In: Neurogenesis Research ISBN: 978-1-62081-723-0
Editors: Gerry J. Clark and Walcot T. Anderson

Chapter V

The Primary Cilium: The Tiny Driver of Dentate Gyral Neurogenesis

James F. Whitfield[1], Balu Chakravarthy[1], Anna Chiarini[2] and Ilaria Dal Prà[2]

[1]Molecular Signaling Group, National Research Council of Canada, Institute for Biological Sciences, Ottawa, Ontario, Canada
[2]Histology and Embryology Section, Department of Life and Reproduction Sciences, University of Verona Medical School, Verona, Italy

Abstract

An emerging picture of the brain is one in which both neurons and astrocytes have an immobile protuberance, a tiny sensory antenna. Each of these antennae is studded with a region-specific selection of receptors and maintains a busy, energy-consuming bidirectional traffic along its microtubular spine (axoneme) of parts of signaling machineries and messages to the cell center from the receptors about mechanical strains and external events. These messages are merged with those from the swarm of synapses on the cell's dendrites to frame appropriate responses. Along with this has come the discovery that neurogenesis in rodents and humans persists in two main regions of the brains of adult rodents and humans, one of which, the subgranular zone of the dentate gyrus, can feed new granule cells into the gyrus's granular layer depending on age and other circumstances. These newborns are at first especially receptive targets for data converging from various neocortical regions onto the cells of the outer entorhinal cortex, the gateway to the hippocampus. Streams of audio, visual, olfactory and other data are sent to the new granule cells' blank-slate dendrites via the glutamatergic axons of the perforant pathway. Now we are learning mainly with mice how signals from the cilia first drive the proliferation of newborn transit-amplifying stem cell progeny and later the postmitotic maturation of these neuroblasts into functional granule cells that can separate the incoming streams of data and synaptogenically "burn" them into their newly emerged dendrites. The streams are then relayed by the granule cells to the recurrently interconnected neurons of the CA3 region for interweaving into a coherent pattern and from there to the pyramidal neurons of the CA1 region for final processing into a

retrievable format and transmission to various regions via the subiculum and the inner levels of the entorhinal cortex, the hippocampal exit gate as well as via the fornix pathway. It is important to extend these murine findings to humans and find out how the neurogenic and other functions of the granule cell cilia are affected by aging and diseases such as Alzheimer's disease.

Introduction

From Disbelief to an Increasingly Crowded Neurogenesis Bandwagon

According to the old neurological canon law there can be no neurogenesis in an adult brain. Obviously such neurogenesis must have been strongly selected against because generating new neurons in a mature brain would scramble, erase or otherwise obstruct established memories. This law was finally challenged first in 1967 by the discovery by Altman and Das of neurogenesis in the postanatal guinea pig dentate gyrus and much later by Nottenbohm's finding adult teststerone –driven neurogenesis in adult male canaries [Nottenbohm, 1980, 1985]. Altman and Das's discovery was vigorously denounced by the defenders of the faith with the expected cries of artefact, but we now know that they had found one of the two principal sites of adult neurogenesis in mammalian brains — the SGZ (the dentate gyrus's subgranular zone [Kempermann, 2011].

Along with this change came the discovery that the deceptively simple single immobile cilia (Latin, *cilium*: eyelash) sticking out of neurons are actually sophisticated signaling antennae, their surfaces bristling with receptors and their narrow interiors crammed with the receptors' signaling machinery and busy kinesin and dynein motors carrying fresh components to the cilial tip and worn-out components and signal mediators from the cilial tip to the cilial base [Blacque et al., 2008; Satir and Christensen, 2008; Scholey, 2008; Verhey et al., 2011]. These cilia provide the neurons with information about the cellular environment to supplement the dendritic flow of synaptic data from other neurons. It has also been discovered that signals from these tiny organelles drive the adult neurogenesis and integration of newborn neurons into the mature granule cell layer of the dentate gyrus.

The Hippocampus Formation, the Mammals' Memory-Recording Device

Any mobile invertebrate or vertebrate must be able to remember the locations of food, potential mates, dangerous things and places. Various arthropods, fish, birds, mice, monkeys and humans of course have different memory-recording devices, but all of them use basically similar neuronal circuitries [Berry et al., 2008; Kempermann, 2011; Sandeman et al., 2011; Strausfeld, 2012].

Running along the medial wall of the temporal horn of each of the lateral ventricles in the human brain is the memory recorder, the two-part hippocampal formation [Duvernoi, 1988] (Figure 1).

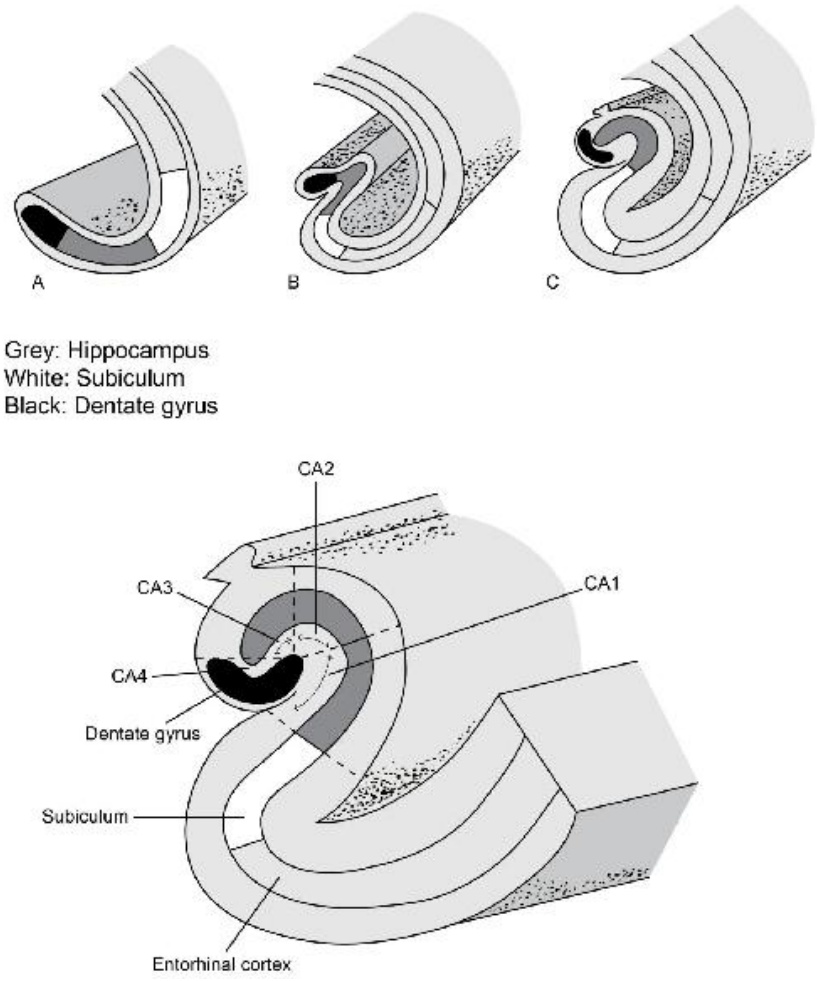

Figure 1. The phylogeny of the hippocampal formation. *A-C.* The flat three-layered reptilian dorsal pallial archeocortex was pushed medially downward in the evolving mammals by the dorsally expanding 6-layered neocortex [Nieuwenhuys, 1994]. It was eventually forced to curl up when it hit the inside edge of the temporal lobe. *D.* Once the archeocortex could be pushed no further, the tubular coiled hippocampal cornu Ammonis emerged with its major CA1 and CA3 fields and the intermediate CA2, and CA4 fields. And with it came the separate, though linked, dentate gyrus with its tooth-like hillocks.

All of the components of the hippocamp proper have the 3-layered cortices of the original doral pallium of ancestral amphibians and reptiles [Nieuwenhuys, 1994; Shepherd, 2011]. The mammalian hippocampus is the curled edge of the parahippocampal gyrus.

It started out in the three-layered reptilian dorsal pallium from which it extended as a flat sheet down the dorsomedial cortex [Nieuwenhuys, 1994]. But then there appeared the novel 6-layered neocortex. As this new structure expanded, it pushed this sheet down further and to curl up against the wall in the shape of a sea horse (e.g. *Hippocampus abdominalis*) [Nieuwenhuys, 1994] (Figure 1).

