival.

Even though the presence of the α7 nAChR promotes maturation and survival of immature neurons, its role in regulating adult neurogenesis remains poorly elucidated. For instance, while it is known that in Alzheimer's disease there is loss of cholinergic cells in the basal forebrain with concurrent loss of innervation of the hippocampus, studies support an increased neurogenesis (Jin et al. 2004; Yu et al. 2009), and an increase in α7 nAChR expression in astrocytes (Teaktong et al. 2003). Loss of the α7 nAChR does result in decreased $GABA_A$ receptors in the hippocampus (Adams et al. 2012), but this opens up the possibility of indirect effects on adult neurogenesis through interneurons. In addition, poor calcium buffering in developing neurons may result in α7 nAChR activation being neurotoxic, rather than neuroprotective, at early stages of adult neurogenesis, as seen when the α7 nAChR is constitutively active in development (Orr-Urtreger et al. 2000). The difficulty in discerning the impact of α7 nAChR activation on adult neurogenesis may lie in the differing and even contradictory actions this receptor has depending on the timing and location of its activation (Gu and Yakel 2011), or potentially by affecting a subset of local cells (Paez-Gonzalez et al. 2014), as occurs in the subventricular zone, another area of adult neurogenesis in the mouse where nAChRs may be important for neuronal proliferation and survival (Mechawar et al. 2004). Neuroprotective functions of α7 nAChRs may only be revealed through stress, which acutely increases ACh release (Imperato et al. 1991). We hypothesized that α7 nAChRs might protect and/or preserve the NSCs and regulate adult neurogenesis. To test this, we used 3D imaging to investigate NSC densities, as well as behavioral performance on a spatial discrimination task to investigate NSC function. We found that blocking or removing the α7 nAChR increased neurogenesis overall but decreased NSC pools and spatial discrimination in adult male, but not female, mice. These results demonstrate a critical role of the α7 nAChRs in the regulation of adult neurogenesis in a sexually dimorphic way.

Materials and methods

Mouse strains and experimental crosses

To identify the NSCs in the subgranular zone of the dentate gyrus, we initially obtained mice from Jackson Laboratory (JAX), including the lines Nestin-Cre/ERT2 (JAX #016261), C57BL/6-Tg(Nes-cre/ERT2)KEisc/J (Lagace et al. 2007). In addition, we obtained ROSATdTomato (JAX #007914, B6.Cg-*Gt(ROSA)26Sor*$^{tm14(CAG-tdTomato)Hze}$/J) as well as α7 nAChR KO (JAX #003232, acra7-B6.129S7-*Chrna7*^{tm1Bay}/J) and α7 $nAChR^{flox}$ mice (JAX #026965, B6(Cg)-*Chrna7*$^{tm1.1Ehs}$/YakelJ (Hernandez et al. 2014)). These mice were later bred and crossed in house in the Comparative Medicine Branch at NIEHS. Mice were not back crossed; instead new breeders were periodically ordered from JAX. Mice were genotyped by tail snip that was submitted to Transnetyx for genotyping. The crosses we used included Nestin-Cre/ERT2 × ROSATdTomato (hemi/het); Nestin-Cre/ERT2 × ROSATdTomato × α7 nAChR KO and Nestin-Cre/ERT2 × ROSATdTomato × α7$nAChR^{flox}$ mice. Both male and female mice were used. In all cases experiments were done using littermate controls.

Mouse housing and treatments

Mice were maintained on a 12-h light:12-h dark cycle with food and water supplied ad libitum except as noted for behavior experiments. All mice were housed in multiple sibling groups. All sibling groups receiving injections were housed in a private cubicle away from common housing. Mice used for behavior were housed in reverse light/dark housing. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human

participants performed by any of the authors. All procedures were approved and performed in compliance with the NIEHS/NIH Humane Care and Use of Animals Protocols.

To induce tdTomato expression in nestin^{+} cells, adult mice hemizygous for Nestin-Cre/ERT2 received 5 consecutive daily intraperitoneal injections (I.P.) of tamoxifen (0.15 mg/g in research grade corn oil). Mice were euthanized a week later. α7nAChR^{flox} cross: Mice were injected with tamoxifen as indicated above to induce the removal of α7 nAChR expression and induce tdTOMATO expression in nestin^{+} cells. Subsequent to injections, mice were maintained in home cages for nine weeks before euthanasia. EdU: EdU was administered in 4 I.P. shots of 41.1 mg/kg body weight given at 2.5 h intervals (Song et al. 2012). Mice were euthanized the following day.

Mice used for behavior began diet restriction and daily handling 10 days prior to behavioral testing. Mice were maintained at 80–85% base weight.

Tissue preparation

Mice were deeply anesthetized using 0.2 ml phenobarbital (FatalPlus). After testing for deep anesthesia, tissue was cleared using ice cold 0.1 M phosphate buffer (PB), pH 7.4, with 0.1% heparin. Mice were transcardially perfused in ice cold 4% paraformaldehyde (PFA). Brains were post-fixed overnight at 4 °C in 4% PFA and then washed in 0.1 M PB. Washed brains were cryoprotected using a 30% sucrose in 0.1 M PB at 4 °C until equilibrated. Brains frozen in tissue freezing medium (TFM; Triangle Biomedical Sciences) were cryosectioned into 50-µm free-floating coronal sections using a Leica CM305S cryostat. Every sixth section of a hippocampus was processed for immunolabeling. Sections were placed in block buffer (0.1 M PBS with 5% goat serum and 0.1% Triton X-100) for 2 h at room temperature or stored at 4 °C until immunofluorescence.

Immunostaining

All immunostaining was done with tissue kept in the dark. 50-µm free-floating sections were incubated with primary antibodies overnight at 4 °C. Negative controls lacking primary were done to confirm specificity of staining. Tissue was triple washed and incubated in secondary antibody for 2 h at room temperature or overnight at 4 °C. After secondary antibody, tissue was triple washed. Where cell nuclei were stained, the final wash contained DAPI (2 µg/ml). Tissue was mounted onto a SuperFrost Plus slide using Prolong Diamond Anti-fade Mounting Media (Molecular Probes). After slides had dried overnight, edges were sealed. Slides were stored at 4 °C in the dark when not being imaged.

Antibodies

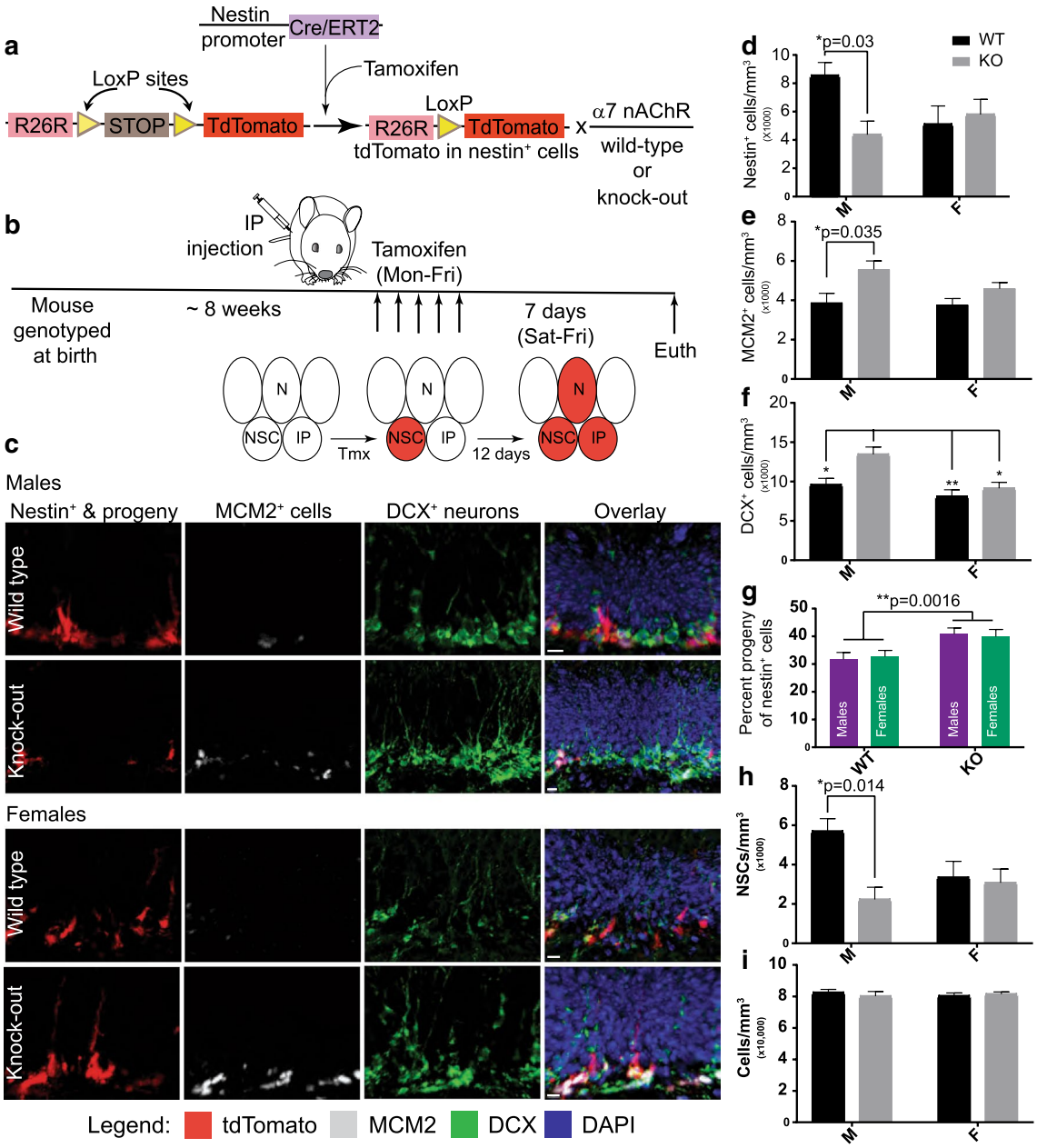
Primary antibodies used include rabbit anti-Prox1 (1:100 cat# MA85654, Millipore, Billerica, MA), guinea pig anti-doublecortin (DCX)(1:500 Cat# AB2253, Millipore, Billerica, MA), rabbit anti-NeuN (1:500 cat# 177487 Abcam, MA, USA), MCM2 (1:500 cat# ab4461 Abcam, Cambridge, MA). Secondary antibodies were Alexa Fluor 488 goat anti-guinea pig (cat#11073, Invitrogen, Carlsbad, CA, USA), Alexa Fluor 647 goat anti-chicken (cat# A21449, Invitrogen, Carlsbad, CA, USA), Alexa Fluor 647 goat anti-rabbit (cat# 150087, Abcam, Cambridge, MA, USA), Alexa Fluor 568 goat anti-guinea pig (cat# ab175714, Abcam, Cambridge, MA), Alexa Fluor 488 goat anti-chicken (cat#150169, Abcam, Cambridge, MA, USA). All secondary antibodies were used at 1:500. Sections were treated for EdU expression using the Click-IT® EdU analysis kits (Thermo Fisher Scientific) with Alexa Fluor 488 antibody. DAPI (cat# 508741, Millipore) was used at 2 µg/ml.

Imaging

Tile scan, Z-stack images of 50-µm-thick brain sections were collected on a Carl Zeiss LSM 710, LSM780, or LSM 880 confocal microscope (Carl Zeiss Inc, Oberkochen, Germany) using one of the following objective lenses: Plan-Apochromat 20 ×/0.8 or the Plan-Apochromat 63 ×/1.4 Oil DIC M27. For the far red channel, a 633-nm laser line was used for excitation of an Alexa 647 secondary antibody using a band pass filter of 640–717. For the red channel, a 561-nm dpss laser was used for excitation of Alexa 568 while a 561–639 filter collected the emission. For the green channel, a 488 nm ArKr laser line was used for excitation of the Alexa 488 secondary while a bandpass 503–552 filter was used for collection of the emission signal. For the blue channel, a 405-nm diode laser line was used for excitation of DAPI while a bandpass 415–503 filter was used for the emission. Furthermore, images were taken with the pinhole of the longest emission wavelength set to 1 airy unit, a zoom setting of 1, and with line averaging set to 2.

