RESPONSIVENESS OF DENTATE NEURONS GENERATED THROUGHOUT ADULT LIFE
DETERMINES SPATIAL MEMORY ABILITY OF AGED RATS

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Running head. New neuron silencing and pathological aging
ABSTRACT

The aging population has a significant impact upon the societal burden of several neurologic disorders such as age-related cognitive decline ranging from mild cognitive impairment to Alzheimer disease. The hippocampus, a key structure in memory, produces neurons throughout life. In old rats, memory deficits have been associated to the exhaustion of cell genesis: rats with preserved spatial memory produced after learning a higher number of new neurons in comparison with animals with memory impairments. However, the contribution of neurons generated earlier in adult life remains undetermined. We explored the hypothesis that a diminution in the responsiveness of neurons generated at different period of adult-life results in memory impairments. By imaging the activation of neurons born in adult (3 month-old), middle-aged (12 month-old) or senescent (18 month-old) rats using the immediate early gene Zif268, we show that these different neuron generations are recruited by learning only in aged-unimpaired rats. In contrast, aged-impaired rats do not exhibit an activity-dependent regulation of zif268 suggesting that neuronal “silencing” leads to memory deficits. These data add to our current knowledge by showing that the aging of memory abilities stems not only from the number but also from the responsiveness of adult-born neurons.

Keywords: Pathological Aging, Adult neurogenesis, Hippocampus, Spatial memory, Plasticity.
INTRODUCTION

The rapidly growing elderly population and the increasing occurrence of cognitive disorders make the maintenance of “successful” (or ‘healthy’ or ‘optimal’) aging a problem of increasing priority in public health [Batles and Batles 1993]. The search for, and the treatment of age-related diseases is hindered mainly because the multifaceted nature of the neuronal disconnection syndrome along the aging-Alzheimer continuum. Neuropathological events featuring early stages of Alzheimer’s disease (AD) and of mild cognitive impairment (MCI) appear in the hippocampus, a key structure in spatial and episodic memory learning and memory. Cellular dysfunction, dendritic changes, synaptodegeneration; cell loss, and alteration of neuroplastic responses have been classically involved in alteration of age-related decline in hippocampal-dependant memory [Dickstein et al. 2013; Mesulam 1999; Petit and Ivy 1988].

More recently, much attention has been devoted to adult hippocampal neurogenesis (ANG). Within the hippocampal formation, the dentate gyrus (DG) has the unique ability to generate new neurons throughout the entire life of an individual [Altman 1962; Gross 2000], humans included [Eriksson et al. 1998; Spalding et al. 2013]. The newly born cells develop into granule neurons that are integrated into functional circuits and play a crucial role in complex forms of learning and memory i.e; pattern separation and relational memory [Aimone et al. 2014; Koehl and Abrous 2011]. In addition, both the addition and the elimination of new neurons in young adult rodent before, during or after learning are important for learning, remembering and forgetting [Dupret et al. 2007; Trouche et al. 2009; Akers et al. 2014].

During aging, an alteration of ANG has also been proposed to be involved in the appearance of spatial relational memory deficits [Abrous et al. 2005; Drapeau and Abrous 2008; Klempin and Kempermann 2007]. Supporting this view, we showed that successful aging —i.e. preserved memory functions— is associated with the maintenance of a relatively high neurogenesis level measured after learning whereas pathological (“unsuccessful”) aging —i.e. memory deficits— is linked to exhaustion of neurogenesis [Drapeau et al. 2003]. A similar relationship between memory and adult hippocampal neurogenesis was evidenced in aged monkeys [Aizawa et al. 2009; Ngwenya et al. 2015] and in humans [Coras et al. 2010]. Moreover, spatial learning in aged animals also influences the survival of newly born cells: in
aged-unimpaired rats, spatial learning increases the survival of cells generated one week before training whereas it eliminates the cells produced during the early phase of training [Drapeau et al. 2007]. In addition, living in an enriched environment [Marlatt et al. 2012; Kempermann et al. 2002] or corticosterone dampening in middle age [Montaron et al. 2006] have a beneficial effect on the rate of neurogenesis and spatial memory measured once the animals have reached senescence. Together, this last set of data raises the fascinating hypothesis that neurons generated throughout adult life could contribute to maintain a normal hippocampal functioning in old age.

To tackle this question, we asked whether that spatial learning abilities in aged animals depends upon the production of new neurons generated only during senescence or earlier in adult life. We took advantage of the existence of inter-individual differences in spatial learning abilities in aged rats and visualized the recruitment of adult-born neurons labeled with analogs of thymidine using Zif268, an Immediate Early Gene (IEG) [Tronel et al. 2015b].
MATERIALS AND METHODS

Animals. In the first experiment, male rats (n=19, OFA, Charles Rivers, France) 16-month old on delivery, were individually housed in transparent cages under a 12h:12h light/dark cycle with ad libitum access to food and water. Temperature (22°C) and humidity (60%) were kept constant. In the second experiment, 2-month-old rats (n=32) were collectively housed in standard cages. When animals reached 600 g, they were individually housed in accordance with the recommendations of the European Union (2010/63/UE). Animals with a bad general health status or tumors were excluded.