The rodent's or primate's two hippocampi are interhemispherically connected via the anterior commissure. Each consists of two separate but interlocking parts. One of these, the *data receive*r, is the dentate gyrus with hillocks running along its length that give it its dentate (toothed) look [Duvernoi, 1988].

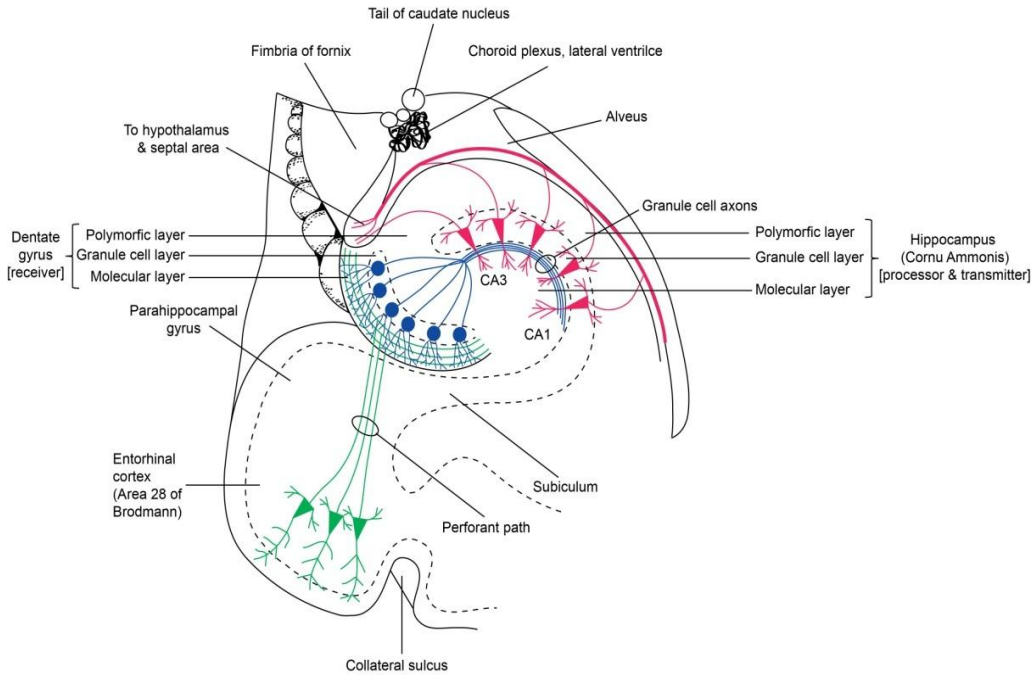

Figure 2. A transverse view of the hippocampal formation and dentate gyrus (DG) of the temporal lobe. Some of the main neuronal pathways linking the entorhinal cortex with the DG and the cornu Ammonis are shown. *Green*, pyramidal neurons of the layer II of the enthorinal cortex, whose axons (the perforant pathway) synapse with the granular neurons of the DG (*blue*). Axons from the DG granules connect with the CA3 pyramidal neurons of the hippocampus (*red*), which in turn connect to the CA1 neurons, the axons of which enter the fornix, the efferent pathway to other limbic system areas, but mainly via the subiculum to layer III of the entorhinal cortex for processing and distribution back to the original cortical data sources.

It extends alongside, and interlocks with, the hippocampus proper. The hippocampus proper combines the data flowing into it from various regions of the brain via the dentate gyrus into retrievable memory packets, which it sends back to these regions (Figure 2). It returns the processed memory packets to the rest of the brain via two routes—the subiculum which in turn extends into the entorhinal and then the parahippocampal cortices and the alveus, fimbria and fornix [Duvernoi, 1988] (Figure 2).

The hippocampus proper is the *data processor and transmitter*, which, because it looked to some like the Egyptian god Ammon's horn, it is said to consist of CA (*C*ornu *A*mmonis) regions (Figures 1 and 2). The 3-layered cortex of the Cornu Ammonis 'fades' into the transitional subiculum with 4, 5, and then 6 layers as it merges with the entorhinal and then the parahippocampal gyrus (Figure 1). At the posterior end of Ammon's horn, pyramidal axons (~60% from subicular cortex carrying data from CA1 and ~40% from CA3) converge into the alveus that covers the hippocampal formation on the ventricular wall. The fibers continue on to form the fimbria that arches up to form the fornix, the main white matter outflow tract from the hippocampus that connects to the mammillary body which in turn targets the anterior thalamic nucleus, the acetylcholinergic basal forebrain neurons, the nucleus accumbens, and the prefrontal cortex (Figure 2). But this is not the place for any further discussion of the very widely ramifying connections of this 3-layered device. But we will summarize the way it might do its memory-encoding job and then some emerging ideas of how it uses primary cilia to do it.

Memory Formation

A Brief Look at a Very Complex but Vital Process

The hippocampus proper's data receiver, the dentate gyrus, is an invention of mammals that projects only to the hippocampus proper (i.e., Ammon's horn) (Figure 2). It receives at specific points along its length on either or both hemispheres streams of data from about different activities from cortical regions, such as the cingulate cortex, sensory association cortices, basolateral amygdala, insular cortex, olfactory bulb and the prefrontal cortex, elicited by an attention-attracting event. The data streams from this event converge on layer II of the entorhinal cortex, a kind of central station and gateway to the hippocampus for cortical data (Figure 2). The importance of the entorhinal station is indicated by the ability of deep-brain focal electrical stimulation during learning trials of the entorhinal cortex, but not direct stimulation of the hippocampus, to enhance spatial memory [Suthana etal., 2012]. The data are then forwarded from the station to the dentate gyrus via the axons of the perforant pathway that synapse on the middle and outer two-thirds of the dentate gyral granule cells' dendrite arbors [Canto et al., 2008; Eichenbaum, 2011; Moscovitch et al., 2005; Small et al., 2011; Toni and Sultan, 2011; Witter, 2007].

The dentate gyrus has 5-10 times more neurons than the entorhinal cortex so the data streams that are initially close together as they leave the entorhinal station are 'deconflicted' by fanning out into the dentate gyrus. Each mature granule cell gets input from thousands of entorhinal neurons, but the strongly inhibitory signaling from GABAergic interneurons allows only strongly stimulated granule cells to project to their CA3 target neurons. However as we shall see below less strongly activated immature newborn granule cells can project sparsely, but yet robustly, to CA3 pyramidal neurons [Aimone et al., 2010].

The fanning out of the data streams is the way to avoid 'collisions' between inflowing and already established patterns in the CA3 networks. So the dentate gyrus's job is to separate the incoming entorhinal data before powerfully and unidirectionally firing a pattern of activity via their potent mossy axons to the CA3 pyramidal neurons with their specialty—inscription-enhancing feed-forward collateral looping. So the granule cells must provide the CA3 cells with streams of data that are sufficiently separated and sparse to avoid interfering with already inscribed data traces thereby enabling the cells to produce a novel pattern of synaptic traces and ultimately retrievable memories [Aimone et al., 2010; Treves, 2009; Treves et al., 2008]. The melding of the dentate data streams into the interleinked nodes of a retrievable memory pattern is achieved by the recycling feedback loops provided by the CA3 pyramidal neurons' recurrent collaterals which do not input new data but reinforce the synaptic inscription of the dentate's mossy input [Treves et al., 2008].

The now-encoded multi-modal memory packet is forwarded via the Schaffer collateral /associational commissural pathway to the larger number of pyramidal neurons in the CA1 region where it is cleaned up and completed without the collateral looping of the CA3 networks. While this is being done, the CA3 neurons are processing the next load of data from the dentate granule cells. Depending on the time separating them, this and the previous one or ones can be sequentially linked by the CA1 into a complete episode and sent off via the subiculum to the entorhinal cortical layer III station and from there back to the original

cortical regions as interlinked modal data nodes as well as to the thalamus, amygdala, basal forebrain and the executive prefrontal cortex via the alveus, fimbria and fornix (Figure 2).

The audio, olfactory, visual, spatial and other components of the episode are stored as the nodes of a cortical network with the hippocampus possibly being a network hub. So if one or more nodes of the cortical network should 'light up' in response to some external event or even to the application of a neurosurgeon's electrode to the cerebral cortex, the resulting activities will reach the hippocampal hub which will connect the rest of the nodes in the cortical regions to produce the conscious recall of events [Penfield and Perot, 1963; Rugg et al., 2008].

