Review

Untold New Beginnings: Adult Hippocampal Neurogenesis and Alzheimer's Disease

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Abstract. Neurogenesis occurs in a limited number of brain regions during adulthood. Of these, the hippocampus has attracted great interest due to its involvement in memory processing. Moreover, both the hippocampus and the main area that innervates this structure, namely the entorhinal cortex, show remarkable atrophy in patients with Alzheimer's disease (AD). Adult hippocampal neurogenesis is a process that continuously gives rise to newborn granule neurons in the dentate gyrus. These cells coexist with developmentally generated granule neurons in this structure, and both cooperative and competition phenomena regulate the communication between these two types of cells. Importantly, it has been revealed that GSK-3 β and tau proteins, which are two of the main players driving AD pathology, are cornerstones of adult hippocampal neurogenesis regulation. We have shown that alterations either promoting or impeding the actions of these two proteins have detrimental effects on the structural plasticity of granule neurons. Of note, these impairments occur both under basal conditions and in response to detrimental and neuroprotective stimuli. Thus, in order to achieve the full effectiveness of future therapies for AD, we propose that attention be turned toward identifying the pathological and physiological actions of the proteins involved in the pathogenesis of this condition.

Keywords: Adult hippocampal neurogenesis, Alzheimer's disease, granule neuron, GSK-3 β , morphology, neuroprotection, tau

ALZHEIMER'S DISEASE: A FEW WORDS ABOUT TWO OF THE MAIN PLAYERS, GSK-3 β AND TAU

Alzheimer's disease (AD) is the most common type of dementia in industrialized countries.

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Although the etiology of the disease remains to be fully elucidated, age, genetic, lifestyle, and environmental factors appear to confer higher susceptibility [1]. AD is characterized by progressive neuronal cell death and atrophy of specific brain areas, including the entorhinal cortex (EC) and the hippocampus, and by a marked impairment of episodic memory [2, 3]. The most relevant histopathological hallmarks of the disease are extracellular senile plaques made of amyloid- β (A β) protein, and neurofibrillary tangles, which are formed mainly by hyperphosphorylated tau

protein. A β and tau have been largely considered to be the cornerstones of AD pathogenesis [4]. Moreover, *in vitro* [5] and *in vivo* [6] studies showed that A β exerts its detrimental actions by activating a key kinase, namely glycogen synthase kinase 3 β (GSK-3 β) [5, 7], thus revealing this kinase as an important player in the amyloid cascade. GSK-3 β is the main kinase that phosphorylates tau [7, 8]. Moreover, an increase in GSK-3 β activity has been observed in the brains of AD patients [9]. These data have confirmed GSK-3 β as a cornerstone of AD pathogenesis and support the notion that this kinase is a crucial molecular link between A β and tau [7, 8, 10–13]. Indeed, a transgenic animal model that overexpresses GSK-3 β in the hippocampus (namely GSK-3 β -oe mouse), generated in our laboratory, has been used to model the cellular and behavioral alterations that occur in AD. In these mice, conditional overexpression (OE) of GSK-3 β results in impaired spatial memory and increased tau phosphorylation in the hippocampus [14–16].

The present work is focused on the hippocampal region, given the relevance of this brain region in AD pathogenesis. It revises the available literature on the effects of GSK-3 β and tau dysregulation on a specific neuronal population of this region, namely granule neurons. Special emphasis is placed on the therapeutic potential of diverse interventions aimed to increase hippocampal plasticity in AD.

A FEW WORDS ABOUT ADULT HIPPOCAMPAL NEUROGENESIS (AHN)

Adult neurogenesis is an infrequent phenomenon in the mammalian brain. Under physiological conditions, only a limited number of human brain regions, including the hippocampus [17], the sub-ventricular zone [18], and the striatum [19], experience this process throughout lifetime. AHN has attracted considerable interest mainly because of the involvement of the hippocampal region in learning and memory and the marked atrophy of this structure in patients with AD [20]. In the hippocampus, adult neurogenesis continuously gives rise to newborn granule neurons (NGNs) throughout life [17, 21–23]. These newly generated cells derive from a special population of radial glial-like precursor cells [24], which undergo several rounds of asymmetric division and generate transiently amplifying progenitors [25]. These cells actively divide in specialized neurogenic niches and go through several development

stages before reaching full maturity [26, 27]. During maturation, NGNs extend their dendritic trees through the granule and the molecular layers (GL and ML) of the dentate gyrus (DG), where they receive afferent innervation from the EC [28] and inhibitory Parvalbumin⁺ interneurons [29]. Moreover, they extend their axonal projections toward the CA3 [30] and the CA2 [31] hippocampal subfields. Importantly, numerous aspects of newborn neuron generation in the hippocampus, including proliferation, maturation, and survival rates, are altered in animal models of AD and in patients with this disease [32, 33].