Thymidine analogue injections. Newly-born cells were labeled by the incorporation of synthetic thymidine analogues (XdU, Table 1). In the first experiment, rats were injected with 5-bromo-2’-deoxyuridine (BrdU) according to a previously described protocol [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous2003;Drapeau, Montaron, Aguerre, and Abrous2007]. These animals received one daily BrdU injection (50 mg/day/ip) for five days when eighteen-month-old, i.e. four months before training. In the second experiment, rats received five injection of 5-chloro-2’-deoxyuridine (CldU) when three-months old and five injection of 5-iodo-2’-deoxyuridine (IdU) when 12 months old [Dupret, Fabre, Dobrössy, Panetier, Rodriguez JJ, Lemaire V, Oliet, Piazza, and Abrous2007], both at equimolar doses of 50mg BrdU/kg.

Water-maze training. Rats were tested in the water-maze when twenty-two months old [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous2003;Drapeau, Montaron, Aguerre, and Abrous2007]. The apparatus consisted of a circular plastic swimming pool (180 cm diameter, 60 cm height) that was filled with water (20 ± 1°C) rendered opaque by the addition of a white cosmetic adjuvant. Before the start of training, animals were habituated to the pool for two days for one minute per day. During training, the Learning group (L) was composed of animals that were required to locate the submerged platform, which was hidden 1.5 cm under the surface of the water in a fixed location, using the spatial cues available within the room. Rats were all trained for four trials per day (90 s with an inter-trial interval of 30 s and released from 3 different start points that varied randomly each day). If an animal failed to locate the platform, it was placed on that platform at the end of the trial. The time to reach the platform was recorded using a video camera that was secured to the ceiling of the room and connected to a computerised tracking system (Videotrack, Viewpoint). Daily results were analyzed in order to rank animals according to their behavioral score calculated over the
last 3 days of training (when performances reached an asymptotic level). Animals were trained for 12 (batch1) or 11 (batch2) days. The behavioral scores (calculated either over the whole training duration or over the last training days) of Aged unimpaired (AU) rats were below the median of the group whereas those of Aged Impaired (AI) animals were above the median of the group. Control groups consisted of animals that were transferred to the testing room at the same time and with the same procedures as trained animals but that were not exposed to the water maze.

**Immunohistochemistry.** Animals were sacrificed 90 min after the last trial (Table 1). The different age-matched control groups were sacrificed within the same period. Free-floating sections (50 µm) were processed using a standard immunohistochemical procedure to visualize the thymidine analogs (BrdU, CldU, IdU) on alternate one-in-ten sections using different anti-BrdU antibodies from different vendors (for BrdU: 1/200, Dako; CldU: 1/500, Accurate Chemical and Scientific Corporation; IdU: 1/200, BD Biosciences) and Zif268 (1:500, Santa Cruz Biotechnology). The number of XdU-immunoreactive (IR) cells in the granule and subgranular layers (gcl) of the DG was estimated on a systematic random sampling of every tenth section along the septo-temporal axis of the hippocampal formation using a modified version of the optical fractionator method. Indeed, all of the X-IR cells were counted on each thick section and the resulting numbers were tallied and multiplied by the inverse of the section sampling fraction (1/ssf=10 for BrdU and IdU-cells that were counted in both side of the DG, 1/ssf=20 for CldU-IR cells that were counted in the left side). The number of Zif268-IER cells (left side) was determined using a 100x lens, and a 60 µm x 60 µm frame at evenly spaced x-y intervals of 350 µm by 350 µm with a Stereo Investigator software (Microbrightfield).