The Dentate Gyrus—The Mammals' Data Receiver

The brains of adult rodents and humans have two principal neurogenic regions, one of which is the SGZ (subgranular zone) of the dentate gyrus where new stimulatory glutamatergic mature granule cells are generated, the other of which is the SVZ (subventricular zone) where new inhibitory GABAergic interneuronal granule cells are generated and sent to the olfactory bulb via the rostral migratory stream [Aimone et al., 2010; Deng et al., 2010; Kempermann, 2011].

The formation of the dentate gyrus starts out traditionally in the developing brain's primary germinative matrix in the wall of the embryo's lateral ventricle. But in mammals a group of precursor cells separates from the ventricular zone and moves toward the hippocampal fissure where they form the outer shell of the future dentate gyrus. Then a second wave of migrating precursors breaks away from the ventricle wall and sets up camp near the shell at the pial surface. From this camp the glia-like precursor cells send radial fibers across the nascent hilus to make a scaffold up which the precursor cells can climb to lay down a second granule cell layer and beneath it a granule-cell-generating zone, the SGZ. For the rest of the human's or rodent's life the SGZ can produce new novelty-recording granule cells, but at a declining rate as the familiarity of the world increases, novelty becomes rarer with age, and the need for new blank-slate granule cells drops [Amrein and Lipp, 2009; Drapeau and Nora Abrous, 2008; Kempermann, 2011; Suh et al., 2009]. Humans have relatively low rates of adult hippocampal neurogenesis compared to other mammals such as rodents and it becomes rudimentary after 30 years of age [Amrein and Lipp, 2009].

But the potential to produce new granule cells when needed is maintained by a pool of neuronal stem cells, the astrocyte-like type 1 radial glial cells, nestling in their special SGZ niche consisting of blood vessel-associated gap-junctionally interconnected astrocytic syncytium [Basak and Taylor, 2009; Goldman and Chen, 2011; Kempermann, 2011; Morrens et al., 2012; Suh et al., 2009]. These type 1 cells, like their lateral ventricular confreres, have the properties of astrocytes, as indicated by their expression of GFAP (glial fibrillary acidic protein), the astrocytic hallmark. They also have vascular end-feet and are gap junctionally coupled [Morrens et al., 2012]. And from the SGZ niche they extend their apical processes through the mature granule cell layer into the molecular layer where they collect information by synapsing with various afferents and stimulation from various neurotrophins. The SGZ niche is specifically instructive for neurogenesis for any neuronal stem cell wherever in the

brain it may originate. While in the niche the neuronal stem cells, like those in other tissues, only initiate a clone-starting cell cycle in order to preserve their precious 'stemness'.

The stem cells' proliferative activation depends on their 'perception' of the level of overlying granule cells' NMDA receptor activity and therefore their functional adequacy [Gould and Cameron, 1996; Nacher et al., 2001; Suh et al., 2009]. If this NMDA activity should drop because of the application of a NMDA receptor antagonist or because of entorhinal cortex damage which reduces the flow of perforant pathway signaling, stem cells will be induced to start a cycle to generate more granule cells and restore the lost NMDA receptor signaling activity [Gould and Cameron, 1996; Nacher et al., 2001; Suh et al., 2009]. An activated stem cell's best option to bot preserve stemness and start a clone of new granule cells is to produce a stem-cell daughter like itself and a daughter that will leave the niche, keep its cell cycle-driving engine operational to initiate a clone starting with rapidly proliferating Type 2 transient amplifying progenitors [Kempermann, 2011].

The first of the stem cell's progenies are known as type 2a neuroblasts [Kempermann, 2011]. These are the most proliferatively active progeny (Figure 3). They lose their parental radial morphology but still express stem cell and radial glial markers such GFAP and the Sox2 gene, the product of which prevents precocious differentiation. These type-2 cells proliferate in clusters around the radial scaffold and capillaries from which they can get a variety of growth and developmental factors such as ADP/ATP, BDNF and VEGF [Antequera et al., 2012; Basak and Taylor, 2009; Goldman and Chen, 2011; Kempermann, 2011]. Indeed, the tight linkage between neurogenesis and angiogenesis in the SGZ is mediated by VEGF, the vascular endothelial growth factor [Suh et al., 2009].

The progeny of the type 2a cells are the less proliferatively active type 2b cells. They mark the shift from the glial phenotype to the neuronal phenotype. They have no radial glia markers and now express neuronal markers such as Neuro D, Thr2, Tis21, and Prox1, the specific hippocampal granule cell marker in the brain (Figure 3). Surprisingly, despite their proliferative competence, they are the first of the progeny to express the Tis21 (PC3) gene, the product of which is a cell cycle inhibitor that operates by inhibiting cyclin D1 transcription [Attardo et al., 2010; Guardavaccaro et al., 2001] (Figure 3). Attardo et al. [2010] have suggested that this Tis21 expression may mark the switch to full synaptic integration and glutamatergic innervations. The first synapses formed by the newborns are mostly with GABAergic hilar basket cells but are not the potent response filters like the GABAergic interneurons impose on the mature granule cells. Unlike mature granule cells the immature cells receive *stimulatory* signaling from GABAergic synapses because these cells express the NKCC1 (Na^+-K^+-$2Cl^-$ 1) transporter, which by increasing the resting cellular Cl^- concentration enables the Cl^- channel-opening GABA [Mejia-Gervacio et al. 2011] to depolarize and thus *stimulate* rather than to hyperpolarize and thus inhibit the cell (Figure 3).

The surviving type 3 progeny of the 2b cells are migratory neuroblasts (Figure 3). But unlike their 2b parents they do not express Tis21, although their proliferative activity has faded [Attardo et al., 2010]. However, they have not definitively shelved their cell cycle-driving machinery because they can still be persuaded by a strong stimulus to start a cell cycle. They move into the lower third of the granule cell layer where they will stay and complete their maturation into functional granule cells. From there they will transiently express their *Tis2* gene, which will definitively shut down their cell cycle engines.