Image analysis

All images captured in Zen Black 2012 were stitched (if tiled) and imported into an Imaris (Bitplane, Oxford Industries) analysis arena. To determine cell densities, three-dimensional (3D) images were first analyzed using a shape function to limit analysis to the dentate gyrus within the section. Cells were then counted using a spots function. Where possible cell counts were batched. Counted cells were confirmed as having a cell nucleus surrounded by the fluorescent marker. Mice in which staining was unclear were eliminated from analysis. Data were recorded in Excel spreadsheets for



further analysis, where densities were calculated. For measuring distance cells had moved inward from the edge of the hilus, regions of interest were selected and maximum intensity projections were imported into ImageJ. Using ImageJ, cells were marked and image was cropped at the edge of the hilus to allow measurement of distance. Measurements were imported into Excel and converted into microns. For tertiary dendrites, 63 × images were imported into Imaris and the filament function was used to manually trace dendritic structure in five randomly selected cells. Total filament length and number of tertiary dendrites were imported into Excel for further analysis. For presentation, images were cropped using Imaris software, and captured via a snapshot feature. Images were modified only by adjusting brightness and contrast to optimize the full dynamic range of the fluorescent signal.

Delayed non-matching to prior (DNTP) task in radial-arm maze

This task was modified from Clelland et al. (2009). Mice were habituated to the testing room and maze one day prior to the task beginning. Habituation consisted of placing the mouse in a clean 8-arm maze (Med Associates) with all arms loaded with chocolate flavored 0.14 mg treats (Lederle et al. 2011) for 8 min. In the afternoon mice had a secondary

◂**Fig. 1** The α7 nAChR regulates adult neurogenesis in adult mice differently in males and females. **a** Administration of tamoxifen to nestin-Cre/ERT2 mice crossed with ROSAtdTomato will induce expression of tdTomato in nestin$^+$ cells, allowing us to label NSCs and their progeny in adult mice. These mice were crossed with α7 nAChR knock-out mice, allowing us to quantify NSCs and their progeny in adult mice in sibling wild type and α7 nAChR knock-outs. **b** Mice were genotyped at birth and sibling knock-out and wild-type mice were raised to adult (8 weeks). At 8 weeks mice were injected with tamoxifen for 5 days (0.15 mg/g in corn oil) to induce tdTomato expression in nestin$^+$ NSCs. Mice were euthanized 1 week after the final injection. The entire timeline was 12 days from first injection until euthanization, which provided sufficient time for a percentage of the initial NSCs fluorescing with tdTomato to divide and differentiate into intermediate progenitors and then immature neurons. **c** 50 µm sections of mouse hippocampi were collected and immunolabeled with rabbit anti-MCM2 (Abcam, 1:500; to mark intermediate progenitors) and guinea pig anti-DCX (Millipore, 1:500; to mark immature neurons), as well as DAPI (2 µg/ml). Images were collected using a Plan-Apochromat × 20/0.8 lens and Zen black software on a Zeiss 710 confocal microscope (Carl Zeiss Inc, Oberkochen, Germany) and analyzed in 3D using Imaris (Bitplane, Oxford industries). Scale bar: 10 µm. **d** Male mice had a significant reduction in nestin$^+$ cells (NSCs and progeny) whereas female mice did not ($p=0.03$). **e** Intermediate progenitors are measured by quantifying MCM2$^+$ cells. There is an overall increase in intermediate progenitors in mice lacking the α7 nAChR ($p=0.0053$), which is largely driven by the increase in progenitors in male mice only ($p=0.035$), since females alone were not significantly different. **f** Immature neurons can be measured by quantifying DCX expression. Immature neurons vary by sex ($p=0.0027$) and genotype ($p=0.0091$). Male mice lacking the α7 nAChR have an increased number of immature neurons ($p=0.024$) compared to male wild-type mice, whereas in females there is no difference in density of immature neurons in wild type versus knock-out mice. **g** Progeny can be measure by colocalization of tdTomato with MCM2 or DCX. There is a significant increase in the percentage of nestin$^+$ cells that had divided and differentiated into intermediate progenitors or immature neurons over the 12 day duration of the experiment in mice lacking the α7 nAChR regardless of sex ($p=0.0016$). **h** Removing progeny from nestin$^+$ cells counts reveal that the non-activated NSC population is decreased significantly in male mice only after loss of the α7 nAChR ($p=0.014$). **i** The overall density of cells in the dentate gyrus remains steady across all sexes and genotypes. Statistical analysis uses two-way ANOVA with Tukey's multiple comparison test and Graphpad Prism 7.0 Software. Experimental groups contained 5–7 mice per group including male wild type, male knock-out, female wild type, female knock-out. In each mouse, four hippocampi sections were analyzed to provide a mean density per mouse. Data expressed as mean ± SEM. Asterisk indicates $*p<0.05$, $**p<0.01$

habituation, consisting of placing the mouse in a single arm of the 8-arm radial maze with 2 baited arms open for 2 min. During the task, mice received sixty (60) two-phase behavior tests over the course of 15 consecutive days (i.e., 4 tests per day). Tests were randomly ordered. In each test, mice were placed in an 8-arm radial maze to find a sample treat. The maze was then thoroughly cleaned. The mouse was returned to the maze within 2 min and tasked with finding a treat in a novel arm at a variable distance from the original arm. In both the sample and the test phase, the mouse was alone in a sound-proofed area under red lighting, while the task was recorded using Ethovision software (Noldus Information Technology). Choice of novel or familiar arm entry was analyzed by sex and genotype. Data were recorded in an Excel sheet for analysis. Statistics were generated using SPSS and Graphpad Prism.

Experimental design and statistical analysis

Power analysis was performed during experimental design. In behavior, male and female sibling groups were run in equal numbers within each group; the order of tasks was randomized. All behavior experiments began at 9 a.m. For image analysis, raw densities were computed from 4 or more dentate gyrus per mouse. Following image analysis by Imaris and Excel, SPSS and/or Graphpad Prism 7.0 software was used for statistical analysis and graphical illustration. Mean data per mouse was computed. A two-tailed Student's *t* test was used to analyze two independent samples. A two-way ANOVA was used to establish statistical significance of data being analyzed for effect of genotype and sex. Post hoc analysis included Sidak's multiple comparison to compare means within each row; Tukey's multiple comparisons to compare all means with every other mean. Data are presented at mean ± SEM. In all cases a value of $p<0.05$ was considered statistically significant.

Results

α7 nAChRs regulates adult neurogenesis in the dentate gyrus of male but not female mice

To determine whether there was an impact on adult neurogenesis after the loss of α7 nAChR, we studied both male and female adult α7 nAChR KO mice that were at least 8 weeks old, a time at which the dentate gyrus has been shown to be stable (Cushman et al. 2012). Using nestin, a marker for neural stem cells in the hippocampus, to establish the presence of neural stem cells, we crossed α7 nAChR KO mice with nestin-Cre/ERT2 × ROSAtdTomato mice and, quantified tdTomato$^+$ cells 12 days after the initial tamoxifen injection (Fig. 1). All the cells marked by tdTomato will have been nestin$^+$ NSCs at the time of the first injection. To verify expression of tdTomato in neural stem cells, at the beginning and periodically throughout the study, nestin-Cre/ERT2 × ROSAtdTomato mice were injected with saline and wild-type siblings were injected with tamoxifen at the same levels used in the experiments; these controls did not show the presence of fluorescent NSCs (data not shown). During these 12 days, a proportion of the nestin$^+$ NSCs will become activated, divide and differentiate first into intermediate progenitor cells and then later into immature neurons. Thus, the labeled cells are nestin$^+$ cells and their progeny.

Since immature neurons go through an activity-dependent critical period from 2 to 4 weeks (Tashiro et al. 2006), this time frame captures adult neurogenesis prior to this critical period.

Our data show that there is a significant decrease in nestin$^+$ NSCs and their progeny in adult male (but not female) mice lacking the α7 nAChR (Fig. 1d; interaction: $F(1,22) = 5.16$, $p = 0.03$, $n = 6$ mwt, 6 fwt, 7 mko, 7 fko; Sidak's, $p = 0.03$ $n = 6$ mwt, 7 mko). Quantification of intermediate progenitor levels, marked by MCM2, reveal there is an overall increase in intermediate progenitors (Fig. 1e: main effect of genetics: $F(1,18) = 10.4$, $p = 0.0053$). This increase is driven by the males (Sidak's, $p = 0.035$, $n = 6$ mwt, 5 mko) since females taken alone do not show any significant difference. Quantification of immature neurons, marked by DCX, reveal a significant difference in densities (Fig. 1f) when considered by sex [$F(1,19) = 11.83$ $p = 0.0027$, $n = 6$ mwt, 6 fwt, 5 mko, 5 fko] or by genotype [$F(1,19) = 2.83$, $p = 0.0091$]. Interestingly, we see an increase in immature neurons in males (Sidak's, $p = 0.024$, $n = 6$ mwt, 5 mko) when comparing knock-out to wild type, but not in females.

Since the progeny of nestin$^+$ NSCs can also be expected to stain positive for MCM2 or for DCX, we were able to first quantify the percentage of nestin$^+$ cells that had been activated and diffierentiated, and then eliminate these cells from our tdTomato$^+$ cell counts. This was done in order to quantify non-activated NSCs. Doing so revealed that there was a significant increase in the percent of progeny of nestin$^+$ cells regardless of sex [Fig. 1g: $F(1,18) = 11.7$, $p = 0.0030$, $n = 6$ mwt, 6 fwt, 5 mko, 5 fko]. In addition, there was a significant decrease in non-activated nestin$^+$ NSCs in males only [Fig. 1h: interaction: $F(1,18) = 5.026$, $p = 0.0378$, $n = 6$ mwt, 6 fwt, 5 mko, 5 fko; Sidak's, $p = 0.014$, $n = 6$ mwt, 5 mko]. Despite these changes, there is no significant impact on the total density of cells in the dentate gyrus by sex or genotype (Fig. 1i).

Taken together these data suggest that the loss of α7 nAChR results in an increase in activation of NSCs in both males and females. In male mice these activated NSCs become intermediate progenitors and then immature neurons. However, in female mice the same mechanism is not evident, despite there being an overall increase in progeny, supporting a more complex pathway.

To test whether lower NSC populations were correlated with an altered level of neurogenesis, we measured cell division using EdU (Fig. 2). In this case, we also considered heterozygote mice. As expected, there was a main effect of sex [Fig. 2c: $F(1,30) = 4.233$, $p = 0.0440$, $n = 6$ mwt, 6 mhet, 6 mko, 6 fwt, 6 fhet, 6 fko]. Analysis of male mice showed that the lower density of NSCs in α7 knock-out mice (Sidak's, $p = 0.028$, $n = 6$ mwt, 6 mko) was dose dependent, with heterozygotes falling midway between knock-out and wild type. In contrast in female mice, there was no effect of genotype. Actively dividing cells labeled with EdU showed no effect by sex, and there was an overall increase in adult neurogenesis in α7 nAChR knock-out mice compared to wild type [Fig. 2d: main effect of genetics: $F(2,30) = 3.94$, $p = 0.03$, $n = 6$ mwt, 6 mhet, 6 mko, 6 fwt, 6 fhet, 6 fko]. In addition, the percent of nestin$^+$ cells that were actively dividing is increased overall [$^{**}F(2,30)$, $p = 0.0043$] as well as when considered separately in both males (Sidak's, $p = 0.039$) and females (Sidak's, $p = 0.029$) (Fig. 2e). In male mice, this would support the hypothesis that NSC populations are decreased because cells are leaving the stem cell pool through increased adult neurogenesis. However in female mice, cell division may be increased but NSC populations remain the same.