**Activation of new cells.** The activation of adult-born cells was examined using immunohistofluorescence. To visualize cells that incorporated thymidine analogues, one-in-ten sections were incubated with different anti-BrdU antibodies (BrdU & CldU, rat primary antibodies at 1/200 Accurate Chemical and Scientific Corporation; IdU, mouse primary antibodies at 1/200, BD PharMingen #347580). Sections were also incubated with Zif268 rabbit (1:500, Santa Cruz Biotechnology). Bound antibodies were visualized respectively with Cy3-goat anti-rat (1:1000, Jackson) or Cy3-goat anti-mouse (1:1000, Jackson) and Alexa-488-goat anti-rabbit antibodies (1:1000, Jackson). CldU- Zif268 and IdU- Zif268 labeling were analyzed on different sections because of some cross reactivity between secondary antibodies made in mice or rat (Fig 1 in [Tronel, Lemaire, Charrier, Montaron, and Abrous2015b]). All BrdU-, CldU- or IdU-labeled cells expressing Zif268 (one side) were
determined using a confocal microscope with HeNe and Arg lasers (Leica, DMR TCSSP2AOBS), with a plane apochromatic 63X oil lens (numerical aperture 1.4; Leica). The percentage of BrdU-, CldU- or IdU-labelled cells that expressed Zif268 was calculated as follow: (Nb of XdU+/IEG+ cells)/(Nb of XdU+/IEG- cells) x 100. All sections were optically sliced in the Z plane using 1 µm interval and cells were rotated in orthogonal planes to verify double labelling.

**Analysis of phenotype.** One-of-ten series was incubated with a rat monoclonal anti-BrdU antibody (1/200 Accurate Chemical and Scientific Corporation) and with a mouse monoclonal anti-NeuN antibody (1:500, Chemicon). Bound anti-BrdU and anti-NeuN antibodies were visualized with a Cy3-goat anti-rat (1:1000, Jackson) and an Alexa 488-labeled goat anti-mouse IgG antibody (1:1000, Jackson). The phenotype of IdU-IR cells and CldU-IR cells was determined using rabbit anti-calbindin antibodies (1/200, Millipore) that were revealed with Alexa 488-labeled goat anti-rabbit IgG antibodies (1/500, Jackson). We also analysed the phenotype of Zif268 cells by incubating one-in ten sections with a rabbit anti-Zif268 antibody (1:500, Santa Cruz Biotechnology) and a mouse monoclonal anti-NeuN antibody (1:500, Chemicon). Bound anti-Zif268 and anti-NeuN antibodies were visualized with a Cy3-goat anti-rabbit (1:1000, Jackson) and an Alexa 488-labeled goat anti-mouse IgG antibody (1:1000, Jackson). Alternate sections were incubated with a rabbit polyclonal anti-Zif268 primary antibody (1/200 Accurate Chemical and Scientific Corporation) and with a mouse monoclonal anti-NeuN antibody (1:500, Chemicon). Bound anti-Zif268 and anti-NeuN antibodies were visualized with a Cy3-goat anti-rabbit (1:1000, Jackson) and an Alexa 488-labeled goat anti-mouse IgG antibody (1:1000, Jackson).

**Statistical analysis.** Data (mean±s.e.m.) were analysed using an ANOVA or Student’s t-test (2 tails) when necessary.
RESULTS

In a first step we sought out to determine whether new neurons born during senescence are recruited by spatial learning. To do so, eighteen-month-old rats were injected with BrdU according to a previously described protocol (Table 1) and were trained four months later in the water maze using a reference memory protocol [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrus2003]. Animals were trained for eleven days (Figure S1a,b) until the aged-unimpaired rats (AU) learned the task (day effect on the Latency: F11,66=2.35, p=0.016; day effect on Distance: F11,66=2.76, p=0.005) and reached asymptotic levels of performances (with no statistical significant differences between the last 3 days). In contrast, the aged-impaired rats did not learn the task although they were searching and finding the platform most of the time (2 or 3 trials out of 4) (day effect on the Latency: F11,66=1.25, p=1.25; day effect on Distance: F11,66=0.96, p=0.48). Ninety minutes after the last trial, animals (and their age-matched control group) were sacrificed for immunohistochemistry. At the time of sacrifice, BrdU-IR cells were 4 months old and the majority was located within the granule cell layer (GCL) (Figure 1a).

These cells were more numerous in the GCL of aged animals with good learning abilities (AU) compared to aged animals with memory deficits (AI) (Figure 2a, F2,16=7.64, p=0.05 with C=AI<AU at p<0.01). This finding is consistent with our previous study showing that the number of neurons generated one month after learning is higher in AU compared to AI [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrus2003] senescent rats. More than fifty percent of BrdU-IR cells in the GCL expressed NeuN (Figure 1b) and neuronal differentiation was not different among groups (Figure 2b, F2,16=2.07, p=0.15).

To determine whether newborn neurons are recruited by learning, we used Zif268 since this IEG is highly expressed in the old DG [Gheidi et al. 2013; Marrone et al. 2011] (Figure 1c). Given that a substantial fraction of cells generated during senescence did not express NeuN, we verify in trained animals that Zif268 expressing cells were expressing NeuN (Figure 1d). We found that the vast majority of activated cells (Zif268) were neurons (NeuN) and that this ratio was similar between good and bad learners (AI: 96.4 ± 0.5; AU: 96 ± 1.3, p>0.05). Then we examined the activation of adult-born cells, meant to be neurons, in response to learning (Figure 1e). We found that the percentage of BrdU-IR cells expressing Zif268-IR in aged animals with good learning abilities was greater than that of aged animals with memory deficits and of untrained control groups (Figure 2c, F2,16=3.70, p=0.05 with
C=AI<AU at p<0.05). In contrast, the total number of Zif268-IR nuclei did not differ between
groups (Figure 2d, F_{2,16}=0.25, p=0.78). These results show that neuronal cells in the
senescent DG are recruited by spatial learning and not by nonspecific effects of training
(swimming, stress) as revealed by the lowest level of recruitment of 4-month-old cells in aged
impaired and control animals.