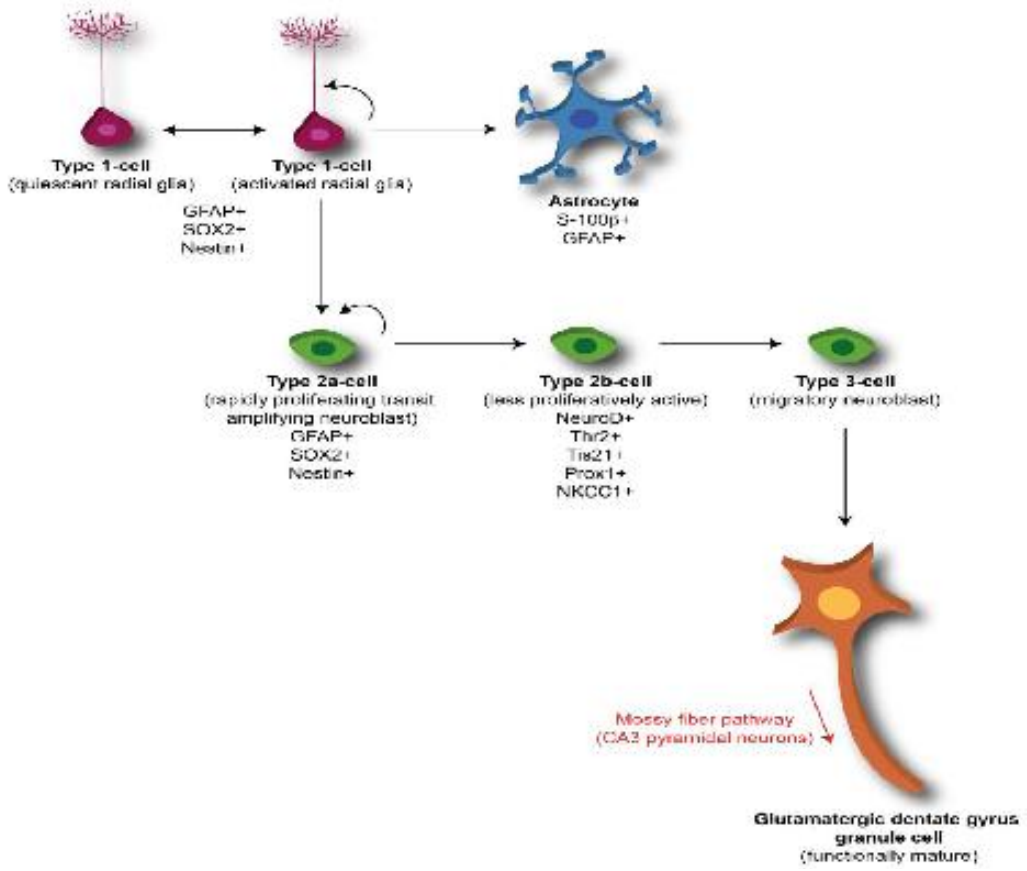

Figure 3. The various stages of adult neurogenesis in the subgranular zone (SGZ) of the dentate gyrus (DG) [Kempermann, 2011]. The precursor cells are shown with their marker genes and other features. See further details in the text. In *red* is the indication that the axons of the mature granule neurons form the mossy fiber pathway that connects with the pyramidal neurons of the CA3 zone in the hippocampal formation. We are not sure which of the newborn neuroblasts initiate ciliogenesis.. According to Amador-Arjona, et al. [2011] the TA neuroblasts must have them because they need cilial signaling to proliferate. But according to Kumamoto et al. [2012] it is the 2-week old migrating neuroblast that starts assembling its cilium in order to drive axonal development and connection to the glutamatergic entorhinal projections.

Now they extend their dendritic trees toward the molecular layer and thus the perforant data streams and project axons along the mossy fiber pathway to reach there their recurrently interconnected, input data recycling, CA3 pyramidal neurons (Figures 2 and 3).

At this point we can begin to see why this adult dentate granule cell neurogenesis was such an important invention. First the newborn granule cells push their new blank-state dendrites through the upper granular layer and into the molecular layer. These newborn dendrites start out with no potentially interfering spines and synapses. But as they mature they start expressing glutamate receptors so they send out very active motile filopodia that are lured to the middle and outer molecular layers by glutamate spilling out of active synapses between perforant entorhinal axons and older dentate granule cells [Toni and Sultan, 2011; Toni et al., 2007]. The immature blank-slate granule cells are hyper-receptive to perforant input because of enhanced synaptic plasticity and a low induction threshold for LTP (*L*ong *T*erm *P*otentiation) that enable them to synaptically inscribe incoming data more readily than

mature granule cells [Mu et al., 2010]. They can relay input to CA3 from weaker synapses than mature granule cells. This is because they don't yet have the inhibitory interneuronal GABAergic filters that allow mature granule cells to send data to the CA3 from only the strongest synapses.

Then they are stronger signalers to CA3 neurons than mature granule cells because, unlike the mature granule cell they do not yet have calbindin [Kempermann, 2011]. The reason for this is that a Ca^{2+} surge triggers neurotransmitter (glutamate) release and the Ca^{2+}-binding calbindin when it appears can restrain or even block this release. Indeed, overexpressing calbindin inhibits mossy fiber-CA3 signaling [Dumas et al., 2004]. However, the novel data-encoding and less filtered data-relaying properties of youth fade and the aging clonal granule cells become increasingly burdened by accumulated dendritic spines and synapses and more restriction by GABAergic interneurons of what they can send to their CA3 targets.

Enter the Primary Cilium

Driving Transit Amplifying Neuroblast Proliferation

Most (70-90%) cells in the adult mouse or rat dentate gyral granule cell layer have a non-motile, ~4µm sensory antenna protruding from their bodies (Figure 4). This is a very busy, energy-consuming tiny organelle, which uses the products of 800 to 1000 genes to be generated and then to be kept intact and functioning [Berbari et al., 2009; Blacque et al., 2008; Nachury et al. 2010; Rohtagi and Snell, 2010; Satir and Christensen, 2008; Scholey, 2008; Verhey et al., 2011]].

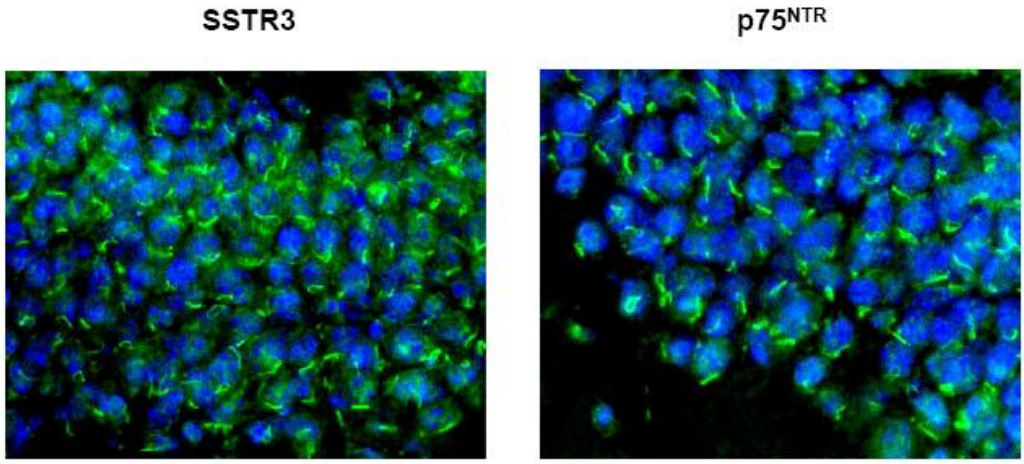

Figure 4. The mature dentate granule cell layer's forest of primary cilia. Between 70% and 90% of the dentate granules cells in 7-18 months old sv129/C57BL6 mice have ~4 µm cilia protruding from their cell bodies. These primary cilia contain the cells' SSTR3 (somatostatin receptor 3) and their $p75^{NTR}$ neurotrophin receptor. The SSTR3 was immunostained with polyclonal antibodies from Gramsch Laboratories (Schwabhausen, Germany). $p75^{NTR}$ was immunostained with rabbit polyclonal antibody (from Alomone Labs Ltd, Jerusalem, Israel) directed against amino acids 188-203 in the receptor's extracellular 'stalk'.

It consists of a spine (axoneme) of 9 microtubule doublets that are templated on the granule cell's mother centriole's 9 microtubule triplets and serve as IFT (intraflagellar transport) trackways for trains of new and replacement cargo-bearing particles being moved to the cilial tip on kinesin-2 motors and nucleus-addressed signal-mediating gene transcription drivers and used parts being carried from the cilial tip to the basal body on dynein motors [Blacque et al., 2008; Satir and Christensen, 2008; Rohtagi and Snell, 2010; Scholey, 2008; Verhey et al., 2011]. Primary cilia are wrapped in a special plasma membrane bristling with various receptors and their tiny interiors are packed with busy transports and the receptors' signaling machinery. Among the receptors in the rodent granule cell cilia are the $p75^{NTR}$ neurotrophin receptor, the somatostatin type 3 receptor and the Shh (Sonic Hedgehog) system's SMO (smoothened) and Ptch (Patched) [Berbari et al., 2008; Chakravarthy et al., 2010; Einstein et al., 2010; Goetz et al., 2009; Händel et al., 1999]. To get into the cilium and its membrane these receptors and their signal- transducing tools must pass through selective ports formed by membranes at the cilial base [Rohtagi and Snell, 2010].

As said above, each type 1 astrocyte-like 'stem' cell has a GFAP-containing radial process, expresses the Nestin and the Sox 2 transcription factor and rarely initiates a cell cycle. Each, when signaled to do so, can start a clone of granule cells starting with a rapidly cycling type 2a TA (transit-amplifying) precursor cell. According to the observations of Kumamoto et al. [2012] these first TA neuroblasts do not have cilia. Using specifically marked retrovirus to label proliferating neuroblasts and then follow their subsequent development, Kumamoto et al. [2012] found that the newborn cells took about 2 weeks to start producing cilia apparently to promote their integration into the granular cell layer and the connection of their dendrite arbors to the glutamatergic entorhinal projections. According to these observations, eliminating cilia should affect the connection to entorhinal projections but not TA neuroblast proliferation. However, according to Amador-Arjona et al., [2011] when SGZ cells were deprived of their cilia by conditionally shutting down the Ift20 (*i*ntra*f*lagellar *t*ransport 20) gene needed for ciliogenesis there was a gradual 50% decrease in SGZ proliferation indicated by a declining fraction of DCX^+, $BrdU^+$, $Ki67^+$ and PCNA $^+$ cycling cells. And *only* TA neuroblasts were significantly affected and must therefore have been ciliated [Amador-Arjona et al., 2011]. This selective, cilium deprivation-related reduction of neuroblast proliferation lowered the number of newborn neurons as well as partially impaired novelty recognition and detection of the escape hole in a Barnes maze [Amador-Arjona et al., 2011]. Thus signals from the primary cilium appear to drive TA neuroblast proliferation in the murine SGZ. Obviously it is important to resolve the seeming conflict between the proliferating ciliated TA neuroblasts of Amador-Arjona et al., [2011] and later-ciliating, maturing newborns of Kumamoto et al [2012].