Campbell et al. (2010) have shown that loss of the α7 nAChR results in delayed maturation of newborn neurons, as well as less overall dendritic length and arborization. To investigate maturation of immature neurons, distance inward from the granule cell-hilus border and dendritic structure was measured through selecting a region of interest, lacking curvature, in the upper arm of the dorsal dentate gyrus (Fig. 3a). Within ImageJ, this region of interest was positioned with the granule cell layer–hilus border at one edge. Distance from the edge of each DCX$^+$ granule cells was calculated, and averaged from four images per mouse. Both the average distance inward from the edge of the hilus into the granule cell layer and the percent of DCX$^+$ neurons that were greater than 10 μm from the edge of the hilus were calculated. No significant difference existed between wild type and α7 nAChR knock-out mice in the distance of DCX$^+$ neurons from the edge of the hilus (Fig. 3b, c).

The tertiary dendritic structure of five randomly selected DCX$^+$ neurons was traced by using the filament function in Imaris on a 3D 63 × z-stacked image (Fig. 3d–f). Accuracy was confirmed through 3D rotation of the image. Cells in which the dendritic structure immediately exited the section were excluded, and total filament length and number of tertiary filaments were measured. We found that there was no significant difference in the number of tertiary dendrites in the α7 knock-out mice [$F(1,22) = 3.67$, $p = 0.0685$, $n = 6$ mwt, 6 fwt, 7 mko, 7 fko; Fig. 3g]. However, there was a significant increase in total dendritic length in α7 nAChR knock-out mice [$F(1,22) = 5.051$, $p = 0350$, $n = 6$ mwt, 6 fwt, 7 mko, 7 fko; Fig. 3h] when both sexes are considered together. One explanation for this unexpected finding is that immature neurons of α7 nAChR knock-out mice, in failing to mature at a normal rate, are retaining DCX expression for longer than their wild-type siblings, resulting in an age differential in neurons selected.

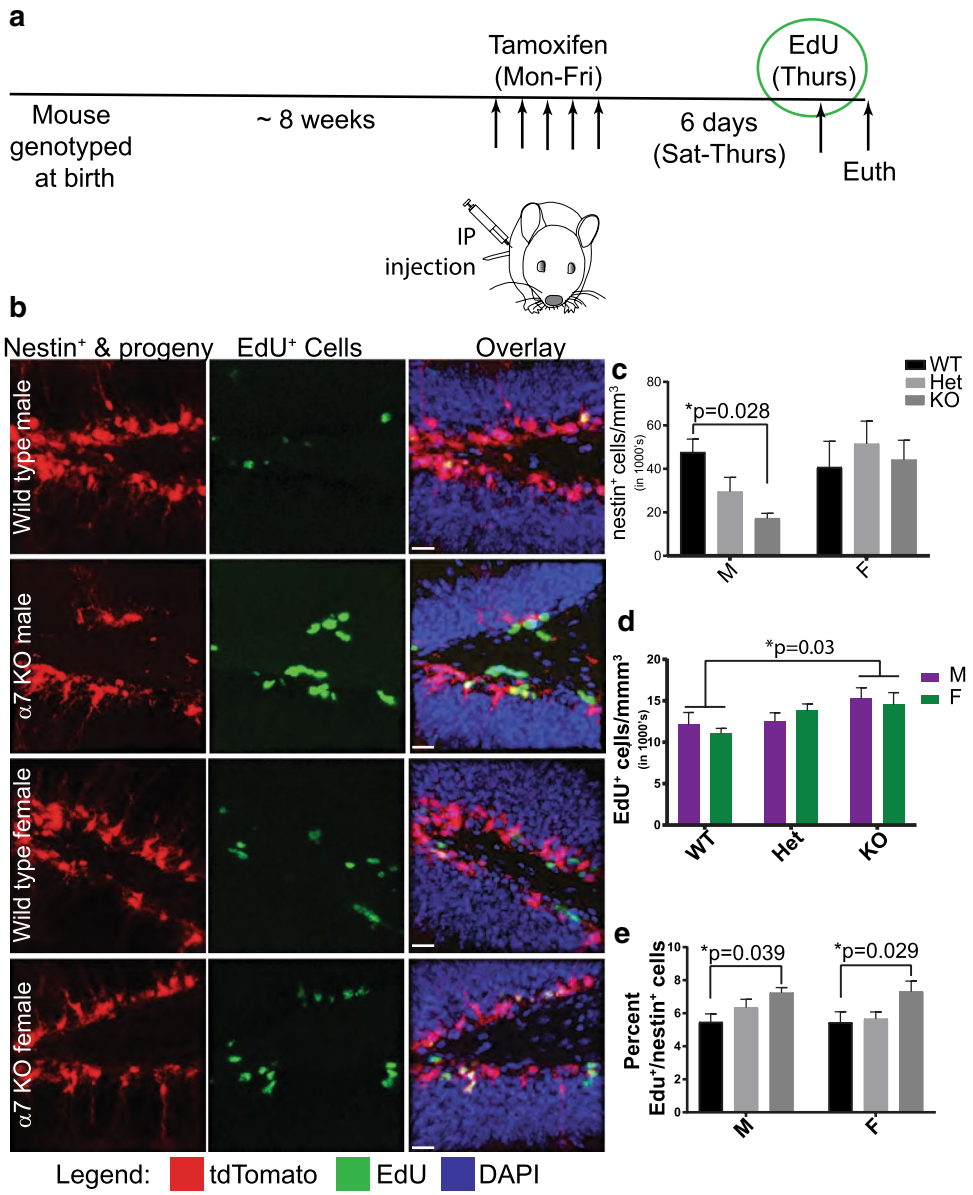


Fig. 2 Adult neurogenesis is increased in adult mice lacking the α7 nAChR, while NSC populations show dose-dependent decrease in males only. **a** Mice were injected with tamoxifen (0.15 mg/g) on the first Monday through Friday after reaching 8 weeks of age. Mice were then dosed with EdU (4 doses 41.1 mg/kg, IP injection at 2.5 h intervals) on the following Thursday, 1 day prior to euthanization. **b** 3-D reconstruction of dentate gyrus from z-stack of free-floating sections of brains of α7 nAChR knock-out mice testing for actively dividing cell numbers (indicating neurogenesis) using EdU. Images are grouped by sex and genotype, including male control mouse, male transgenic mouse, female control mouse and female transgenic mouse (heterozygotes not shown). TdTomato was expressed in NSCs following tamoxifen injections, Alexa 488 green fluorescence was used to label EdU within actively dividing cells, and nuclei were identified by DAPI (2 µg/ml). Scale bar: 20 µm. **c** Analysis shows that males and female differ ($p=0.0440$, $n=35$). Males show a significant decrease in NSC populations ($p=0.027$, $n=17$) as the copies of α7 nAChR declined; and knock-out mice have a significantly lower density of NSCs than wild type ($p=0.021$, $n=5$ wt, 6 ko). Female mice show no relationship between loss of the α7 nAChR and NSC numbers ($p=0.766$, $n=18$). **d** The loss of α7 nAChR caused an overall increase in actively dividing cells with no effect of sex ($p=0.0465$, $n=35$, two-way ANOVA). **e** Both male ($p=0.039$) and female ($p=0.029$) mice showed an increase in the number of nestin$^+$ cells that were also identified with EdU as currently undergoing cell division. *Asterisk* indicates $*p<0.05$. Statistics analysis using two-way ANOVA with Tukey's post hoc using SPSS and Graphpad Prism 7.0 Software. Data in bar graph expressed as mean ± SEM

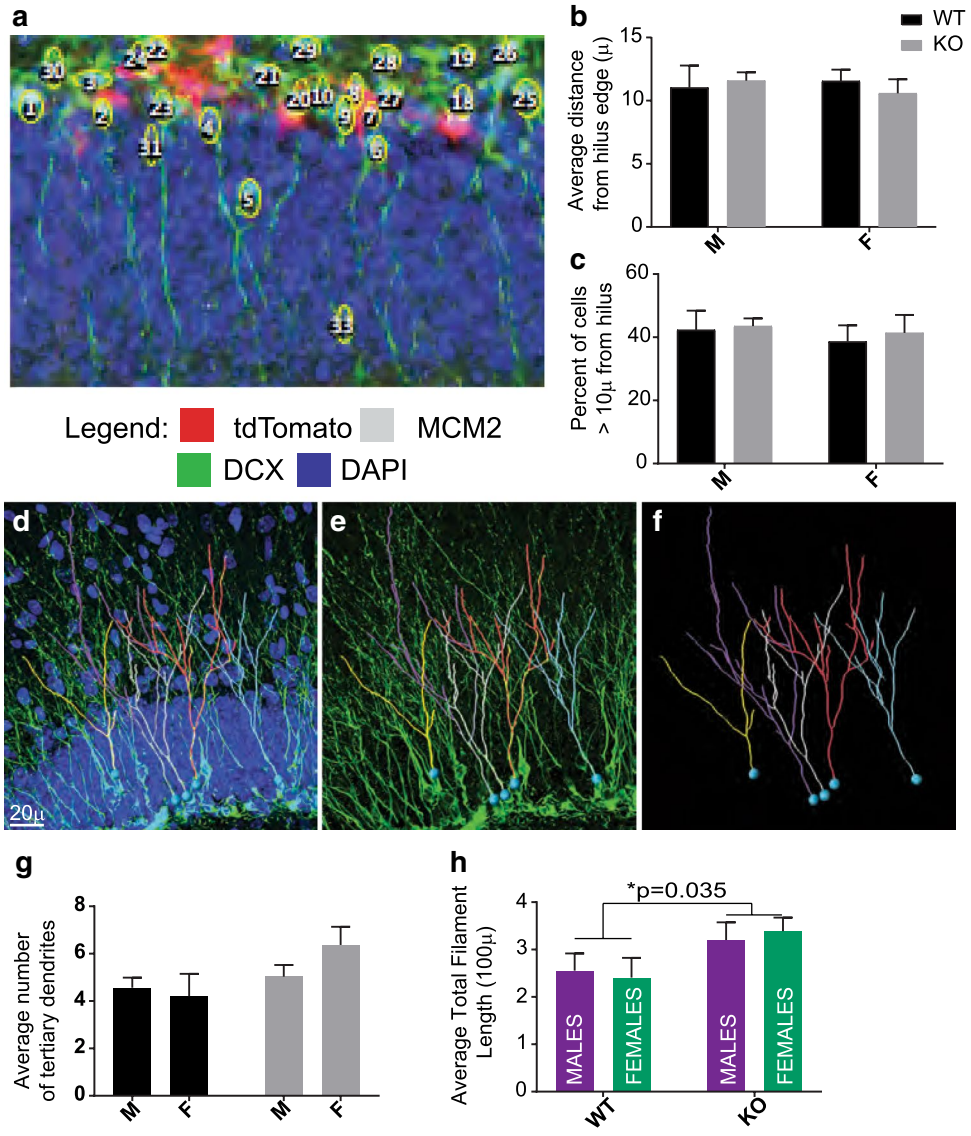


Fig. 3 Effect of loss of α7 nAChRs on maturation of neurons. **a** The distance that immature neurons had moved in from the hilus was measured to determine if maturation was increasing. To do this, maximum projection images were analyzed in ImageJ by marking all visible cell bodies also stained with DCX. When the edge of the image was aligned with the edge of the hilus, the inward movement of cells could be measured. **b** No difference was seen in knock-out mice of either sex in average inward mobility of immature neurons. ***c*** There was no difference in the percent of cells that had moved inward from the edge of the hilus by more than 10 μm in male or female mice of either genotype. **d–f** In order to examine the effect of loss of α7 nAChRs on dendritic structure, 63X images were captured and analyzed using Imaris Filament function. These images delineate the process. **d** A region of interest was randomly selected from the upper arm of the dentate gyrus in the dorsal region of the hippocampus and imaged at 63X. **e** Five DCX cells were randomly selected from all cells present in the image. Cells were not selected if the dendritic structure traveled out of the plane created by the section. Cells were manually traced using the filament function on Imaris. Cells were checked for accuracy in 3D. **f** Tracings were used to count tertiary filaments and measure overall filament length. **g** There was no significant difference in the number of tertiary dendrites ($p=0.068$). **h** Compared with wild-type mice, mice that lacked the α7 nAChR had an overall increase in total filament length [$F(1,22)=5.051$, $p=0350$, $n=6$ mwt, 6 fwt, 7 mko, 7 fko] when both sexes are considered together. Statistical analysis uses two-way ANOVA with Sidak's multiple comparison test and Graphpad Prism 7.0 Software. Experimental groups contained 5–7 mice per group including male wild type, male knock-out, female wild type, female knock-out. In each mouse, four hippocampi sections were analyzed to provide a mean density per mouse. Data expressed as mean ± SEM. *Asterisk* indicates $*p<0.05$