Then we asked whether neurons born earlier, i.e. in middle-age or young adulthood, are
also recruited by learning during aging. For this purpose, animals were injected with CldU
when three months old, and with IdU when middle-aged (at twelve months old; Table 1).
Animals were trained ten months later for eleven days until the AU learned the task (day
effect on the Latency: F_{10,100}=22.08, p<0.001; day effect on Distance: F_{10,100}=18.77, p<0.001)
and reached three days of stable performances (Figure S1c,d). In this batch, the AI showed a
dramatic improvement of their performances on the last training day (day effect on the
Latency: F_{10,100}=6.67, p<0.001; day effect on Distance: F_{10,100}=22.08, p<0.001). Trained
animals (and their age-matched control group) were sacrificed 90 minutes after the last trial.
At the time of sacrifice IdU cells were ten months old (Figure 1f). Their number was not
influenced by training or by the cognitive status of the animals (Figure 3a, F_{2,29}=0.87,
p=0.43). More than eighty percent of IdU cells expressed the neuronal marker calbindin
(Figure 1g, 3b, F_{2,28}=4.21, p=0.02 with C=AI<AU at p=0.02). The percentage of neurons
born during middle age and expressing Zif268 was greater in the AU group than that
measured in AI and C groups (Figures 1h, 3c, F_{2,29}=4.87, p=0.02 with C=AI<AU at p<0.01
and p<0.05 respectively).

CldU-IR cells examined in the same animals were nineteen months old (Figure 1i). Their number was not influenced by training or by the cognitive status of the animal (Figure 4a,
F_{2,29}=0.52, p=0.6). By analysing phenotype of CldU cells with calbindin, we found that that
exposure to the water maze slightly increased neuronal differentiation (Figure 1j,4b, C: 82.8
± 1 AI: 86.9 ± 0.8; AU: 86.2 ± 0.7; F_{2,29}=6.54, p<0.01 with C<AI=AU at p=0.01). Again, we
found that the percentage of CldU-IR cells expressing Zif268 was greater in the AU group than that measured in AI and C groups (Figures 1k, 4c, F_{2,29}=6.96, p=0.004 with C=AI<AU
at p<0.01 and p<0.05 respectively). The total number of cells expressing Zif268-IR (C:
29270.02 ± 2360.54: AI: 26068.94 ± 2366.78; AU: 28739.22 ± 3095.74, F_{2,29}=0.42, p=0.65)
did not differ between groups.
DISCUSSION

To determine whether neurons generated during adult life participate to learning abilities in old age, the expression of the IEG Zif268 in new neurons was assessed. We found that cells generated during young adulthood, middle age and senescence survive for a long period of time and are functionally integrated into the dentate network. When taking into account individual differences in memory abilities, we highlight that although the number of new cells generated in 12-month-old animals is decreased tenfold compared to 3 months old rats, the total number of CdU-IR or IdU-IR cells measured when animals reached senescence is similar between AU and AI and not different from untrained control animals.

Between middle age and senescence the number of cells is further decreased, but then a difference among the AU and AI groups appears. Based on our previous data, it is likely that the emergence of such a difference results from a difference in cell proliferation [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous2003] most probably linked to changes in the neurogenic niche [Abrous, Koehl, and Le Moal2005;Drapeau and Abrous2008]. In addition, difference in neuronal differentiation might be at play since the percentage of cells expressing Calbindin in AU was increased compared to the other groups. This observation is not unprecedented [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous2003] albeit not systematic [Drapeau, Montaron, Aguerre, and Abrous2007] and could be related to the role of Zif268 in neuronal maturation. Indeed, Zif268 has been recently shown to control the time-frame of maturation of adult born neurons during the critical period within which they are selected to survive [Veyrac et al. 2013].