What Cell Cycle Drivers Are Housed in the TA Cell's Primary Cilium?

It has been known for ~7 years that the murine granule cell cilia house the Shh (Sonic Hedgehog) machinery [Corbit et al., 2005; Han et al., 2008]. Indeed Goetz et al. [2009] have called the primary cilium a "hedgehog signal transduction machine". And Breunig et al. [2008] and Han et al. [2008] have shown that primary cilia regulate hippocampal neurogenesis via Shh signaling. The proliferogenic process starts when Shh, stimulated by a cell cycle driver, binds to Ptch (Patched), and pulls it out of the cilial membrane and into the

main cell membrane [Goetz and Anderson, 2010]. This releases Smo from its cytoplasmic cage [Goetz and Anderson, 2010]. It can then enter the cilium and be carried to the cilial tip by the kinesin 2 motor. There Smo stops a Ptch-promoted basal complex from preventing Gli from entering the cilium a well as promoting a mechanism at the cilial tip that would process Gli to Gli-R (Gli-Repressor) [Goetz and Anderson, 2010]. Along with the liberation of Smo and the dispersal of the cilium-access-blocking complex Gli is now carried to the cilial tip where it is processed into the Gli-A transcription activator and carried by the dynein transporter down to the basal body gateway through which it is released and enters the nucleus [Goetz and Anderson, 2010]. In the nucleus Gli-A targets the genes producing the G1 cyclins (cylins D1, D2, E) that when combined with their appropriate CDKs (cyclin-dependent protein kinases) drive the build-up to DNA replication and TA neuroblast proliferation [Goetz et al., 2009; Kenney and Rowitch 2000] (Figure 5).

Armato et al. [2011] have suggested a role for cilial Shh in the ability of the 16 kDa cytokine-hormone leptin to stimulate TA neuroblast proliferation in the murine dentate gyrus and to improve memory in Alzheimer's disease (AD)-model mice reported by Garza et al. [2008], Greco et al. [2010] and Pérez-González et al. [2011]. Leptin could be therapeutic for AD because it has been safely and daily injected subcutaneously for as long as 10 years to treat obesity in humans [Paz-Filo et al. 2011].

This leptin story begins in the brain but not with neurogenesis. Leptin is best known as a controller of the level of body energy reserves stored as white fat [Levin et al., 2011; Li, 2011]. When leptin arrives in the hypothalamus after being secreted by white-fat adipocytes, it induces arcuate nuclear anorexigenic proopiomelanocortin (POMC)-expressing neurons and orexigenic neuropeptide Y/Agouti-related peptide (NPY/AgRP)-expressing neurons to suppress appetite and prevent hyperphagic obesity by respectively stimulating and silencing these two types of neuron [Levin et al., 2011; Li, 2011]. It does this by via JAK2/STAT3 signaling from Lep-Rb, its long isoform receptor.

But where is Lep-Rb in leptin-target neurons? There is convincing evidence of Lep-Rb being housed in primary cilia. Thus, interfering with ciliogenesis and function causes mice and humans to become hyperphagic and obese [Davenport et al., 2007; Gupta et al., 2009; Mok et al., 2010; Satir, 2007; Seo et al., 2009; Wang et al., 2009]. Yet despite this no one has actually seen Lep-Rb in the cilia of leptin target cells such as the hypothalamic arcuate neurons. Indeed according to Funahashi et al. [2003] the receptor is located not in the cilia of arcuate neurons but in the cytoplasmic membranes. Stratigopoulos et al. [2011] have also reported that cultured murine arcuate neurons' Lep-Rb receptors are in the cytoplasmic membrane. But exposing these cells to leptin produced Lep•Lep-Rb complexes that clustered around the cilial basal bodies where they presumably sent strong signals to the cilia but apparently without entering them.

It follows that the most likely mechanism of leptin action is that it binds to Lep-Rb in the cell membrane to form Lep•Lep-Rb complexes that send a signal that activates a cilial mechanism that mediates the response. In the case of leptin-stimulated SGZ TA neuroblasts that leptin-triggered cilium-mediated mechanism could be the Shh-induced production of Gli-A that invades the neuron's nucleus and stimulates the cell cycle-drivers' genes (Figure 5). So could leptin stimulate Shh? The answer is almost certainly "*yes*" because of Choi et al. [2010]'s report that leptin activates the hedgehog mechanism in hepatic stellate cells by stimulating Shh expression.

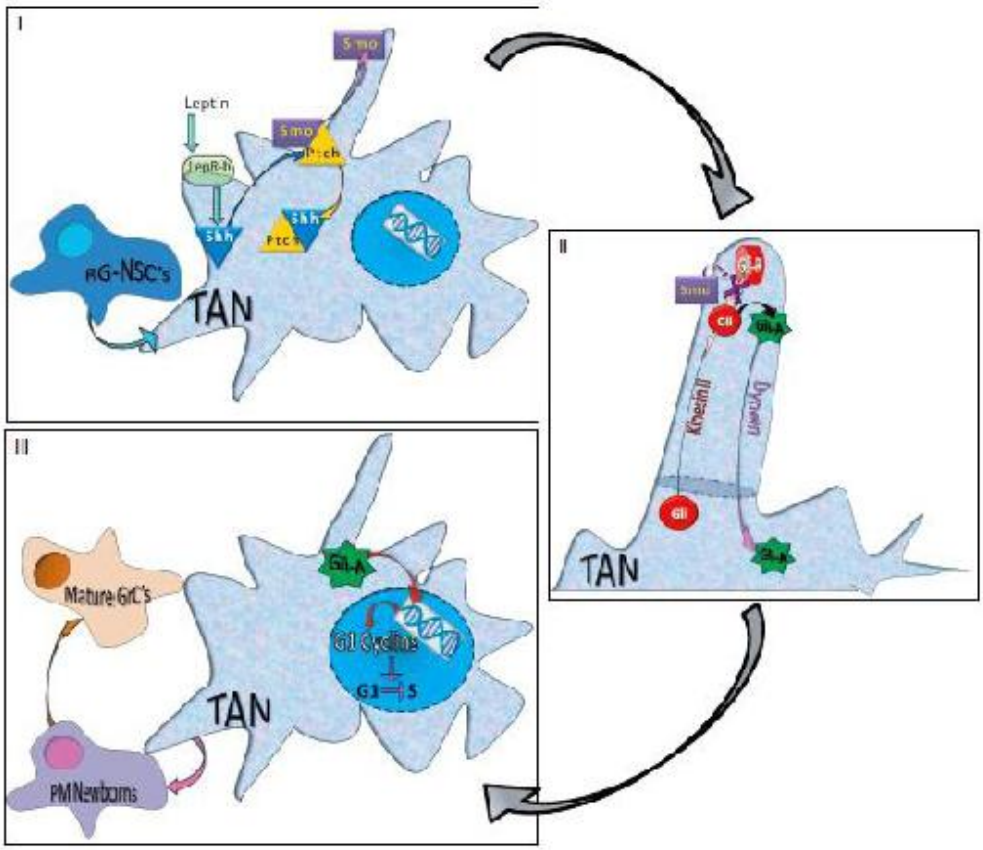

Figure 5. How Lep-induced signals from LepR-b's stationed in the cell membrane could stimulate the primary cilium-dependent proliferation of TANs (transit amplifying neuroblasts) generated by radial glial stem cells in the dentate gyral SGZ (subgranular zone). *(I)* Signals from Lep•LepR-b complexes stimulate the production of Sonic hedgehog (Shh), which in turn causes Ptch (Patched) to leave the cilium. The key player in this mechanism is Gli, which is carried by the kinesin-II transporter along the cilial microtubule trackway to the cilial tip where the Gli-processing machinery is located. *(II)* In the presence of Ptch before the appearance of Shh, Gli is processed by the tip machinery into Gli-R, its transcription repressor form. But Gli is also prevented from entering the cilium by a Gli-destroying complex. The Shh-driven exit of Ptch releases Smos (Smootheneds), which move up to the cilium's tip and enable the formation of Gli-A, the gene-activating form of Gli that is then carried down the trackway by the dynein transporter and passes through the cilium's basal barrier. *(III)* Gli-A then moves to the nucleus, where it stimulates the expression of the cyclins D and E for the cyclin-dependent protein kinase engines that drive the key stages of the pre-replicative build-up to DNA replication. Upon reaching the end of the transit amplifying part of the neuronal maturation program, the accumulated TANs shut down their proliferative cycling machinery and become post-mitotic newborns, which progressively mature, and, if lucky enough to survive, enter the granule cell layer and join the veteran granule cells.