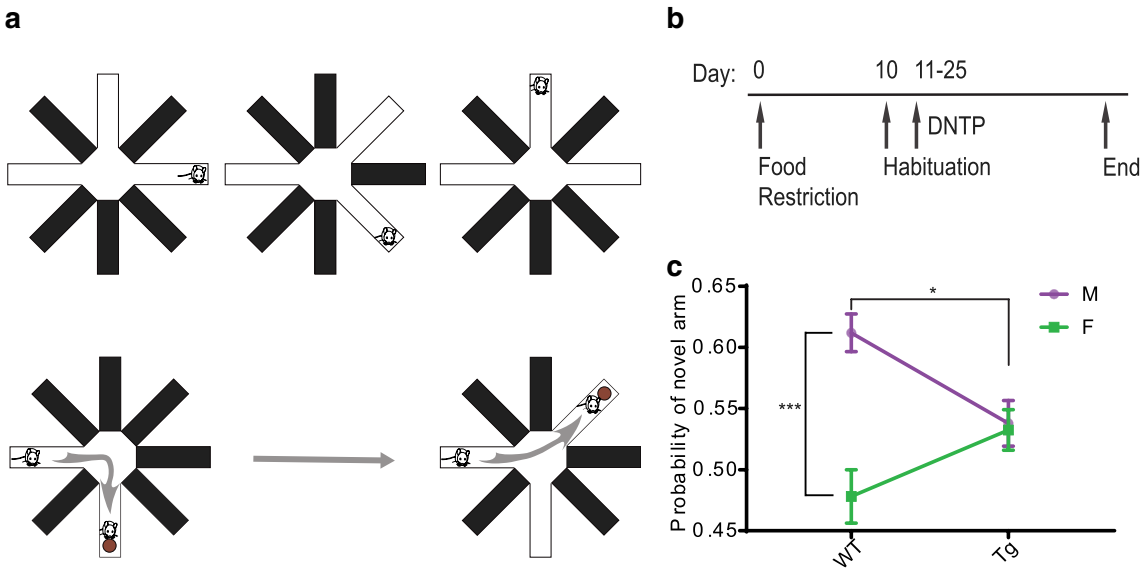


Fig. 4 Loss of α7 nAChRs impairs performance in DNTP task in male mice and results in lost sexual dimorphism. Schematic (**a**) and timeline (**b**) of DNTP task. Mice were kept in 12 h light:12 h dark reverse light dark and placed on restricted diet beginning 10 days prior to experiment, and handled and weighed daily during this time. Prior to the experiment, mice were habituated to the room and maze. Habituation included 8 min free exploration of the maze with reward pellets at the end of each arm, and 2 min restricted exploration of the maze with reward pellets at the end of 2 open arms. During the two-phase task, mice were placed in one arm of an eight-arm radial maze and retrieved a reward pellet placed in another arm (sample arm). After a short delay, a novel arm was loaded with a reward pellet. Mice were placed in the maze in the original arm and allowed to retrieve the treat. We measured whether the mouse chose the novel arm or the familiar arm to enter first. ***c*** Our results show that there is a sex by genotype interaction in this task ($p=0.0025$, $n=53$), with wild-type males preferring the novel arm while females did not ($p=0.0009$, $n=10$ m, 8 f). This sexual dimorphism disappears when the α7 nAChR is knocked out ($p=0.9957$; $n=19$ m, 16 f). Transgenic male mice performed significantly closer to chance than wild-type males ($p=0.0408$, $n=10$ wt, 19 tg) while females do not show a significant change ($p=0.2780$, $n=8$ wt 16 tg) in their choice of novel or familiar arm. Asterisk indicates $*p<0.05$, $**p<0.01$, $***p<0.001$. Statistical analysis using a two-way ANOVA and Sidak's post hoc using SPSS and Graphpad Prism 7.0 software. Data expressed as mean ± SEM

α7 nAChR regulation of delayed non-matching to prior (DNTP) task in radial-arm maze reveals sexually dimorphic dentate gyrus function

We investigated whether the increased neurogenesis in the absence of α7 nAChRs (using the global knock-out) correlated with changes in behaviors associated with adult neurogenesis. To do this, we used a DNTP task in an 8-arm radial maze (Fig. 4a) that had been previously used to measure a behavioral phenomenon thought to assess pattern separation processes (Clelland et al. 2009). Mice ran each of three possible tasks, with each task having two phases. The first (sample) phase involved placing the mouse in the maze and allowing it to find the reward pellet. The second phase, occurring after a short delay, recorded the mouse re-entering the maze and retrieving a new reward pellet from a novel arm. Of interest was whether the mouse entered the novel or familiar arm first. This task was run on mice beginning 10 days after food restriction began, and occurred over 15 consecutive days, with 4 tasks per day per mouse (Fig. 4b). Our results show there was a sex by genotype interaction [$F(1,49)=10.16$, $p=0.0025$, $n=53$, two-way ANOVA, Fig. 4c]. The DNTP radial-arm maze task was sexually dimorphic in wild-type mice; while male mice preferred the novel arm, wild-type female mice did not prefer the novel arm (Tukey's, $p=0.0009$, $n=10$ m, 8 f). However, male and female mice lacking the α7 nAChR did not show any difference in performance on this task ($p=0.9957$; $n=19$ m, 16 f). Individual analysis of mice by sex showed that the loss of the α7 nAChR in male mice caused a significant decrease in probability of entry into the novel arm ($p=0.0408$, $n=10$ wt, 19 tg). However, in female mice there was no significant difference between wild type and transgenic α7 nAChR mice ($p=0.2780$, $n=8$ wt 16 tg). Therefore, while sexual dimorphism is apparent in performance of this task in wild-type mice, male mice have a decrease in performance associated with the loss of the α7 nAChR in this spatial discrimination task, but female mice do not; this supports the hypothesis that the α7 nAChR is important in hippocampal decision-making strategies, and suggests a sexually dimorphic function.

α7 nAChRs on nestin$^+$ cells or their progeny are involved in regulation of NSC populations in males

There are multiple cell types in the hippocampus that express functional α7 nAChRs, including interneurons, astrocytes, microglia as well as immature granule cells (John et al. 2015; Jones and Yakel 1997; King et al. 2017; Sharma and Vijayaraghavan 2001; Shen and Yakel 2012; Shytle et al. 2004). We investigated if the loss of α7 nAChRs in nestin$^+$ NSCs prior to or during adult neurogenesis was responsible