The main finding of our study is that the ability for newborn cells to be recruited by learning in aged rats depends upon their memory abilities. Indeed, the percentage of adult-born cells expressing Zif268 was higher in animals that learned the task compared to animals that did not. This finding is in accordance with our previous data showing that i) when compared to control rats (naïve rats or rats trained to find a visible platform), adults required to use an allocentric mapping strategy in the water maze (or the dry maze) exhibit an increased percentage of mature adult-born neurons expressing Zif268 [Tronel et al. 2015a;Tronel, Lemaire, Charrier, Montaron, and Abrous2015b], and ii) ablating mature adult-born neurons generated four months before training (when animals where 3 months old) delays the ability of rats to learn such a task [Lemaire et al. 2012]. In the present experiment the percentage of adult-born cells expressing Zif268 in each experimental group was similar for the three neuronal populations studied. It was thus independent of the age of the animals at
the time of labeling (3, 12, and 18 months) and of the age of the cells at the time of training (4, 10, and 19 months). It was also independent of whether or not the total number of XdU cells differed between AU and AI groups.

It could be argued that neurons born during development, which represent a major part of the DG, are also involved in differences in spatial memory abilities in old age. However, three arguments seem to rule out this hypothesis. First, the total number of granule cells is similar between AU and AI groups [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous 2003; Drapeau, Montaron, Aguerre, and Abrous 2007; Rapp and Gallagher 1996]. Second, we have shown that neurons born in neonates (first postnatal week) are activated in different memory processes when they are mature compared to neurons of the same age born in adults. Indeed, the former are not recruited by spatial learning in the water maze when animals are tested at seven months old [Tronel, Lemaire, Charrier, Montaron, and Abrous 2015b]. Third, if neurons generated during development (pre- and post-natal periods) were activated by spatial learning, given their high numbers, differences in the total number of Zif268 cells should have emerged as a function of cognitive status. However, additional experiments are required before ruling out a potential involvement of developmentally-born neurons.

One question that we did not address is whether the three neuronal populations studied participate to the same extent to learning. To address this point, sophisticated models that allow to selectively tag new neurons generated within a defined period of time (adulthood, middle-age or senescence) and to ablate them during training performed at senescence, are required. One possibility would be to take advantage of the recently developed pharmacogenetic approach of DREADD (Designer Receptor Exclusively Activated by Designer Drug) [Rogan and Roth 2011] in order to tag specifically new neurons.

A previous study has shown that 4-month-old neurons generated in old rats exhibiting spatial memory deficits are recruited in response to spatial exploration behavior with the same probability than 4-month-old neurons generated in aged good learners or in young adult rats [Marrone et al. 2012] From this dataset it was concluded that disrupted information processing at old age may be linked to a reduced number of adult-generated granule cells, and not to a deficit in their functionality. However, in this study the activation of adult-generated neurons was evaluated in response to a simple form a learning (spatial exploration). Taking the present data into consideration, we rather suggest that adult-born neurons in aged-impaired rats are sufficiently connected to integrate simple stimulations generated during
simple form of learning but insufficiently integrated to process the complex stimulations generated during spatial navigation.

Zif268 is known to be regulated in an activity-dependent manner by learning (for review see [Veyrac et al. 2014]). It is overexpressed in response to different types of learning in distinct structures and circuits that are processing the ongoing information and several arguments indicate that it is required for memory consolidation and reconsolidation through epigenetic regulations. Although the mechanisms are not fully understood, the activation of Zif268 may strengthen the memory trace. It can be hypothesized that during learning the activation of Zif268 in adult-born neurons of AU rats may be involved in the formation, stabilization and reactivation of place cells in the hippocampal network, events known to support spatial learning [O'Keefe J 1978].

Here we hypothesize that adult-born neurons that do not exhibited activity-dependent regulation of zif268 become functionally silent in the course of aging, leading to memory deficits. Although the firing patterns that are sufficient to induce Zif268 in adult-born neurons in “behaving” animals are so far unknown, adult-born neurons silencing may have several origins. It may result from a loss of synaptic inputs [Geinisman et al. 1986] from the entorhinal cortex and/or the septum [Fischer et al. 1987;Smith et al. 2000] and/or to an inability to fire properly [Ahlenius et al. 2009] as a consequence of methylation-induced transcriptional repression [Penner et al. 2010;Penner et al. 2011]. This suggests that the beneficial effect of living in an enriched environment, or of lowering corticosterone levels [Marlatt, Potter, Lucassen, and van 2012;Kempermann, Gast, and Gage 2002;Montaron, Drapeau, Dupret, Kitchener, Aurousseau, Le, Piazza, and Abrous 2006] from middle age, on memory abilities at old age result from decreasing age-related silencing of adult-born neurons, a hypothesis that await to be tested.