ORCHESTRATION OF AHN BY GSK-3 β AND TAU

Regulation of the rate of AHN

In the DG, GSK-3 β increases the proliferation of neuron precursors and prevents them from acquiring a neuronal fate [34]. Moreover, GSK-3 β overactivation increases the apoptosis of mature granule neurons and blocks the differentiation of neuroblasts [35]. Interestingly, an increased number of Doublecortin (DCX)⁺ neuroblasts is observed in GSK-3 β -oe mice [35]. In this regard, the successive stages of AHN are featured by the expression of specific markers, and a stereotyped pattern of expression is thought to drive the maturation of these cells [25, 26, 36]. For instance, DCX expression is switched off at 3–4 weeks of cell age under physiological conditions. However, we found that its expression was aberrantly prolonged until the sixth week of cell age in GSK-3 β -oe mice [35], thus rendering an increased number of neuroblasts whose maturational progression was blocked. As will be further discussed, this blockade has important consequences for NGN functionality in GSK-3 β -oe mice.

As previously mentioned, tau is one of the main downstream targets of GSK-3 β and is considered a capital regulator of AHN [37, 38]. In this regard, both the expression and the post-transcriptional modifications of tau are tightly regulated during the maturation of NGNs [37–39]. For instance, the tau isoform featured by the presence of three-repeat microtubule-binding domains (namely, 3R-tau) is transiently expressed during the NGN neuroblast stage [38]. In rodents, individual NGNs show the highest expression of 3R-tau at 2 weeks of cell age [39], and the expression of this molecule is maintained until 4 weeks, a time point at which 3R-tau

Fig. 1. Tracings of Golgi-stained granule neurons of control subjects (A) and patients with Alzheimer's disease (AD) (B). It should be noted how granule neurons of control subjects display a single primary apical dendrite emerging from the soma, thus resembling a "Y-shape", whereas granule neurons of AD patients show several primary apical dendrites that are poorly branched in the distal domains, thus acquiring a "V-shape".

is replaced by the four-repeat microtubule-binding domain form of the protein (4R-tau) [38]. By using an antigen retrieval protocol, we showed that 3R-tau is a transient marker of NGN axons [39]. Noteworthy, this isoform of tau confers the cytoskeleton with greater plasticity than the 4R-tau isoform and the expression of 3R-tau is coincident with the period of time in which NGNs exhibit highest plasticity [40]. Although the 3R-tau/4R-tau ratio in humans and rodents appears to differ [41], the absence of reliable human 3R-tau markers for immunohistochemistry determinations has hindered the detailed study of these regulatory mechanisms in the context of human AHN to date. However, it has been proposed that an imbalance in the aforementioned ratio underlies certain neurodegenerative diseases [42]. This notion deserves further exploration in the context of AD.

Despite the predominant role that tau is assumed to play in AHN regulation, tau knock-down does not cause alterations in the proliferation, differentiation, or survival rates of neural precursors in the DG *in vivo* [43]. In this regard, compensatory mechanisms exerted by other microtubule-associated proteins (MAPs), such as MAP-1A and MAP-1B [44, 45], occur in the absence of tau. In contrast to the apparent absence of alterations showed by tau knock-out (tau^{-/-}) mice regarding the basal rate of AHN, we and others have demonstrated that various animal models of tauopathies in which pathogenic forms of tau are overexpressed exhibit marked alterations in AHN [37, 46]. In this regard, the overexpression of a pathogenic form of the protein is not only characterized by its lack of physiological functions but also by the gain of toxic functions. A recent study by our group also revealed that stereotaxic injection of a soluble form of tau has devastating effects on the structural plasticity of granule neurons and impairs pattern separation ability [47]. These data might be

relevant for the field of neurodegenerative disorders, since they contribute to shedding light on novel pathological roles played by distinct tau species *in vivo*.