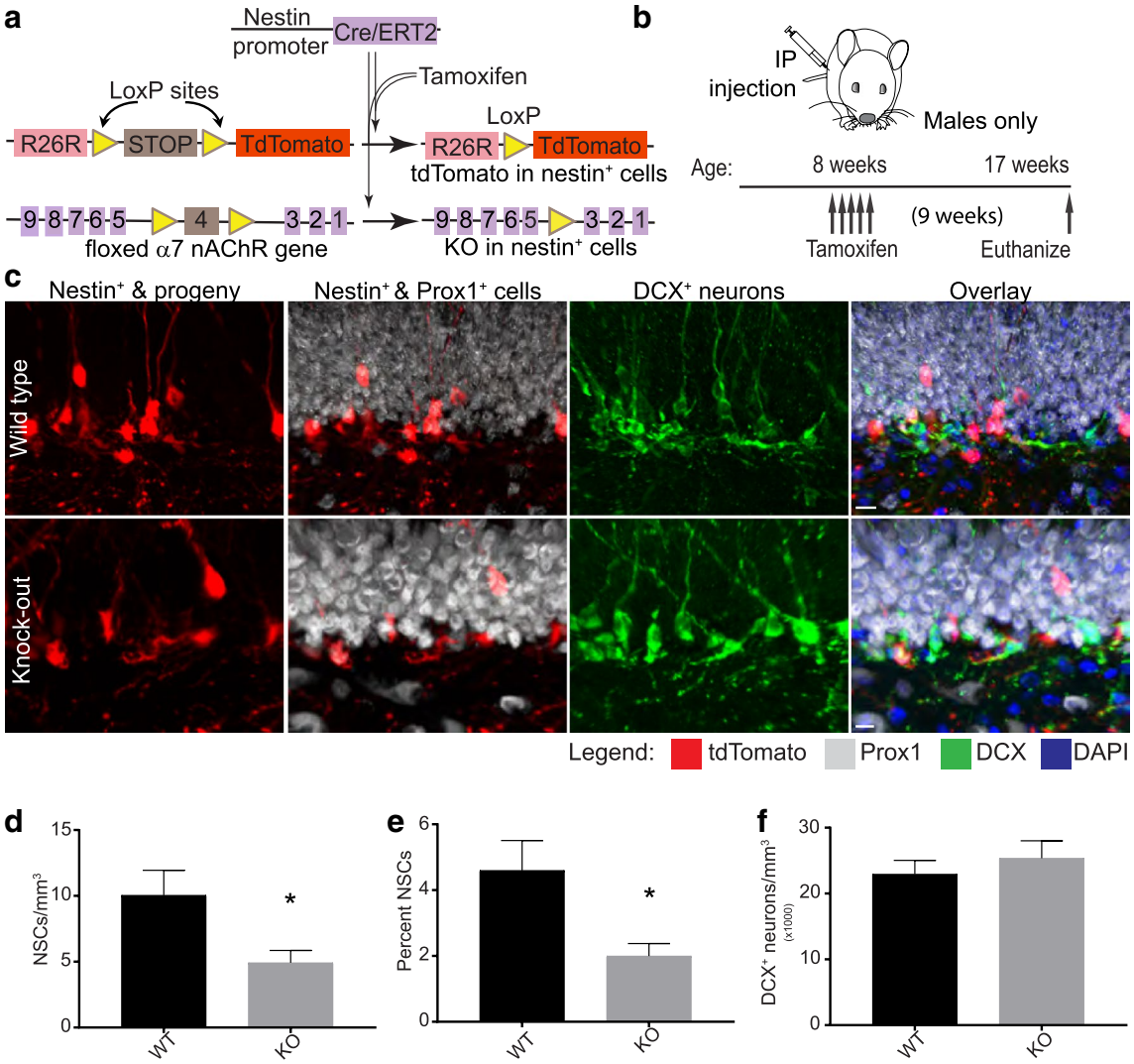


Fig. 5 Loss of α7 nAChRs in nestin$^+$ cells results in decreased NSCs in males. **a** Administering tamoxifen to nestin-CreER/T2 × ROSAtdTomato × α7 nAChR^{flox} male mice results in the loss of α7 nAChR expression in nestin$^+$ NSCs and their progeny of male mice homozygous for floxed α7 nAChR, as well as the expression of tdTomato expression in nestin$^+$ cells and their progeny. In this experiment females were not tested. **b** Male mice were dosed with tamoxifen through intraperitoneal injection in 5 consecutive doses (0.15 mg/g) at 8 weeks of age. Mice then aged 9 weeks and were euthanized at 17 weeks, allowing sufficient time for α7 nAChR$^-$/nestin$^+$ cells to divide, differentiate and mature into the dentate gyrus granule cell layer. ***c*** 3-D images were collected of dentate gyrus of α7 nAChR^{flox} × nestin-Cre/ERT2 × ROSATdTomato wild-type and knock-out mice euthanized 9 weeks after final tamoxifen injection. This delay allows activated NSCs to divide and mature without α7 nAChR expression. Images show NSCs, immature neurons (gp-antiDCX 1:500, Gt-antiGp Alexa 488 1:500), and granule cells (Rb-antiProx1 1:500, Gt-antiRb Alexa 647 1:500), as well as DAPI (2 µg/ml). Scale bar: 10 µm. **d** The NSC population was significantly decreased ($p=0.0416$, $n=5$, 5). This is comparable to the result seen in whole body knock-out of the α7 nAChR, in which there is an approximate 50% decline in NSCs in the knock-out compared to the wild type. **e** The percent of NSCs compared to the overall population of cells in the dentate gyrus was significantly decreased ($p=0.0468$, $n=5$, 5). **f** There were no significant differences in numbers of immature neurons in these mice ($p=0.495$, $n=5$, 5). This is in contrast to the whole body knock-out of α7 nAChR, where male mice showed a significant increase in immature neurons. Female mice were not analyzed. Asterisk indicates $*p<0.05$. Statistical analysis with unpaired Student's *t* test calculated using Graphpad Prism 7.0. Data in bar graph expressed as mean ± SEM

for the changes in adult neurogenesis we had seen in male (but not female) mice; that is whether these changes are a result of cell autonomous or simple feed-back regulation. In this investigation we used only male mice. To do this, we selectively eliminated the α7 nAChRs from nestin$^+$ cells and all their progeny after male mice had reached adulthood (8 weeks). Since nestin is widely expressed during development, we used the nestin-Cre/ERT2 × ROSAtdTomato-inducible mouse (to be able to limit the loss of α7 nAChR to only adult male mice) and crossed it with the α7 nAChR^{flox} mouse (Hernandez et al. 2014). This cross (Fig. 5a) allowed us to knock out the α7 nAChRs in nestin$^+$ cells only in the adult male mice when administered tamoxifen. Nine weeks after tamoxifen (Fig. 5b), tissue was imaged (Fig. 5c), recording the presence of nestin$^+$ cells, DCX$^+$ cells (immature granule cells), Prox1$^+$ cells (mature granule cells) and DAPI. By subtracting out progeny (cells that were DCX$^+$/nestin$^+$ or Prox1$^+$/nestin$^+$) from nestin$^+$ cell counts, we could establish the numbers of nestin$^+$ NSCs present. We found a 51 ± 9% decrease in density of NSCs in the dentate gyrus of male mice with conditional α7 nAChR deletion compared to wild type ($p = 0.0416$, $n = 5$, 5 unpaired Student's t test; Fig. 5d), as well as a decline in the percent of cells in the dentate gyrus that are NSCs by 52 ± 9% ($p = 0.0468$, $n = 5$, 5, unpaired Student's t test; Fig. 5e). This is similar to the whole body α7 nAChR knock-out, in which there was a similar decline of NSCs in male mice. In this case the decrease was 61% ± 18% decrease in NSCs, whereas in the whole body knock-out there was 51% ± 9% decrease in NSCs. Interestingly, the loss of α7 nAChRs had no impact on immature neuron numbers ($p = 0.495$, $n = 5$, 5 unpaired Student's t test; Fig. 5f). This is in contrast to the whole body knock-out of α7 nAChRs, where there was a significant increase in DCX$^+$ immature neurons. This indicates that while the loss of α7 nAChRs selectively from the nestin$^+$ cells in adult male mice during the development of granule cells results in a decrease in NSCs pools, it is only partially responsible for the overall changes seen in the α7 nAChR knock-out mice since there is no apparent overall increase in neurogenesis.

To investigate maturation of immature neurons, distance inward from the granule cell–hilus border and dendritic structure was recorded. Distance from the granule cell–hilus border was measured by selecting a region of interest, lacking curvature, in the upper arm of the dorsal dentate gyrus, and positioning the image with the granule cell layer–hilus border at one edge (Fig. 6a). Distance of selected DCX$^+$ cells from the image edge was calculated by ImageJ and converted to microns in Excel. The average distance was calculated for that image and four images were averaged per mouse. The average inward distance from the edge of the hilus was significantly greater in α7 nAChR knock-out than wild-type mice ($p = 0.0288$, $n = 6,6$, unpaired Student's

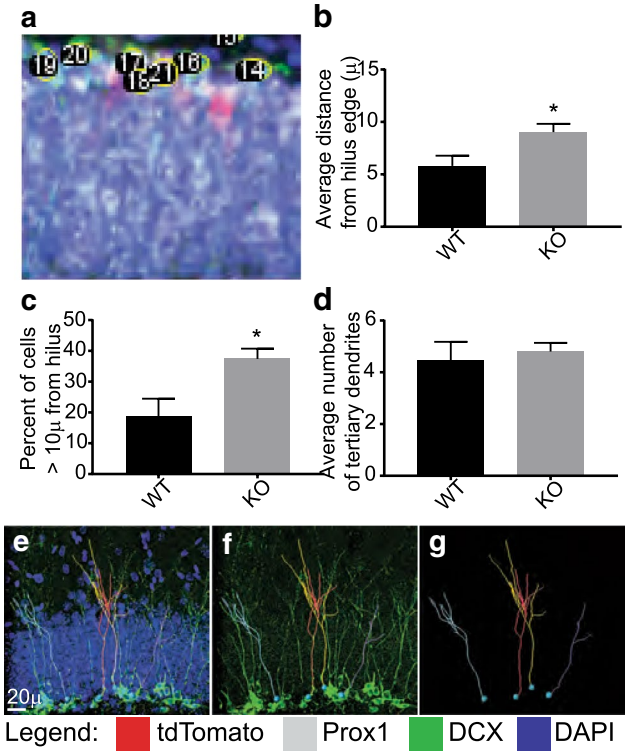


Fig. 6 Loss of α7 nAChRs in nestin$^+$ cells results in greater inward mobility in males. **a** The average distance inward from the hilus edge that cells had traveled was measured by taking a maximum intensity projection and importing it into ImageJ. **b** Results indicated that loss of the α7 nAChR in NSCs was associated with greater inward mobility of these cells from the hilus prior to their maturation into adult neurons ($p = 0.0288$, $n = 6,6$). **c** Loss of α7 nAChRs from NSCs results in a larger percentage of cells that have moved more than 10 μm from the edge of the hilus as immature neurons ($p = 0.0168$, $n = 6,6$). **d** Tertiary dendrites were counted by imaging regions of interest in dorsal dentate gyrus and analyzing using filament feature in Imaris. Loss of α7 nAChRs does not impact on tertiary dendrite numbers. **d–f** In order to examine the effect of loss of α7 nAChRs on dendritic structure, images were captured and analyzed using Imaris Filament function. These images delineate the process. **d** A region of interest was randomly selected from the upper arm of the dentate gyrus in the dorsal region of the hippocampus and imaged. **e** Four DCX cells were randomly selected from all cells present in the image. Cells were manually traced using the filament function on Imaris and checked for accuracy in 3D. **f** Tracings were used to count tertiary filaments and measure overall filament length. Female mice were not analyzed. Asterisk indicates *$p < 0.05$. Statistical analysis with unpaired Student's t test calculated using Graphpad Prism 7.0. Data in bar graph expressed as mean ± SEM

t test; Fig. 6b), and the percent of cells that were greater than 10 μm from the edge of the hilus was greater in α7 nAChR knock-out than wild-type mice ($p = 0.0168$, $n = 6,6$, unpaired Student's t test; Fig. 6c). This supports a dysregulation in the maturation process among the immature neurons of the dentate gyrus in the α7 nAChR knock-out mice. However, neither tertiary dendrites (Fig. 6d) nor total filament length was significantly different in the α7 nAChR knock-out mice

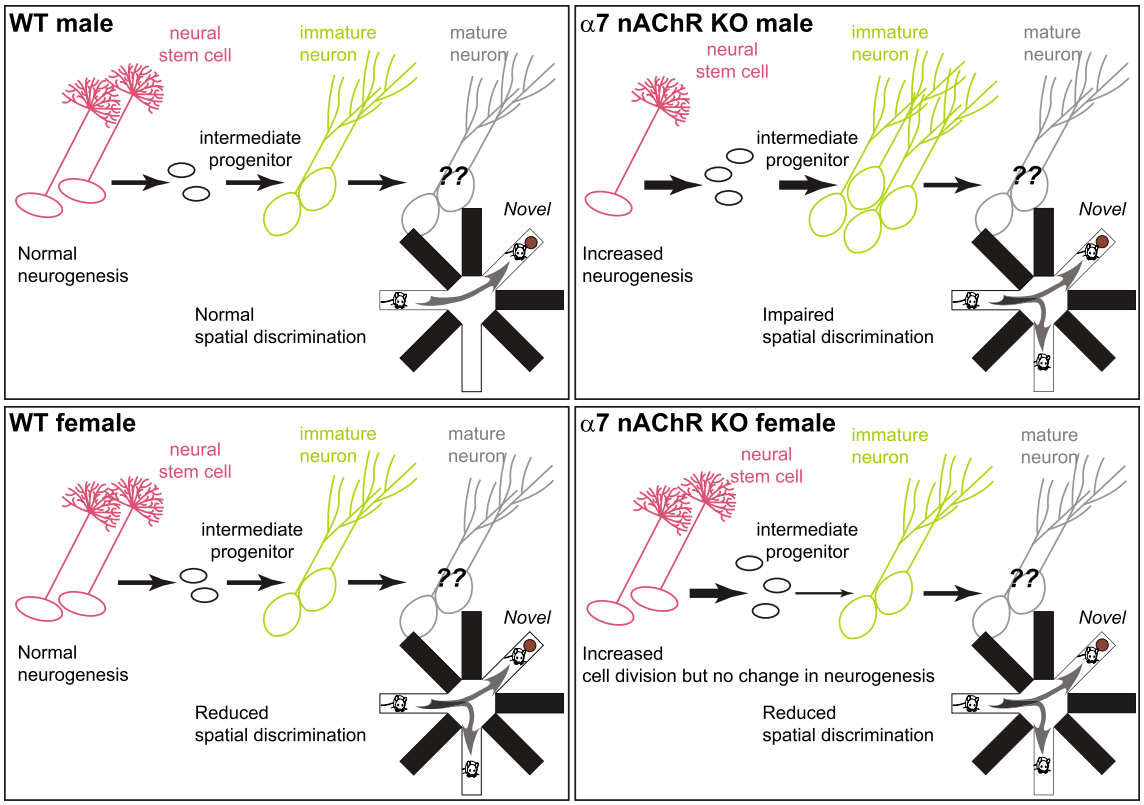


Fig. 7 Effects of α7 nAChRs by sex and genotype. Schematic shows a summary of the effects of loss of α7 nAChRs by sex and genotype. Overall, cell divisions increased in α7 nAChR knock-out mice (shown by a larger arrow), with an increase in intermediate progenitor cells. In adult male mice only, loss of α7 nAChRs also led to a decrease in NSCs and an increase in immature neurons, supporting the idea that adult neurogenesis was increased in these mice after the loss of the α7 nAChR. Despite their apparent higher levels, we would anticipate poor survival for these immature neurons, and a loss of neurons at maturity. Future studies will test survival. In addition, the loss of performance in the DNTP radial-arm maze task is consistent with reduced functionality of the dentate gyrus, supporting the hypothesis that the observed neurogenesis is dysregulated and aberrant, rather than healthy. Wild-type adult female mice demonstrate a lack of preference for novelty in the DNTP radial-arm maze task, with no significant change in mice lacking the α7 nAChR. In addition, in adult female mice levels of NSCs and immature neurons remain unchanged, indicating that increased cell division is not having an easily identifiable lasting impact on adult neurogenesis

(data not shown). Tertiary structure was measured in Imaris, by tracing filaments in 3D (Fig. 6e–g). This may indicate that although more cells are entering the immature cell phase, they are failing to survive.