Aging is also accompanied in some individuals by the emergence of mood disorders [Karel 1997] that have been associated with increased HPA axis activity and decreased hippocampal mineralocorticoid and/or glucocorticoid receptor gene expression [Sapolsky 1992]. Given that ANg is involved in mood disorders [Pittenger and Duman 2008;Yassa and Stark 2011;Revest et al. 2009] and that chronic treatment with antidepressant from middle age onward prevents the appearance of depression (and memory disorders) when animals reached senescence [Yau et al. 2002], it is tempting to propose that the age-related appearance of mood disorders also results from the silencing of neurons born during adult life. Our results thus offer a new target for the therapeutic use of antidepressants to prevent the occurrence of mood (as well as memory) disorders among populations of aged individuals.
Our results may have a huge impact for human well-being if our animal-based investigations are validated in a non-demented elderly population. Recently, the rate of cell genesis and the number of immature neurons expressing double-cortin or PSA-NCAM in human hippocampi have been reported to decrease to very low levels [Sorrells et al. 2018], an observation consistent with rodents’ data [Drapeau, Mayo, Aurousseau, Le Moal, Piazza, and Abrous2003; Drapeau, Montaron, Aguerre, and Abrous2007; Lemaire et al. 1999; Montaron et al. 1999; Montaron, Drapeau, Dupret, Kitchener, Aurousseau, Le, Piazza, and Abrous2006]}. Taking into consideration that adult-born neurons need several weeks to be recruited by spatial learning [Kee et al. 2007], a property extending over several months [Lemaire, Tronel, Montaron, Fabre, Dugast, and Abrous2012; Tronel, Charrier, Sage, Maitre, Leste-Lasserre, and Abrous2015a; Tronel, Lemaire, Charrier, Montaron, and Abrous2015b] or even years (present data), the very low rate of neurogenesis at old age does not ruled out the implication of neurones born throughout adult-life in hippocampal function of aging subjects.

In conclusion, our results highlight the importance of neurons born throughout adult-life in memory processing when animals have reached senescence. Whether the responsiveness of adult-born granule neurons in cognitively preserved animals allow to preserve hippocampal functioning (the maintenance hypothesis[Nyberg et al. 2012]) or provide resilience to age related pathology (the reserve hypothesis[Stern 2002]) remains to be disentangled. But clearly, our results reveal a novel perspective for developing therapies to prevent age-related disorders by acting throughout adult life on adult-born dentate neurons.
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The authors declare no conflict of interest.
Reference List

Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. Physiol Rev 2005; 85: 523-569.

Ahlenius H, Visan V, Kokaia M, Lindvall O, Kokaia Z. Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. J Neurosci 2009; 29: 4408-4419.

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH. Regulation and Function of Adult Neurogenesis: From Genes to Cognition. Physiol Rev 2014; 94: 991-1026.

Aizawa K, Ageyama N, Yokoyama C, Hisatsune T. Age-dependent alteration in hippocampal neurogenesis correlates with learning performance of macaque monkeys. Exp Anim 2009; 58: 403-407.

Akers KG, Martinez-Canabal A, Restivo L et al. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science 2014; 344: 598-602.

Altman J. Are new neurones formed in the brains of adult mammals? Science 1962; 135: 1127-1128.

Batles PB, Batles MM. Successfull aging : Perspectives from the behavioral sciences. Cambridge University Press; 1993.

Coras R, Siebzehnrubl FA, Pauli E et al. Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans. Brain 2010; 133: 3359-3372.

Dickstein DL, Weaver CM, Luebke JI, Hof PR. Dendritic spine changes associated with normal aging. Neuroscience 2013; 251: 21-32.

Drapeau E, Abrous DN. Role of neurogenesis in age-related memory disorders. Aging Cell 2008; 7: 569-589.

Drapeau E, Mayo W, Aurourseau C, Le Moal M, Piazza PV, Abrous DN. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc Natl Acad Sci 2003; 100: 14385-14390.

Drapeau E, Montaron MF, Aguerre S, Abrous DN. Learning-induced survival of new neurons depends on the cognitive status of aged rats. J Neurosci 2007; 27: 6037-6044.

Dupret D, Fabre A, Dobroissy M et al. Spatial learning depends on both the addition and removal of new hippocampal neurons. PLOS Biology 2007; 5: 1683-1694.

Eriksson PS, Perfilieva E, Bjork-Eriksson T et al. Neurogenesis in the adult human hippocampus. Nat Med 1998; 4: 1313-1317.

Fischer W, Wictorin K, Bjorklund A, Williams LR, Varon S, Gage FH. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. Nature 1987; 329: 65-68.
Geinisman Y, Toledo-Morrell L, Morrell F. Loss of perforated synapses in the dentate gyrus: morphological substrate of memory deficit in aged rats. Proc Natl Acad Sci U S A 1986; 83: 3027-3031.

Gheidi A, Azzopardi E, Adams AA, Marrone DF. Experience-dependent persistent expression of zif268 during rest is preserved in the aged dentate gyrus. BMC Neurosci 2013; 14: 100.