There is another potent proliferogen, $p75^{NTR}$, housed in the primary cilia of *almost all* of the dentate granule cells in mice of all ages [Chakravarthy et al., 2010] (Figures 4 and 6). This, along with the earlier finding of $p75^{NTR}$ in murine CA1 pyramidal cells [Woo et al., 2005], shatters the strongly held belief that there are no $p75^{NTR}$-expressing hippocampal cells. According to this belief any $p75^{NTR}$ seen in the dentate gyrus should only be *on* axons from BFCSNs (basal forebrain cholinergic septal neurons) attracted to the dentate gyrus by the BDNF, NGF and NT-3 neurotrophins produced by granule cells and hilar neurons and

terminating on granule cell dendrites in the inner molecular layer and hilus, but, carefully note, *not on the cilium-bearing granule cell bodies* [Bruel-Jungerman et al., 2011; Makuch et al., 2001].

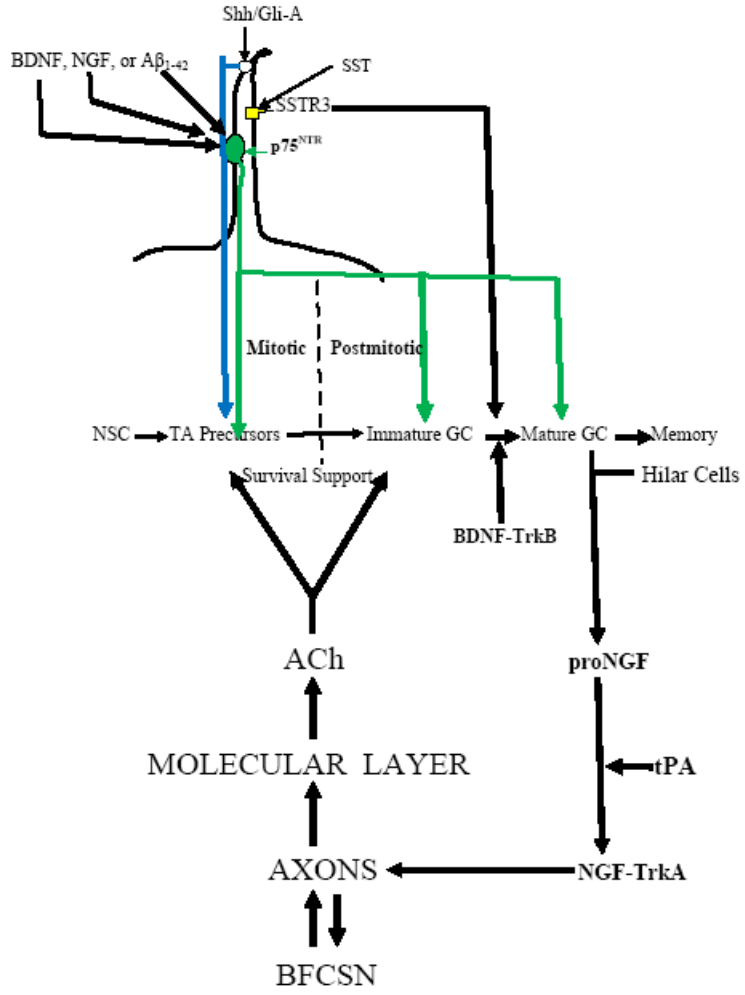

Figure 6. The likely three roles of cilial $p75^{NTR}$ in the generation and maturation of dentate gyral granule cells. In this scheme, neurotrophins (BDNF or NGF) in the subgranular niche activate cilial $p75^{NTR}$ the signals from which could mediate the selective cilial-dependent proliferation of transit-amplifying (TA) neuroblasts decribed by Amador-Arjona, et al. [2011]. After several rounds of proliferation, the newborn cells migrate toward the granule cell layer where they extend and refine their dendrite arbors for fully connecting to the glutamatergic entorhinal projections. The migration, aborization and 'lifetime' cilial maintenance could involve ceramide produced by $p75^{NTR}$-stimulated sphingomyelinase. Postmitotic newborn granule cell maturation is also stimulated by signaling from the SSTR3 receptors and BDNF•TrkB complexes. When the new granule cells enter the granule cell layer and serve transiently as high-LTP-generating, super-sensitive encoders of novel data from the entorhinal projections. Normally half or more of the newborn cells may die without reaching the granule cell layer. But NGF produced in the dentate gyrus from proNGF by tPA /plasmin forms NGF•Trk A complexes that retrogradely reach and stimulate BFCSNs to make ACh that promotes newborn granule cell survival and thus neurogenesis and memory formation. Eventually the successful neurons lose their hyper-responsiveness and become synaptically burdened with old 'memories', Then they retire and are replaced with new recorders. GC, granule cell; NSC, neuronal stem cell; SST, somatostatin; SSTR3, somatostatin receptor 3; tPA, tissue pkasminogen activator.

The progenitors and TA neuroblasts in the SGZ are in synaptic contact with the BFCSNs' cholinergic axons and they express ACh (acetylcholine) receptors, which enable them to respond to the diffuse availability of ACh [Bruel-Jungerman et al., 2011]. The BFCSN axons release ACh, which promotes progenitor neuron proliferation and the survival and maturation of TA neurons [Bruel-Jungerman et al., 2011; Van der Borght et al., 2005]. Conversely, inhibiting ACh activity or selectively killing BFCSNs inhibits the production in the SGZ of new neurons [Bruel-Jungerman et al., 2011; Van der Borght et al., 2005] (Figure 6).

But what might the cilial $p75^{NTR}$ be doing along with Shh and ACh for the SGZ granule cell production? Key clues to one of $p75^{NTR}$'s functions are: 1) proliferating (i.e., BrdU-expressing) cells in the SGZ express $p75^{NTR;}$ 2) knocking out $p75^{NTR}$ reduces the proliferating cells and hippocampal neurogenesis by 59%-79% [Bernabeu and Longo, 2010; Colditz et al., 2010]. Although we do not know whether the progenitor cells in the other principal adult neurogenesis region, the SVZ [Kemperman, 2011], the $p75^{NTR}$ signaling induced by BDNF, NGF, or perhaps surprisingly by the Alzheimer's disease–driving $A\beta_{1-42}$ peptide drives their proliferation [Sotthibundhu et al., 2009; Young et al., 2007]. Therefore, the cilial $p75^{NTR}$ may be the driver of TA neuroblast proliferation (Figure 6). And the BDNF, NGF and NT-3 produced by the mature granule cells and hilar neurons could be the stimulators of TA neuroblast proliferation via the cilial $p75^{NTR}$ (Figure 6).

Of course a reader might ask about the relation between $p75^{NTR}$ and the Shh mechanism in the driving of TA neuroblast proliferation. Both are known to be needed to produce new granule cells at least in the murine SGZ, but we do not know how they might be linked. So far there seems to be only one report of a linkage between $p75^{NTR}$ and Shh signaling and that is in murine medulloblastoma cells [Küchler et al., 2011].

What Do the Cilia Do after Driving Neuroblast Proliferation?