In summary (Fig. 7), our results show that in adult male mice, the loss of the α7 nAChR increases adult neurogenesis with an increase in intermediate progenitors and immature neurons, and a loss of NSCs. However, the overall decrease in NSCs and their progeny suggests that these cells are transient and do not survive to integrate into the granule cell layer. In addition, the impaired performance of male knock-out mice in the DNTP task suggest that neurogenesis is dysregulated and pathogenic, in contrast to the health-associated increases seen with environmental stimulus. Interestingly, female mice do not show this same pattern. To begin with, they do not show the same preference for novelty as the male mice do in the DNTP task, and loss of the α7 nAChR does not impair their performance. In addition, knock-out female mice show an increase in intermediate progenitors and in cell division, but no change in NSC numbers or in immature neurons. This indicates that there is no increase in adult neurogenesis in female mice lacking the α7 nAChR, and increased cell divisions do not either survive beyond the intermediate progenitor stage or revert to NSCs.

Discussion

Impaired adult neurogenesis is present in many neuropsychiatric and neurodegenerative disorders (Aimone et al. 2014; Besnard and Sahay 2016; Burket et al. 2017; Kang et al. 2016; Lazarov and Marr 2010), and it has been proposed that improving adult neurogenesis could be therapeutic (Jin et al. 2004). α7 nAChR dysfunction has been linked to many of these disorders (Dineley et al. 2015). For instance, α7 nAChRs are increased in the hippocampus in

Alzheimer's disease patients (Teaktong et al. 2003), and are linked with impaired auditory sensory gating seen in patients with schizophrenia (Martin and Freedman 2007). Clinical studies suggest treatment of schizophrenia with α7 nAChR agonists and positive allosteric modulators has resulted in improved cognition and reduced negative symptoms (Freedman 2014). Studies in rodent models of neuropsychiatric and neurodegenerative disorders have also implicated the α7 nAChR (Gass et al. 2016; Lykhmus et al. 2015; Papouin et al. 2017; Plitman et al. 2017). In addition, α7 nAChRs have been shown to regulate aggression through activation of dentate granule cells (Lewis et al. 2017). Furthermore, prenatal stress decreases adult neurogenesis (Belnoue et al. 2013), and also affects the levels of α7 nAChRs in adult rodents (Baier et al. 2015). Adult neurogenesis in the human brain primarily originates with NSCs located in the subgranular zone of the dentate gyrus (Altman and Das 1965; Spalding et al. 2013) within the hippocampus. These quiescent cells self-replicate, or when activated may differentiate into intermediate progenitors which then produce neurons.

Our main finding is that loss of α7 nAChRs results in a sex-dependent dysregulation of adult neurogenesis. In male mice, there are fewer NSCs inclusive of their progeny or alone, while in both sexes the amount of cell division, the number of intermediate progenitors and the percent of nestin$^+$ cells that differentiated into other cells were increased, supporting an increase in neurogenesis overall. This increase is unlikely to be due to increased levels of survival, since survival in the first 2 weeks is expected to remain the same for α7 nAChR knock-out and wild-type mice (Campbell et al. 2010). The activation of the NSCs could also lead to an increase in newly formed astrocytes, resulting in a permanent decline in neurogenesis (Encinas et al. 2011); exploring this possibility is important and the focus of future research. Here, we found that there is an increase in immature neurons in males only, with no overall increase in total density of DG cells. This would be consistent with poor survival of DG cells previously seen in an α7 nAChR knock-out model (Campbell et al. 2010). However, when we measured dendritic arborization, we see an increase in dendritic arbors in α7 nAChR knock-out mice compared to their sibling controls. This is contrary to previous findings that show an overall loss of dendritic arborization in α7 nAChR knock-out mice (Campbell et al. 2010). One possible explanation is that lack of the α7 nAChR is causing the immature neurons, which have been shown to retain immature features (Campbell et al. 2010), to retain expression of DCX for longer than their sibling wild-type controls. This might result in substantially differently aged neurons. Another possibility lies in the fact that our cells were all chosen from the upper arm of the dorsal dentate gyrus; it is possible that the lower arm or the ventral dentate gyrus might show a different effect. It is important to note that the increase we see occurs in the full group of male and female mice; when considered alone neither individual group shows a significant difference; this implies that any increase is likely to be subtle. Loss of the α7 nAChR in male mice had functional consequences in a behavior task thought to assess pattern separation processes. We further show that loss of the α7 nAChR solely in nestin$^+$ cells and their progeny, the adult-born neurons, also results in a decrease in number of NSCs.

Consistent with Dranovsky et al. (2011), we show that at 12 days post-initial injection approximately 30% of all nestin$^+$ NSCs will have divided and differentiated into intermediate progenitors or immature neurons in wild-type mice. This time point allowed for maximal expression of tdTomato after tamoxifen administration and encapsulated neurogenesis occurring before an activity-dependent critical period (Tashiro et al. 2006), which impacts on total numbers of cells present in α7 nAChR knock-out mice (Campbell et al. 2010). The significant increase in this percentage seen in α7 nAChR null mice supports the idea that adult neurogenesis is increased in these mice, as does the increase in intermediate progenitors. Although it has been shown that there is no difference in survival of neurons at 2 weeks in α7 nAChR null mice (Campbell et al. 2010), not only is this time point 2 days later but this could be accounted for by an increased adult neurogenesis being counterbalanced by a decreased survival of immature neurons beyond this point. In addition, the previous study did not distinguish male from female mice. The fact that in male mice there is also an increase in immature neurons (DCX$^+$) supports the idea of continued proliferation at least to this point. Some literature suggests that lack of α7 nAChR in the immature neurons of the α7 nAChR knock-out mice is protective early on, due to a lower calcium burden on cells that had not yet developed calcium chelators. Similarly, overactivation of the α7 nAChR can result in neuronal loss due to calcium toxicity (Orr-Urtreger et al. 2000). These same cells, however, may be less likely to survive to adulthood due to a delayed maturation, an extended period of GABAergic depolarization, and poor development of dendritic structure (Campbell et al. 2010). Unexpectedly, our results showed an increased dendritic arborization in α7 nAChR knock-out mice compared to sibling controls. In our study, we were analyzing DCX$^+$ cells. It is possible that delayed maturation also causes a delay in the loss of expression of DCX, resulting in our measuring cells of differing relative ages. Further research would be needed to disentangle the possible interpretations of this result. Delayed maturation of adult-born neurons might result in a transient increase, followed by a lasting decline, as NSCs leave the existing pool at an increased rate, but fail to survive into maturity.

Since female mice do not show this same increase, it is likely that the excess intermediate progenitors are not

differentiating into neurons, suggesting they are either failing to survive or are instead reverting to NSCs, which would stabilize numbers of NSCs present. Differences between males and females are consistent with studies suggesting that the dentate gyrus is a sexually dimorphic structure (Galea 2008). Many factors could be important in this difference between male and female mice, including but not limited to estrus cycling, which was not measured in this study. In addition, it has been shown that estrogen can modulate acetylcholine release in the hippocampus (Gabor et al. 2003), as well as having other effects (Spencer et al. 2008) that impact cholinergic function.

The overall decrease in nestin$^+$ cells inclusive of their progeny within a 12-day time frame in a global α7 nAChR knock-out adult male mouse supports the idea these cells are not surviving to maturity, compatible with α7 nAChRs having a neuroprotective role during the differentiation and maturation process of adult-born neurons in male mice, and with previous literature (Campbell et al. 2010). Early life stress can lead to both fewer α7 nAChRs and less neurogenesis (Glenn et al. 2007). On the other hand, prenatal choline administration, potentially activating the α7 nAChR, results in increased neurogenesis into adulthood (Velazquez et al. 2013), again supporting the α7 nAChRs being neuroprotective (Hernandez and Dineley 2012; Voytenko et al. 2015). Denervating the dentate gyrus of cholinergic neurons results in a loss of new neurons and an increase in cell death in the dentate gyrus (Cooper-Kuhn et al. 2004). Interestingly, we found that the loss of the α7 nAChRs increased adult neurogenesis, suggesting that the signaling that results in cell death may be inclusive of an overall deregulation of adult neurogenesis. Other research, conducted in male mice, has suggested that by 4 weeks post-neurogenesis there will be a decline in surviving adult-born neurons in α7 nAChR knock-out mice (Campbell et al. 2010). In our study, only male mice showed a decrease in NSCs, while females did not. Possible alternative explanations for this finding include the differing baseline levels in adult NSC populations seen for wild-type females, the potential for differing levels of expression of neuronal markers, differing metabolism of tamoxifen in female mice, or a differing means of regulating adult neurogenesis. Disentangling these possibilities would provide interesting further research, and may also suggest that this as an interesting line of enquiry to pursue when researching neuropsychiatric and neurodegenerative disorders associated with loss of α7 nAChRs that have a sexually dimorphic presentation (Guebel and Torres 2016; Martinez-Pinilla et al. 2016).

The combination of changes in cell densities in the male α7 nAChR knock-out mice suggest that the α7 nAChR normally acts as a brake on adult neurogenesis. It has recently been found that there is a decreased calcium signaling cascade in induced pluripotent stem cells and neural progenitor cells derived from persons with both increased and decreased α7 nAChR expression (Gillentine et al. 2017). Since excitation has been shown to increase adult neurogenesis (Deisseroth et al. 2004), but excitation through constitutive activation of the α7 nAChR has been shown to be neurotoxic during development (Orr-Urtreger et al. 2000), our results suggest a dysregulation rather than any activity-dependent increase. Loss of the α7 nAChR can also reduce inhibitory function in the hippocampus and $GABA_A$ receptors in male mice (Adams et al. 2012), which would lead to decreased activity-dependent GABA release. In either case, activity-dependent changes could not be the sole cause.

NSCs are quiescent while bathed in spill-over GABA from nearby parvalbumin$^+$ interneurons (Song et al. 2012, 2013), and do not respond to bath applied acetylcholine. By contrast, when NSCs differentiate into immature granule cells, their progeny express fully functional α7 nAChRs (John et al. 2015). Furthermore, although constitutive activation of the α7 nAChR results in apoptosis in the developing CNS (Orr-Urtreger et al. 2000), galantamine, an acetylcholinesterase inhibitor, increases survival of immature neurons through an α7 nAChR-dependent mechanism (Kita et al. 2014). In addition, α7 nAChRs regulate the maturation and integration of immature neurons into the dentate gyrus (Campbell et al. 2010). If maturation, integration, and survival are impaired in granule cells lacking α7 nAChR, this could result in increased compensatory proliferation; e.g., with increased, but impaired, levels of neurogenesis seen in Alzheimer's patients (Jin et al. 2004; Lazarov and Marr 2010), acting in a cell autonomous fashion. When we knocked out the α7 nAChR selectively in nestin$^+$ cells in adult male mice, we saw a reduction in density of NSC pools and a decrease in the percent of nestin$^+$ cells, however there was no overall change in density of immature neurons. This would be consistent with a failure of differentiated neurons to survive, but only partially supports a purely cell autonomous mechanism driving the dysregulation seen in α7 nAChR knock-out mice.