The "V-shape" phenotype of newborn granule neurons in AD

As previously mentioned, NGNs go through a multi-stage development process before they reach maturity [25]. Although fully mature newly and developmentally generated granule neurons were initially described to be undistinguishable [48], growing evidence indicates that they show both functional and anatomical differences [49]. Regarding the latter, these two types of granule neurons differ in their positioning with respect to the GL [49, 50]. In this respect, NGNs are located in the inner third of the GL, whereas developmentally generated granule neurons are found in the two outer thirds of this layer [50]. Moreover, both types of cell show morphological particularities worthy of further discussion, the most remarkable difference being the number of primary apical dendrites. Under physiological conditions, most newly generated granule neurons show a single primary apical dendrite, which is extensively branched in the ML, thus resembling a "Y-shape" [49]. In contrast, developmentally generated granule neurons show several primary apical dendrites emerging from the soma [51]. In contrast to the scenario observed under physiological conditions, we showed that GSK-3 β OE causes a dramatic change in the morphology of NGNs by triggering the presence of several primary apical dendrites, thereby conferring them a "V-shape" [16]. The relevance of this observation lies in the fact that the same morphology is observed in the granule neurons of AD patients [16]. Representative neuronal tracings corresponding to Golgi-stained granule neurons belonging to

control subjects and AD patients are shown in Fig. 1. To the best of our knowledge, our work was the first to describe the occurrence of this phenomenon in AD patients; however, subsequent studies by other authors have revealed a similar “V-shape” phenotype, including the appearance of several primary apical dendrites in various pathological conditions [52–55]. Further efforts should address the molecular mechanisms involved in the appearance of this particular phenotype. In this regard, several molecules associated with the cytoskeleton may drive the development of dendritic branches and should be further explored in the context of neurodegenerative diseases [56].

Despite the observed marked effects of GSK-3 β OE on the morphology of mouse NGNs, a critical question for the AD research field is whether these effects are a *cell-autonomous* consequence of GSK-3 β OE or whether they represent a *non-cell-autonomous* indirect effect derived from massive cell death or neuroinflammation. This question is particularly relevant in the context of AHN, given that the promoter used to drive GSK-3 β OE, namely *CamKII*, is active only in mature cells [16]. In order to address this point, we developed an innovative system to selectively drive GSK-3 β OE in our target cells, namely NGNs [57, 58]. This system takes advantage of the capacity of retroviruses to exclusively transduce proliferating cells and of the fact that proliferation is restricted mostly to NGNs in the DG [30]. This novel system is based on the stereotaxic injection of a retrovirus encoding the reverse Tetracycline activator (rtTA) element into the hippocampus of tetR-GSK-3 β mice. These animals carry a bi-directional Tetracycline repressor (TetR) promoter followed by a GSK-3 β cDNA in one direction and a cDNA encoding β -Galactosidase (β -Gal) fused to a nuclear localization signal in the other. By using this methodology, GSK-3 β OE occurs only in those NGNs infected by the retrovirus and after the administration of Doxycycline. Thus, our novel methodology has three major advantages over traditional transgenic mouse systems. The first is the use of an rtTA element instead of the classical tTA element. This approach renders a tet-ON system in which GSK-3 β OE is selectively triggered by the administration of Doxycycline—a feature that allows precise temporal control of GSK-3 β OE. The second advantage is that the system allows tight regulation of the age and type of cell populations that overexpress this protein by means of Doxycycline administration during the desired periods. Finally, the system allows the double monitoring of GSK-3 β OE by means of

two reporter proteins, namely EGFP (encoded by the retroviral genome) and β -Gal (encoded by the murine genome). As previously mentioned, this methodology allowed us to achieve rapid and selective GSK-3 β OE in the NGNs infected by the retrovirus [57]. Using this strategy, we demonstrated the *cell-autonomous* character of the alterations triggered by GSK-3 β OE in NGNs, which showed a “V-shape” phenotype only under tet-ON conditions.