While the signals from neurotrophins-activated cilial $p75^{NTR}$ are likely to be proliferogenic for TA neuroblasts cells in the adult murine SGZ [Amador-Arjona et al., 2011], what would these signals be used for in postmitotically locked mature granule cells, virtually all of which still have it in their primary cilia? The first clue to a possible answer is the observation of Kumamoto et al. [2012] that newborn murine SGZ granule cells use their cilia to extend their dendrite arbors and refine their glutamatergic connections to the entorhinal projections. Bieberich [2011] and Wang et al. [2009] have provided a second clue to this function—ceramide. Ceramide is part of a ring-shaped pericentrioler compartment, the 'sphingosome', that is attached to the cilial basal body and is involved in ciliogenesis and cell migration the newborn granule cell activities described by Kumamoto et al [2012]. And it has also been shown to promote dendrite arborization by cultured rat embryonic hippocampal neurons [Brann et al., 1999; Schwarz and Futerman, 1997].The linkage of ceramide to cilial $p75^{NTR}$ in mature granule cells is the fact that neurotrophin (e.g. NGF)-activated $p75^{NTR}$ produces ceramide by stimulating membrane-associated neutral sphingomyelinase [Dobrowsky and Carter, 1998]. Thus cilial $p75^{NTR}$ signaling may promote ciliogenesis, dendritic arborization, migration of newborn cells into the mature granule cell layer and then 'lifetime' cilium maintenance, (Figure 6).

Another receptor is packed into the dentate granule cells primary cilia along with $p75^{NTR}$. This is the non-proliferogenic SSTR3, the somatostatin type 3 receptor, which, unlike the four

other SSTRs, is specifically addressed to primary cilia by a specific amino acid sequence [Berbari et al., 2008; Einstein et al., 2010; Händel et al., 1999]. SSTR3 is localized to virtually all mature dentate gyral granule cells [Chakravarthy et al., 2010]. And it is needed for memory encoding because knocking it out severely impairs the ability of mice to recognize novel objects [Einstein et al., 2010] (Figure 6).

Could a Failure of Primary Cilia Be Involved in the Devastating Cognitive Decline of Alzheimer's Disease (AD)?

The common, non-mutationally based, form of AD (> 95 % of the cases), LOAD (*l*ate-*o*nset AD), begins in brains perhaps as young as 30-40 years-old and spreads imperceptibly with advancing age until it clinically surfaces decades later with a shrinking hippocampus and an alarmingly failing memory. The two important AD targets are the entorhinal cortex and the dentate gyrus. But what targets them?

Active neurons in a healthy brain produce the aggregation-prone $A\beta_{1\text{-}42}$ peptide which is cut out of a membrane-associated protein known as APP (amyloid precursor protein) but kept at a safe—picomolar—level by being destroyed by proteases, being swept up by microglia, or by being cleared from the brain and dumped into the circulation by transporters such as LRP1 [Selkoe et al., 2012]. But if the non-toxic, physiologically functioning $A\beta_{1\text{-}42}$ monomers should exceed the safety level because of declining clearance mechanisms in the aging brain, they will aggregate into toxic soluble oligomers and protofibrils that start the brain on its journey to AD [Benilova et al., 2012; Selkoe et al., 2012]. The accumulation of these toxic peptides is believed to disrupt neuronal networks in the entorhinal cortex and the dentate gyrus [Benilova et al., 2012; Selkoe et al., 2012]. They may do this at least in part by binding to, and activating, the CaSR (Ca^{2+}-sensing receptor), which by stimulating GSK-3β (glycogen synthase kinase-3β) hyperphosphorylates the microtubule-associated tau protein [Armato et al., 2012].

This signaling along with Ca^{2+} surges delocalizes tau from its normal association with axonal microtubules and axonal traffic [Selkoe et al., 2012]. But the delocalized phospho-tau can collect into the AD hallmark NFTs (neurofibrillary tangles) and also invade the dendrites where it destroys spines and synapses [Selkoe et al., 2012]. Moreover, the Aβ-activated CaSR also stimulates astrocytes to start a kind of intercellular contagion by making and secreting $A\beta_{1\text{-}42}$ oligomers onto their tightly associated neurons [Armato et al., 2012].

But what might dentate primary cilia have to do with this? $A\beta_{1\text{-}42}$, like neurotrophins such as BDNF and NGF, can also bind and activate $p75^{NTR}$ and presumably cilial $p75^{NTR}$ [Chiarini et al., 2006; Sotthibundhu et al., 2009] (Figure 7). Therefore, this could explain a mysterious aspect of adult neurogenesis in the early, $A\beta_{1\text{-}42}$-accumulating stages of AD.

Adult neurogenesis normally declines with age in rodents and humans, and it would be expected to drop even faster with the approach of AD. Although neurogenesis certainly drops in AD, there are indications of increased proliferation in the early, $A\beta_{1\text{-}42}$-oligomers-accumulating, stages of AD, but the newborn neurons do not mature and neurogenesis consequently drops despite the surge of proliferative activity which without the shut-down feedback from mature granule cells could deplete or even empty the stem cell pool [Shruster et al., 2010; Waldau and Shetty, 2008; Yu et al., 2009].

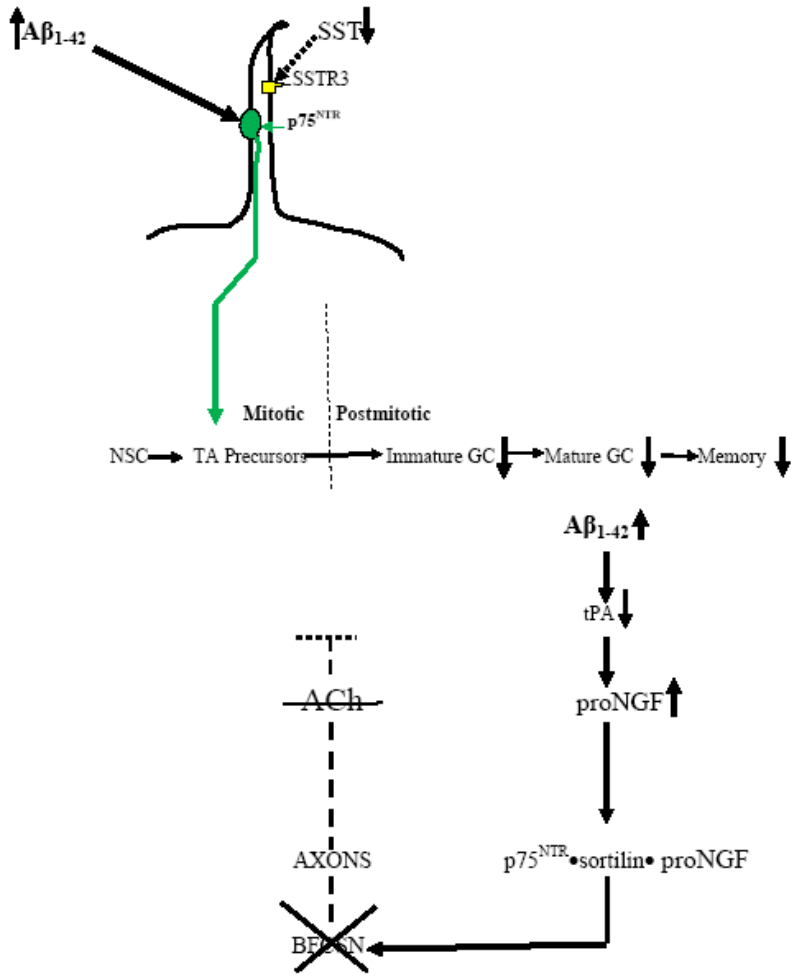

Figure 7. The Aβ peptides accumulating in the early stages of AD also stimulate cilial $p75^{NTR}$ like neurotrophins and stimulate TA cell proliferation. But the additional neurogenesis expected from this stimulation fails and neurogenesis drops because proNGF can no longer be converted into NGF because of a shortage of plasmin resulting from a fall of tPA. But proNGF preferentially binds and activates the $p75^{NTR}$• sortilin complex instead of NGF•TrkA in the BFCSN axons. The retrograde flow of $p75^{NTR}$• sortilin signals down the axons kill the BFCSNs which cuts off the supply of ACh that promotes the survival of the newborn granule cells. Furthermore, newborn cells that do survive are prevented from maturing by the lack of somatostatin (SST) that characterizes AD brains and the consequent silencing of SSTR3 signaling. The abbreviations are the same as in Figure 6.