If not cell autonomous, it is possible hippocampal interneurons expressing α7 nAChRs (Jones and Yakel 1997), could be responsible for the changes we observed. Recent studies show that loss of α7 nAChRs can change cell composition in the hippocampus, causing an increase in parvalbumin$^+$ interneurons in C3H mice (Bates et al. 2014), or an overall decrease in interneurons in the hippocampus (Adams et al. 2012). Parvalbumin$^+$ interneurons in the subgranular zone act to inhibit neurogenesis through spill-over of GABA (Song et al. 2012). In addition, a small subset of somatostatin$^+$ interneurons with cell bodies in the hilus and dendrites that project to the molecular layer inhibit neurons locally and in the medial septum and diagonal band of Broca (MS/DBB) (Yuan et al. 2017) which could be impacting adult neurogenesis. Interestingly, parvalbumin$^+$ interneurons

receive input from the MS/DBB (Bao et al. 2017), and loss of this input causes activation of NSCs with a decline in NSC pools. While the largest input onto these interneurons appears to be GABAergic, the MS/DBB also have cholinergic neurons both modulating GABAergic locally and projection neurons in intraseptal circuitry (Damborsky et al. 2017), as well as directly activating interneurons in the hippocampus (Dannenberg et al. 2015). It is also possible that immature neurons expressing GAD67 (Cabezas et al. 2013) are contributing to the effect. In short, there are several possible sources of cholinergic input to the NSCs via interneurons as well. It will be important in the future to determine the source of cholinergic regulation of neurogenesis.

Our wild-type mice show a sexual dimorphism in the DNTP radial-arm maze task. Though unexpected, other studies have also shown varying degrees of sexual dimorphism in hippocampal structure and function (Cushman et al. 2012; Galea 2008; Luine et al. 2017). While the DNTP radial-arm maze task has been shown to be both hippocampal- and neurogenesis-dependent, conflicting views exist on whether it can properly assess pattern separation processes (Anacker and Hen 2017; Besnard and Sahay 2016; Clelland et al. 2009; Santoro 2013), or more broadly spatial discrimination or other hippocampal functions. However, the fact that sexual dimorphism disappears in this assessment when the α7 nAChR is removed remains interesting. That male, but not female, mice show both loss of NSCs and decreased performance in the task subsequent to the loss of the α7 nAChR is an intriguing correlation, but more research is needed to determine whether the effect seen is the direct result of changes in neurogenesis after loss of α7 nAChRs. Multiple previous studies show an association between levels of adult neurogenesis and performance in tasks measuring pattern separation, spatial discrimination, or cognitive flexibility (Clelland et al. 2009; Cushman et al. 2012; Hollands et al. 2017; Pan et al. 2012; Sahay et al. 2011). Previous studies in rats and voles have shown the hippocampus to be a sexually dimorphic structure (Galea 2008; Luine et al. 2017). For instance, estrogen increases neurogenesis during proestrus in female rats (Tanapat et al. 1999). In addition, a previous report has shown that complete loss of all post-natal neurogenesis produces sex-dependent changes in hippocampus-dependent cognitive function (Cushman et al. 2012). It remains to be determined how the α7 nAChR is regulating performance in the DNTP task in male mice.

The ability to adapt appropriately to a changing environment is essential for mental health and is lacking in many neurological disorders including Alzheimer's disease and schizophrenia. One commonality of these diseases is involvement of α7 nAChR dysfunction. Our primary focus was to determine whether α7 nAChRs regulate adult neurogenesis in the dentate gyrus, and if this resulted in behavioral consequences. Our data support the hypothesis that α7 nAChR activation regulates adult neurogenesis. While increased neurogenesis occurred both in male and female mice in the absence of α7 nAChRs, loss of NSCs and decrease in performance on a DNTP task was only observed in male mice. While our data suggest that α7 nAChR expression on NSCs is important, interneurons, astrocytes and/or microglia may also be involved. In future studies, we will further address how this receptor is regulating neurogenesis, and how this regulation differs in male and female mice, and whether this regulation is exacerbated by stress, and/or alleviated by environmental enrichment. Understanding how NSCs are regulated by ligands acting on the α7 nAChR may provide a potential therapeutic strategy for treating a wide variety of neurodegenerative and neurological diseases.

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Compliance with ethical standards

Ethical statement All procedures were approved and performed in compliance with the NIEHS/NIH Humane Care and Use of Animals Protocols.

Informed consent This article does not contain any studies with human participants performed by any of the authors. All procedures were approved and performed in compliance with the NIEHS/NIH Humane Care and Use of Animals Protocols.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors. All procedures were approved and performed in compliance with the NIEHS/NIH Humane Care and Use of Animals Protocols.

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams CE, Yonchek JC, Schulz KM, Graw SL, Stitzel J, Teschke PU, Stevens KE (2012) Reduced Chrna7 expression in mice is associated with decreases in hippocampal markers of inhibitory function: implications for neuropsychiatric diseases. Neuroscience 207:274–282. https://doi.org/10.1016/j.neuroscience.2012.01.033

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH (2014) Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev 94:991–1026. https://doi.org/10.1152/physrev.00004.2014

Albuquerque EX, Pereira EF, Alkondon M, Schrattenholz A, Maelicke A (1997) Nicotinic acetylcholine receptors on hippocampal neurons: distribution on the neuronal surface and modulation of receptor activity. J Recept Signal Transduct Res 17:243–266. https://doi.org/10.3109/10799899709036607

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335

Anacker C, Hen R (2017) Adult hippocampal neurogenesis and cognitive flexibility—linking memory and mood. Nat Rev Neurosci 18:335–346. https://doi.org/10.1038/nrn.2017.45

Baier CJ, Pallares ME, Adrover E, Monteleone MC, Brocco MA, Barrantes FJ, Antonelli MC (2015) Prenatal restraint stress decreases the expression of alpha-7 nicotinic receptor in the brain of adult rat offspring. Stress 18:435–445. https://doi.org/10.3109/10253890.2015.1022148

Bao H et al (2017) Long-range GABAergic inputs regulate neural stem cell quiescence and control adult hippocampal neurogenesis. Cell Stem Cell 21:604–617 e605. https://doi.org/10.1016/j.stem.2017.10.003

Bates RC, Stith BJ, Stevens KE, Adams CE (2014) Reduced CHRNA7 expression in C3H mice is associated with increases in hippocampal parvalbumin and glutamate decarboxylase-67 (GAD67) as well as altered levels of GABA(A) receptor subunits. Neuroscience 273:52–64. https://doi.org/10.1016/j.neuroscience.2014.05.004

Belnoue L, Grosjean N, Ladeveze E, Abrous DN, Koehl M (2013) Prenatal stress inhibits hippocampal neurogenesis but spares olfactory bulb neurogenesis. PLoS One 8:e72972. https://doi.org/10.1371/journal.pone.0072972

Besnard A, Sahay A (2016) Adult hippocampal neurogenesis, fear generalization stress. Neuropsychopharmacology 41:24–44. https://doi.org/10.1038/npp.2015.167

Boldrini M et al (2018) Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 22:589–599 e585. https://doi.org/10.1016/j.stem.2018.03.015

Bonaguidi MA, Song J, Ming GL, Song H (2012) A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus. Curr Opin Neurobiol 22:754–761. https://doi.org/10.1016/j.conb.2012.03.013

Burket JA, Urbano MR, Deutsch SI (2017) Sugarcoated perineuronal nets regulate "GABAergic" transmission: bittersweet hypothesis in autism spectrum. Disord Clin Neuropharmacol 40:120–130. https://doi.org/10.1097/WNF.0000000000000209

Cabezas C, Irinopoulou T, Cauli B, Poncer JC (2013) Molecular and functional characterization of GAD67-expressing, newborn granule cells in mouse dentate gyrus. Front Neural Circ 7:60. https://doi.org/10.3389/fncir.2013.00060

Campbell NR, Fernandes CC, Halff AW, Berg DK (2010) Endogenous signaling through alpha7-containing nicotinic receptors promotes maturation and integration of adult-born neurons in the hippocampus. J Neurosci 30:8734–8744. https://doi.org/10.1523/JNEUROSCI.0931-10.2010

Chawla MK et al (2005) Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. Hippocampus 15:579–586. https://doi.org/10.1002/hipo.20091

Clelland CD et al (2009) A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325:210–213. https://doi.org/10.1126/science.1173215

Cooper-Kuhn CM, Winkler J, Kuhn HG (2004) Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. J Neurosci Res 77:155–165. https://doi.org/10.1002/jnr.20116

Cushman JD et al (2012) Juvenile neurogenesis makes essential contributions to adult brain structure and plays a sex-dependent role in fear memories. Front Behav Neurosci 6:3. https://doi.org/10.3389/fnbeh.2012.00003

Damborsky JC, Smith KG, Jensen P, Yakel JL (2017) Local cholinergic-GABAergic circuitry within the basal forebrain is modulated by galanin. Brain Struct Funct 222:1385–1400. https://doi.org/10.1007/s00429-016-1283-0

Dannenberg H et al (2015) Synergy of direct and indirect cholinergic septo-hippocampal pathways coordinates firing in hippocampal networks. J Neurosci 35:8394–8410. https://doi.org/10.1523/JNEUROSCI.4460-14.2015

Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC (2004) Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron 42:535–552

Dickinson-Anson H, Winkler J, Fisher LJ, Song HJ, Poo M, Gage FH (2003) Acetylcholine-secreting cells improve age-induced memory deficits. Mol Ther 8:51–61

Dineley KT, Pandya AA, Yakel JL (2015) Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol Sci 36:96–108. https://doi.org/10.1016/j.tips.2014.12.002

Dranovsky A et al (2011) Experience dictates stem cell fate in the adult hippocampus. Neuron 70:908–923. https://doi.org/10.1016/j.neuron.2011.05.022

Encinas JM et al (2011) Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. Cell Stem Cell 8:566–579. https://doi.org/10.1016/j.stem.2011.03.010

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317. https://doi.org/10.1038/3305

Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci 25:10074–10086. https://doi.org/10.1523/JNEUROSCI.3114-05.2005

Freedman R (2014) alpha7-nicotinic acetylcholine receptor agonists for cognitive enhancement in schizophrenia. Annu Rev Med 65:245–261. https://doi.org/10.1146/annurev-med-092112-142937

Gabor R, Nagle R, Johnson DA, Gibbs RB (2003) Estrogen enhances potassium-stimulated acetylcholine release in the rat hippocampus. Brain Res 962:244–247

Galea LA (2008) Gonadal hormone modulation of neurogenesis in the dentate gyrus of adult male and female rodents. Brain Res Rev 57:332–341. https://doi.org/10.1016/j.brainresrev.2007.05.008

Gass N et al (2016) An acetylcholine alpha7 positive allosteric modulator rescues a schizophrenia-associated brain endophenotype in the 15q13.3 microdeletion, encompassing CHRNA7. Eur Neuropsychopharmacol 26:1150–1160. https://doi.org/10.1016/j.euroneuro.2016.03.013

Ge S, Yang CH, Hsu KS, Ming GL, Song H (2007) A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 54:559–566. https://doi.org/10.1016/j.neuron.2007.05.002

Gillentine MA, Yin J, Bajic A, Zhang P, Cummock S, Kim JJ, Schaaf CP (2017) Functional consequences of CHRNA7 copy-number alterations in induced pluripotent stem cells and neural progenitor cells. Am J Hum Genet 101:874–887. https://doi.org/10.1016/j.ajhg.2017.09.024