This system shows great versatility and potential utility in the field of neurodegenerative diseases, since it has proved highly suitable for the study of the *cell-autonomous* effects of other pathogenic proteins involved in these conditions [58].

Synaptic integration of newborn granule neurons

Although the functional consequences of the morphological alterations previously described remain to be fully elucidated, we have proposed that a reduced dendritic mass in the ML decreases the afferent connectivity of NGNs by reducing the likelihood of distal dendrites receiving afferent contacts from the EC. This hypothesis is strongly supported by the clear detrimental effect of GSK-3 β at the synapse [59]. In this regard, long-term depression downregulates long-term potentiation through GSK-3 β activation [60, 61], a phenomenon which is followed by synapse elimination.

By using a PSD95:GFP-expressing retrovirus [62], we demonstrated that the NGNs of GSK-3 β -oe mice exhibit a marked reduction in the number and size of postsynaptic densities (PSDs), thus revealing impaired afferent connectivity [16, 63]. Special mention should be given to the important consequences of selective impairment of the synaptic integration of NGNs on the whole trisynaptic circuit. In this regard, during the period in which NGNs are *young and excitable* [40], they are thought to play a key role in information processing along the hippocampal circuit [64, 65]. In fact, it has been proposed that newly and developmentally generated granule neurons cooperate to create an accurate and complex representation of new memories. Developmentally generated granule neurons are believed to be involved mostly in pattern completion (an ability based on generalization) [66], whereas the lower activation threshold and higher excitability of NGNs favor their involvement in hippocampal pattern separation (a phenomenon consisting of the production of two differentiated outputs in response to very similar inputs) [67, 68]. The continuous re-definition of

this elegant hypothesis proposed by Sahay [68] and McHugh [67] is continuously revealing the existence of additional complex orchestration mechanisms. For instance, competition phenomena between newborn and developmentally generated neurons to establish synaptic contacts with afferent fibers from the EC may also drive the intricate communication between this structure and the DG [69, 70]. This notion has been further supported by an elegant study by Sahay et al., in which the authors observed that reducing the connectivity of mature granule neurons dramatically increases the survival, maturation, and number of NGNs and maturity of synaptic connections of these cells [71]. These observations may have important consequences in the context of pathological conditions involving an imbalance between the synaptic integration of newly and developmentally generated granule neurons. In this regard, we found a similar imbalance in a murine model of AD overexpressing GSK-3 β (GSK-3 β -oe mice) in the hippocampus [51]. Granule neurons of these animals show a generalized reduction in the number of dendritic spines and synaptic contacts [51]. However, detailed morphometric analysis of these structures revealed that NGNs show a reduction in PSD volume (which in turn revealed a decrease in the synaptic strength of their afferent connections), whereas the dendritic spines of developmentally generated neurons show a marked enlargement (thereby suggesting a strengthening of the afferent connections of these cells). According to the competition hypothesis, these findings suggest the selectively impaired synaptic integration of NGNs in this animal model of AD. On the basis of the higher plasticity conferred by these cells to the hippocampal circuit, we have proposed that their reduced synaptic integration favors greater stability of old synaptic connections and a generalized lack of plasticity at the hippocampal level. Noteworthy, GSK-3 β -oe mice exhibit alterations in hippocampal-dependent behaviors requiring high behavioral flexibility, such as in pattern separation [55].

There is intense debate in the field as to whether competition phenomena established between newly and developmentally generated NGNs are a physiological mechanism aimed to compensate the reduced capacity of the oldest neurons to cope with insults, or whether the natural *purpose* of NGNs is to gradually replace the oldest cells in order to favor their withdrawal from the hippocampal circuit [72, 73]. Nevertheless, the observation of these competition phenomena acquire particular relevance in the context of neurodegenerative diseases, given that

impairments in pattern separation and in AHN have been found in AD patients and in animal models of this disease [32]. These observations and the extraordinary regenerative potential of AHN have placed this process in the spotlight of therapeutic strategies aimed to tackle neurodegenerative diseases.