Gross CG. Neurogenesis in the adult brain: death of a dogma. Nat Rev Neurosci 2000; 1: 67-73.

Karel MJ. Aging and depression: vulnerability and stress across adulthood. Clin Psychol Rev 1997; 17: 847-879.

Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat Neurosci 2007; 10: 355-362.

Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002; 52: 135-143.

Klempin F, Kempermann G. Adult hippocampal neurogenesis and aging. Eur Arch Psychiatry Clin Neurosci 2007; 257: 271-280.

Koehl M, Abrous DN. A new chapter in the field of memory: adult hippocampal neurogenesis. Eur J Neurosci 2011; 33: 1101-1114.

Lemaire V, Aurousseau C, Le Moal M, Abrous DN. Behavioural trait of reactivity to novelty is related to hippocampal neurogenesis. Eur J Neurosci 1999; 11: 4006-4014.

Lemaire V, Tronel S, Montaron MF, Fabre A, Dugast E, Abrous DN. Long-lasting plasticity of hippocampal adult-born neurons. J Neurosci 2012; 32: 3101-3108.

Marlatt MW, Potter MC, Lucassen PJ, van PH. Running throughout middle-age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. Dev Neurobiol 2012; 72: 943-952.

Marrone DF, Ramirez-Amaya V, Barnes CA. Neurons generated in senescence maintain capacity for functional integration. Hippocampus 2011.

Marrone DF, Ramirez-Amaya V, Barnes CA. Neurons generated in senescence maintain capacity for functional integration. Hippocampus 2012; 22: 1134-1142.

Mesulam M-M. Neuroplasticity Failure in Alzheimer's Disease: Bridging the Gap between Plaques and Tangles. Neuron 1999; 24: 521-529.

Montaron MF, Drapeau E, Dupret D et al. Lifelong corticosterone level determines age-related decline in neurogenesis and memory. Neurobiol Aging 2006; 27: 645-654.

Montaron MF, Petry KG, Rodriguez JJ et al. Adrenalectomy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus. Eur J Neurosci 1999; 11: 1479-1485.
Ngwenya LB, Heyworth NC, Shwe Y, Moore TL, Rosene DL. Age-related changes in dentate gyrus cell numbers, neurogenesis, and associations with cognitive impairments in the rhesus monkey. Front Syst Neurosci 2015; 9: 102.

Nyberg L, Lovden M, Riklund K, Lindenberger U, Backman L. Memory aging and brain maintenance. Trends Cogn Sci 2012; 16: 292-305.

O'Keefe J NL. The hippocampus as a cognitive map. Oxford: Oxford University Press; 1978.

Penner MR, Roth TL, Barnes CA, Sweatt JD. An epigenetic hypothesis of aging-related cognitive dysfunction. Front Aging Neurosci 2010; 2: 9.

Penner MR, Roth TL, Chawla MK et al. Age-related changes in Arc transcription and DNA methylation within the hippocampus. Neurobiol Aging 2011; 32: 2198-2210.

Petit TD, Ivy O. Neuroplasticity . A Lifespan approach. New York: A.R.Liss,Inc; 1988.

Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008; 33: 88-109.

Rapp PR, Gallagher M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. Proc Natl Acad Sci USA 1996; 93: 9926-9930.

Revest JM, Dupret D, Koehl M et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry 2009; 14: 959-967.

Rogan SC, Roth BL. Remote control of neuronal signaling. Pharmacol Rev 2011; 63: 291-315.

Sapolsky RM. Stress, the aging brain, and the mechanisms of neuron death. Cambridge, Massachusset, London, England: A bradford book, The MIT Press, 1992.

Smith TD, Adams MM, Gallagher M, Morrison JH, Rapp PR. Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J Neurosci 2000; 20: 6587-6593.

Sorrells SF, Paredes MF, Cebrian-Silla A et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 2018; 555: 377-381.

Spalding KL, Bergmann O, Alkass K et al. Dynamics of hippocampal neurogenesis in adult humans. Cell 2013; 153: 1219-1227.

Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc 2002; 8: 448-460.

Tronel S, Charrier V, Sage C, Maitre M, Leste-Lasserre T, Abrous DN. Adult-born dentate neurons are recruited in both spatial memory encoding and retrieval. Hippocampus 2015a.

Tronel S, Lemaire V, Charrier V, Montaron MF, Abrous DN. Influence of ontogenetic age on the role of dentate granule neurons. Brain Struct Funct 2015b; 220: 645-661.
Trouche S, Bontempi B, Roullet P, Rampon C. Recruitment of adult-generated neurons into functional hippocampal networks contributes to updating and strengthening of spatial memory. Proc Natl Acad Sci U S A 2009; 106: 5919-5924.