Glenn MJ, Gibson EM, Kirby ED, Mellott TJ, Blusztajn JK, Williams CL (2007) Prenatal choline availability modulates hippocampal neurogenesis and neurogenic responses to enriching experiences in adult female rats. Eur J Neurosci 25:2473–2482. https://doi.org/10.1111/j.1460-9568.2007.05505.x

Goncalves JT, Schafer ST, Gage FH (2016) Adult neurogenesis in the hippocampus: from stem. Cells Behav Cell 167:897–914. https://doi.org/10.1016/j.cell.2016.10.021

Graham AJ, Martin-Ruiz CM, Teaktong T, Ray MA, Court JA (2002) Human brain nicotinic receptors, their distribution and participation in neuropsychiatric disorders. Curr Drug Targ CNS Neurol Disord 1:387–397

Gu Z, Yakel JL (2011) Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. Neuron 71:155–165. https://doi.org/10.1016/j.neuron.2011.04.026

Guebel DV, Torres NV (2016) Sexual dimorphism and aging in the human hippocampus: identification, validation, and impact of differentially expressed genes by factorial microarray and network analysis. Front Aging Neurosci 8:229. https://doi.org/10.3389/fnagi.2016.00229

Hasselmo ME (2006) The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16:710–715. https://doi.org/10.1016/j.conb.2006.09.002

Hernandez CM, Dineley KT (2012) alpha7 nicotinic acetylcholine receptors in Alzheimer's disease: neuroprotective, neurotrophic or both? Curr Drug Targ 13:613–622

Hernandez CM, Kayed R, Zheng H, Sweatt JD, Dineley KT (2010) Loss of alpha7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease. J Neurosci 30:2442–2453. https://doi.org/10.1523/JNEUROSCI.5038-09.2010

Hernandez CM et al (2014) Research tool: validation of floxed alpha7 nicotinic acetylcholine receptor conditional knockout mice using in vitro and in vivo approaches. J Physiol 592:3201–3214. https://doi.org/10.1113/jphysiol.2014.272054

Hollands C, Bartolotti N, Lazarov O (2016) Alzheimer's disease and hippocampal adult neurogenesis exploring shared mechanisms. Front Neurosci 10:178. https://doi.org/10.3389/fnins.2016.00178

Hollands C et al (2017) Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer's disease by compromising hippocampal inhibition. Mol Neurodegener 12:64. https://doi.org/10.1186/s13024-017-0207-7

Holmes MM (2016) Social regulation of adult neurogenesis: a comparative approach. Front Neuroendocrinol 41:59–70. https://doi.org/10.1016/j.yfrne.2016.02.001

Imperato A, Puglisi-Allegra S, Casolini P, Angelucci L (1991) Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. Brain Res 538:111–117

Jessberger S, Parent JM (2015) Epilepsy and adult neurogenesis. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a020677

Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA (2004) Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci USA 101:343–347. https://doi.org/10.1073/pnas.2634794100

John D, Shelukhina I, Yanagawa Y, Deuchars J, Henderson Z (2015) Functional alpha7 nicotinic receptors are expressed on immature granule cells of the postnatal dentate gyrus. Brain Res 1601:15–30. https://doi.org/10.1016/j.brainres.2014.12.041

Jones S, Yakel JL (1997) Functional nicotinic ACh receptors on interneurones in the rat hippocampus. J Physiol 504(Pt 3):603–610

Jung MW, McNaughton BL (1993) Spatial selectivity of unit activity in the hippocampal granular layer. Hippocampus 3:165–182. https://doi.org/10.1002/hipo.450030209

Kang E, Wen Z, Song H, Christian KM, Ming GL (2016) Adult neurogenesis and psychiatric disorders. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a019026

Khiroug SS, Harkness PC, Lamb PW, Sudweeks SN, Khiroug L, Millar NS, Yakel JL (2002) Rat nicotinic ACh receptor alpha7 and beta2 subunits co-assemble to form functional heteromeric nicotinic receptor channels. J Physiol 540:425–434

King JR, Gillevet TC, Kabbani N (2017) A G protein-coupled alpha7 nicotinic receptor regulates signaling and TNF-alpha release in microglia. FEBS Open Biol 7:1350–1361. https://doi.org/10.1002/2211-5463.12270

Kita Y, Ago Y, Higashino K, Asada K, Takano E, Takuma K, Matsuda T (2014) Galantamine promotes adult hippocampal neurogenesis via M(1) muscarinic and alpha7 nicotinic receptors in mice. Int J Neuropsychopharmacol 17:1957–1968. https://doi.org/10.1017/S1461145714000613

Kotani S, Yamauchi T, Teramoto T, Ogura H (2006) Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. Neuroscience 142:505–514. https://doi.org/10.1016/j.neuroscience.2006.06.035

Lagace DC et al (2007) Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. J Neurosci 27:12623–12629. https://doi.org/10.1523/JNEUROSCI.3812-07.2007

Lazarov O, Marr RA (2010) Neurogenesis and Alzheimer's disease: at the crossroads. Exp Neurol 223:267–281. https://doi.org/10.1016/j.expneurol.2009.08.009

Lederle L et al (2011) Reward-related behavioral paradigms for addiction research in the mouse: performance of common inbred strains. PLoS One 6:e15536. https://doi.org/10.1371/journal.pone.0015536

Levin ED (2012) alpha7-Nicotinic receptors and cognition. Curr Drug Targ 13:602–606

Lewis AS, Pittenger ST, Mineur YS, Stout D, Smith PH, Picciotto MR (2017) Bidirectional regulation of aggression in mice by hippocampal alpha-7 nicotinic acetylcholine receptors. Neuropsychopharmacology. https://doi.org/10.1038/npp.2017.276

Luine V, Gomez J, Beck K, Bowman R (2017) Sex differences in chronic stress effects on cognition in rodents. Pharmacol Biochem Behav 152:13–19. https://doi.org/10.1016/j.pbb.2016.08.005

Lykhmus O et al (2015) alpha7 Nicotinic acetylcholine receptor-specific antibody induces inflammation and amyloid beta42 accumulation in the mouse brain to impair memory. PLoS One 10:e0122706. https://doi.org/10.1371/journal.pone.0122706

Martin LF, Freedman R (2007) Schizophrenia and the alpha7 nicotinic acetylcholine receptor. Int Rev Neurobiol 78:225–246. https://doi.org/10.1016/S0074-7742(06)78008-4

Martinez-Pinilla E, Ordonez C, Del Valle E, Navarro A, Tolivia J (2016) Regional and gender study of neuronal density in brain during aging and in Alzheimer's disease. Front Aging Neurosci 8:213. https://doi.org/10.3389/fnagi.2016.00213

Mechawar N et al (2004) Nicotinic receptors regulate the survival of newborn neurons in the adult olfactory bulb. Proc Natl Acad Sci USA 101:9822–9826. https://doi.org/10.1073/pnas.0403361101

Orr-Urtreger A, Broide RS, Kasten MR, Dang H, Dani JA, Beaudet AL, Patrick JW (2000) Mice homozygous for the L250T mutation in the alpha7 nicotinic acetylcholine receptor show increased neuronal apoptosis and die within 1 day of birth. J Neurochem 74:2154–2166

Paez-Gonzalez P, Asrican B, Rodriguez E, Kuo CT (2014) Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. Nat Neurosci 17:934–942. https://doi.org/10.1038/nn.3734

Pan YW, Chan GC, Kuo CT, Storm DR, Xia Z (2012) Inhibition of adult neurogenesis by inducible and targeted deletion of ERK5 mitogen-activated protein kinase specifically in adult neurogenic regions impairs contextual fear extinction and remote fear memory. J Neurosci 32:6444–6455. https://doi.org/10.1523/JNEUROSCI.6076-11.2012

Papouin T, Dunphy JM, Tolman M, Dineley KT, Haydon PG (2017) Septal cholinergic neuromodulation tunes the astrocyte-dependent gating of hippocampal NMDA receptors to wakefulness. Neuron 94:840–854 e847. https://doi.org/10.1016/j.neuron.2017.04.021

Plitman E et al (2017) Kynurenic acid in schizophrenia: a systematic review and meta-analysis. Schizophr Bull 43:764–777. https://doi.org/10.1093/schbul/sbw221

Rubboli F, Court JA, Sala C, Morris C, Chini B, Perry E, Clementi F (1994) Distribution of nicotinic receptors in the human hippocampus and thalamus. Eur J Neurosci 6:1596–1604

Sahay A et al (2011) Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472:466–470. https://doi.org/10.1038/nature09817

Santoro A (2013) Reassessing pattern separation in the dentate gyrus. Front Behav Neurosci 7:96. https://doi.org/10.3389/fnbeh.2013.00096

Schmidt-Hieber C, Jonas P, Bischofberger J (2004) Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 429:184–187. https://doi.org/10.1038/nature02553

Sharma G, Vijayaraghavan S (2001) Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. Proc Natl Acad Sci USA 98:4148–4153. https://doi.org/10.1073/pnas.071540198

Shen JX, Yakel JL (2012) Functional alpha7 nicotinic ACh receptors on astrocytes in rat hippocampal CA1 slices. J Mol Neurosci 48:14–21. https://doi.org/10.1007/s12031-012-9719-3

Shytle RD et al (2004) Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. J Neurochem 89:337–343. https://doi.org/10.1046/j.1471-4159.2004.02347.x

Song J et al (2012) Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature 489:150–154. https://doi.org/10.1038/nature11306

Song J et al (2013) Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. Nat Neurosci 16:1728–1730. https://doi.org/10.1038/nn.3572

Sorrells SF et al (2018) Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555:377–381. https://doi.org/10.1038/nature25975

Spalding KL et al (2013) Dynamics of hippocampal neurogenesis in adult humans. Cell 153:1219–1227. https://doi.org/10.1016/j.cell.2013.05.002

Spencer JL, Waters EM, Romeo RD, Wood GE, Milner TA, McEwen BS (2008) Uncovering the mechanisms of estrogen effects on hippocampal function. Front Neuroendocrinol 29:219–237. https://doi.org/10.1016/j.yfrne.2007.08.006

Tanapat P, Hastings NB, Reeves AJ, Gould E (1999) Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci 19:5792–5801

Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH (2006) NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature 442:929–933. https://doi.org/10.1038/nature05028

Teaktong T et al (2003) Alzheimer's disease is associated with a selective increase in alpha7 nicotinic acetylcholine receptor immunoreactivity in astrocytes. Glia 41:207–211. https://doi.org/10.1002/glia.10132

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270. https://doi.org/10.1038/6368

Velazquez R et al (2013) Maternal choline supplementation improves spatial learning and adult hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome. Neurobiol Dis 58:92–101. https://doi.org/10.1016/j.nbd.2013.04.016

Voytenko LP et al (2015) Hippocampal GABAergic interneurons coexpressing alpha7-nicotinic receptors and connexin-36 are able to improve neuronal viability under oxygen-glucose deprivation. Brain Res 1616:134–145. https://doi.org/10.1016/j.brainres.2015.04.061

Wu J, Liu Q, Tang P, Mikkelsen JD, Shen J, Whiteaker P, Yakel JL (2016) Heteromeric alpha7beta2 nicotinic acetylcholine receptors in the brain. Trends Pharmacol Sci 37:562–574. https://doi.org/10.1016/j.tips.2016.03.005

Yu Y et al (2009) Increased hippocampal neurogenesis in the progressive stage of Alzheimer's disease phenotype in an APP/PS1 double transgenic mouse model. Hippocampus 19:1247–1253. https://doi.org/10.1002/hipo.20587

Yuan M et al (2017) Somatostatin-positive interneurons in the dentate gyrus of mice provide local- and long-range septal synaptic inhibition. Elife. https://doi.org/10.7554/eLife.21105