By using the same retroviral approach previously mentioned, we demonstrated that GSK-3 β OE increases tau phosphorylation in single NGNs in a *cell-autonomous* manner, thus revealing tau as a key mediator of the alterations in NGN functionality caused by GSK-3 β OE. In this regard, despite the classical association of tau with the axonal compartment, growing evidence strongly supports the notion that this protein is present and involved in various functions of the somatodendritic compartment. In 2011, Ittner et al. [74] elegantly demonstrated the participation of tau in cortical synapses. In that study, the authors showed that tau depletion is neuroprotective against synapse destabilization caused by the presence of A β . More recently, these authors have demonstrated that tau phosphorylation finely tunes the synaptic roles played by tau [75]. It has been proposed that tau modulates the synaptic localization of *Fyn* kinase, which favors synapse stabilization [74]. On the other hand, tau phosphorylation by GSK-3 β increases tau aggregation and impedes several of its actions at the synapse. A detailed schematic diagram showing these regulatory mechanisms is shown in Fig. 2. In agreement with previous observations in other neuronal populations [74], our data reveal that the absence of tau impairs the synaptic integration of NGNs [43]. These alterations are particularly apparent in the most distal part of the dendritic tree, where the NGNs of tau-/- mice show the most drastic reduction in the number of PSDs, accompanied by a marked decrease in the volume of these structures [43]. Importantly, these data indicate that the synaptic integration of NGNs in tau-/- mice is impaired, as a result of a reduction in the connectivity of the outer parts of their dendritic trees with afferent fibers from the EC.

Nevertheless, the general consensus in the field is that the absence of tau confers a certain degree of neuroprotection in response to exposure to various toxic agents. In this regard, we demonstrated, for the first time, that the absence of tau conferred NGNs with extraordinary resistance to stress [43]. However, our results also showed that the absence of tau completely blocked the stimulatory actions exerted by environmental enrichment (EE) on NGNs. Of note, EE is one of the most potent positive regulators of AHN

Fig. 2. Schematic model proposed to explain the mechanisms through which GSK-3 β and tau regulate the synaptic integration of granule neurons. A) Under basal conditions, tau plays a central role at the synapses through its interaction with Fyn kinase. On the other hand, tau stabilizes microtubules, thus allowing the dendritic transport of synaptic receptors to the synapses, where they are stabilized through their interactions with the scaffold protein PSD95. GSK-3 β exerts tau-dependent and -independent regulatory actions at the synapse. B) Under pathological conditions, GSK-3 β overexpression increases tau phosphorylation. This phenomenon decreases tau affinity to bind microtubules, which are destabilized. On the one hand, this compromises dendritic transport. On the other hand, tau phosphorylation triggers its aggregation, which further impairs cell function. In addition, synapses are destabilized by tau-dependent and tau-independent mechanisms. C) The chronic maintenance of these pathological conditions leads to the disappearance of the synapse.

[76]. It increases the survival, maturation, and plasticity of NGNs in WT mice [77]. However, we did not observe these stimulatory effects in tau-/- NGNs [43]. Our results therefore indicate that tau depletion has a negative impact on neurodegenerative diseases by further decreasing the plasticity of especially sensitive neuronal populations. These data should be taken into account during the development of strategies aimed to ameliorate neurodegeneration caused by aberrant forms of tau. Special emphasis should be placed on identifying the physiological roles played by the various species of tau in specific neuronal populations, given that not all of these functions can be compensated by other MAPs.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Our current perception of AHN is continuously being modified by the addition of new brush-strokes that illustrate particular aspects of the orchestration mechanisms underlying AHN. Given the enormous complexity of the regulatory mechanisms driving the synaptic integration of NGNs into the pre-existing trisynaptic circuits, future efforts should be focused on unraveling the fine boundary between the neuroprotective and detrimental actions exerted by GSK-3 β and tau. In this regard, we have observed that the absence of tau blocks the stimulatory actions of EE on NGNs. On the other hand, we have found that GSK-3 β OE prevents some of the stimula-

tory actions of EE and physical exercise on NGN connectivity. Thus, both an increase in and blockade of the activity of certain proteins, such as GSK-3 β or tau, may have equivalent detrimental effects on the structural plasticity of NGNs, a crucial cell population that shows marked alterations in animal models of AD models and patients with this condition. Hence, to achieve effectiveness, therapeutic strategies aimed to ameliorate the symptoms or to prevent the progression of AD should be particularly careful in the choice of pathological target and should ensure the maintenance of the essential physiological functions of these proteins.

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