Veyrac A, Besnard A, Caboche J, Davis S, Laroche S. The transcription factor Zif268/Egr1, brain plasticity, and memory. Prog Mol Biol Transl Sci 2014; 122: 89-129.

Veyrac A, Gros A, Bruel-Jungerman E et al. Zif268/egr1 gene controls the selection, maturation and functional integration of adult hippocampal newborn neurons by learning. Proc Natl Acad Sci U S A 2013; 110: 7062-7067.

Yassa MA, Stark CE. Pattern separation in the hippocampus. Trends Neurosci 2011; 34: 515-525.

Yau JL, Noble J, Hibberd C et al. Chronic treatment with the antidepressant amitriptyline prevents impairments in water maze learning in aging rats. J Neurosci 2002; 22: 1436-1442.
Figure Legends

Figure 1. Newborn neurons in the DG of aged rats. (a) Illustration of 4-month-old BrdU-IR neurons in an animal with preserved memory. (b) Confocal photomicrographs of 4-month-old BrdU-IR cells (blue) expressing NeuN (green). (c) Illustration of Zif268-IR cells in the DG of senescent rat. Confocal photomicrographs of (d) neurons (NeuN, green) expressing Zif268 (blue) and of (e) 4-month-old BrdU-IR cells (red) expressing Zif268 (green). (f) Illustration of 10-month-old IdU-IR neurons. Confocal photomicrographs of IdU-IR cells (red) expressing (g) Calbindin (green) or (h) Zif268 (green). Illustration of 19-month-old CldU-IR neurons. (i) Confocal photomicrographs of CldU-IR cells (red) expressing (j) Calbindin (green) or (k) Zif268 (green). Bar scale for a,f,i= 20µm. Bar scale for c: 100µm. Bar scale for b,d,e,g,h,j,k=10µm.

Figure 2. Neurons produced during old age are activated by spatial learning. Top: Experimental design. (a) The number of BrdU-IR cells is higher in the aged rats that learned the task (AU) compared to those with spatial memory deficits (AI) or to control animals (C). (b) The percentage of cells differentiating into neurons (BrdU-IR cells expressing NeuN) is similar between the three groups. (c) The expression of Zif268 in BrdU-IR cells generated in senescent DG is increased in AU compared to AI rats and C rats. (d) The number of neurons expressing Zif268 is similar between the three groups. *: p<0.05, **: p<0.01 compared to AU.

Figure 3. Neurons produced during middle-age are activated by spatial learning in aged rats. Top: Experimental design. (a) The numbers of IdU-IR cells -generated at mid-age- are independent of the memory abilities measured when rats reached senescence. (b) The percentage of cells differentiating into neurons (IdU-IR cells expressing Calbindin) is slightly increased in AI (compared to C and AU). (c) The expression of Zif268 in IdU-IR cells is increased in AU rats compared to AI rats and C rats. *: p<0.05, **: p<0.01 compared to AU. °: p<0.05 compared to C.

Figure 4. Neurons produced during young adulthood are activated by spatial learning in aged rats. Top: Experimental design. (a) The numbers of CldU-IR cells generated when animals are young adult is independent of the memory abilities measured when rats reached
senescence. (b) The percent of CldU-IR cells expressing calbindin is increased by training. (c) The expression of Zif268 in CldU-IR cells generated in young adult DG is increased in AU compared to AI rats and C rats. °: p<0.05 compared to AU, +: p<0.05 compared to AI. *: p<0.05, **: p<0.01 compared to AU.

**Figure S1. Spatial memory abilities of aged rats in the water maze.** Learning performances are expressed as the mean latency (a,c) and mean distance travelled (b,d) to find the submerged platform for the first (a,b) and second (c,d) cohort of senescent rats.
**Table 1**: Summary of the procedures

| Batch | Experiment                        | XdU | Rats’ age at XdU injections | Neurons’ age at time of sacrifice | Group size |
|-------|-----------------------------------|-----|-----------------------------|-----------------------------------|------------|
| 1     | Recruitment of 4-month-old neurons | BrdU| 18 months                   | 4 months                          | C = 5      |
|       |                                   |     |                             |                                   | AI = 7     |
|       |                                   |     |                             |                                   | AU=7       |
| 2     | Recruitment of 10-month-old neuron | IdU | 12 months                   | 10 months                         | C = 10     |
|       |                                   |     |                             |                                   | AI = 11    |
|       |                                   |     |                             |                                   | AU=11      |
|       | Recruitment of 19-month-old neurons | ClU | 3 months                    | 19 months                         | C = 10     |
|       |                                   |     |                             |                                   | AI = 11    |
|       |                                   |     |                             |                                   | AU=11      |
Montaron et al., Figure 1
Montaron et al., Figure 2
IdU

22 M

12 M

10-months-old cells

Montaron et al., Figure 3
Montaron et al., Figure 4