The reason for this surge of neuroblast proliferation could be stimulation of the cilial $p75^{NTR}$ by accumulating $A\beta_{1-42}$ (Figure 7). The inability of the newborn granule cells to survive could be due at least to two things. First, it is the well-known decline of SST in the AD brain and therefore the loss of cilial SSTR3 signaling needed for granule cell maturation and functioning [Burgos-Ramos et al., 21008; Einstein et al., 2010] (Figure 7). Second, it is the profound loss of basal forebrain cholinergic neurons in AD brains and with them their ACh that normally promotes the survival and maturation of TA neurons [Van der Borght et al., 2005] (Figure 7).

Conclusion

We have come to the end of our brief voyage through the hippocampal formation and the dentate gyrus with its granule cell layer covered with a forest of receptor-loaded immobile primary cilia (Figure 4). We know that these tiny cilia are crammed with the machinery for producing the receptors' signals that drive the proliferation of TA neuroblasts and then their postmitotic maturation and functioning as memory recorders. But these tiny devices are a challenge to isolate and directly analyze, so we rely on arguably specific immunostaining and gene knock-downs and knock-ups to find out what they might do and how they might do it.

Also nearly all of our considerable knowledge of adult neurogenesis enshrined in Kempermann [2011] plus the indications that primary cilial signaling drives it have necessarily been obtained virtually entirely from mice.

We simply do not know whether human dentate gyral granule cells are ciliated or whether human, like murine TA neuroblast proliferation, maturation and integration into to the mature granule cell layer, are also driven from primary cilia. Hopefully we will soon be able to extend our ideas about adult neurogenesis and the roles of primary cilia in it from mice to humans. Indeed, we have taken some very small steps towards this goal by finding ciliated cells in samples of hippocampi from octogenarian normal and AD humans (not shown) and in phenotypically normal astrocytes from adult human cerebral cortices (Figure 8).

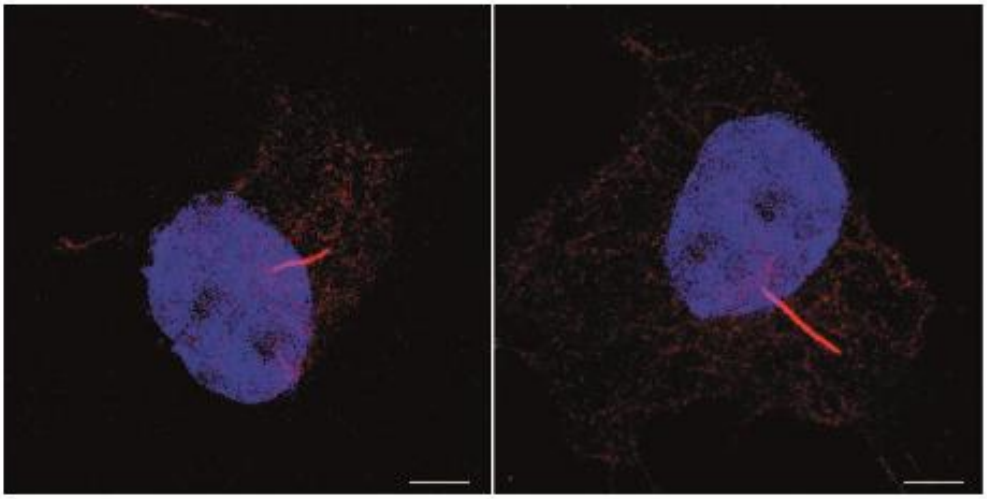

Figure 8. Examples of the primary cilia protruding from cultured normal adult cerebral human atrocytes. The astrocytes shown here were set into culture according to Dal Prà et al. [2011]. The cells were fixed with 4% paraformaldehyde for 30-min at room temperature, and next challenged with an antibody against acetyl-tubulin at a final concentration of 1.0 μg ml^{-1}. Specific secondary antibodies conjugated with Alexa Fluor-488 or -568 (either at 5.0 μg ml^{-1}) were then applied. DNA was stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, 1.0 ng ml^{-1} for 10-min). The cells were examined under a Leica TCS-SP5 confocal microscope and photographed at 630X original magnification. The primary cilium is clearly visible in or close to the nuclear zone of the cells. *Blue*, DNA; *red*, acetyl-tubulin. *Bar*, 10 μm.

Acknowledgment

We are very grateful to Drs. Raffaella Pacchiana and Clara Bonafini of the Verona Group for preparing Figs.1, 2, 3 and 5.

References

Aimone, J.B., Deng, W. and Gage, F.H. (2010). *Adult neurogenesis: integrating theories and separating functions*. Trends Cogn. Sci. 14, 325-337.

Altman, J. and Das, G.D. (1967). *Postnatal neurogenesis in the guinea pig.* Nature 214, 1098-1010.

Amador-Arjona, A., Elliott, J., Miller, A., Ginbey, A., Pazour, G.J., Enikolopov, G., Roberts, A.J. and Terskikh, A.V. (2011). *Primary cilia regulate proliferation of amplifying progenitors in adult hippocampus: implications for learning and memory.* J. Neurosci. 31, 9933-9944.

Amrein, I. and Lipp, H.-P. (2009). *Adult hippocampal neurogenesis of mammals: evolution and life history.* Biol. Lett.5, 141-144.

Antequara, D., Portero, A., Bolos, M., Orive, G., Hernández, R.M., Pedraz, J.LK. and Carro, E. (2012). *Encapsulated VEGF-secreting cells enhance proliferation of neuronal progenitors in the hippocampus of AβPP/Ps1 mice.* J. Alzheimer's Dis. Jan 9. Epub ahead of print.

Armato, U., Chakarvarthy, B., Chiarini, A., Dal Prà, I. and Whitfield, J.F. (2011). *Leptin, sonic hedgehogs, and neurogenesis—a primary cilium's tale.* J. Alzheimer's Dis. Parkinson. 2:105e doi: 10.4172/2161-0460.1000e105.

Armato, U., Bonafini, C., Chakravarthy, B., Chiarini, A., Whitfield, J.F. and Dal Prà, I. (2012). *The calcium-sensing receptor: a novel Alzheimer's disease crucial target*. J. Neurol. Sci., in press.

Attardo, A., Fabel, K., Krebs, J., Haubensak, W., Huttner, W.B. and Kempermann, G. (2010). *Tis21 expression marks not only populations of neurogenic precursor cells but also new postmitotic neurons in adult hippocampal neurogenesis.* Cereb. Cortex 20, 304-314.

Basak, O. and Taylor, V. (2009). *Stem cells of the adult mammalian brain and their niche*. Cell Mol. Life Sci. 66, 1057-1072.

Benilova, I., Karran, E. and De Strooper, B. (2012). *The toxic Aβ oligomer and Alzheimer's disease: an emporer in need of clothes.*

Bernabeu, R.O. and Longo, F.M. (2010). *The p75 neurotrophin receptor is expressed by adult mouse dentate progenitor cells and regulates neuronal and non-neuronal cell genesis.* BMC Neurosci. 11,136.

Berbari, N.F., Johnson, A.D., Lewis, J.S., Askwith, C.C. and Mykytyn, K. (2008). *Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors.* Mol. Biol. Cell 19, 1540-1547.

Berbari, N.F., O'Connor, A.K., Haycraft, C.J. and Yoder, B.K. (2009). *The primary cilium as a complex signaling center.* Curr. Biol. 19, R526-R535.

Berry, J., Krause, W.C. and Davis, R.L. (2008). *Olfactory memory traces in Drosophila. Progr.* Brain Res. 169, 293-304.

Biberich, E. (2011). *Ceramide stem cell differentiation and embryo development: novel functions of a topological cell-signaling lipid and the concept of ceramide compartments*. J. Lipids, doi:10.1155/2011/610306.

Blacque, O.E., Cevik, S. and Kaplan, O.I. (2008). *Intraflagellar transport: from molecular characterisation to mechanism.* Front. Biosci. 13, 2633-2652.

Brann, A.B., Scott, R., Neuberger, Y., Abulafia, D., Boldin, S., Fainzilber, M. and Futerman, A.H. (1999). *Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons*. J. Neurosci. 19, 8199-8206.

Breunig, J.J., Sarkisian, M.R., Arellano, J. I., Morozov, Y.M., Ayoub, A.E., Sojitra, S., Wang, B., Flavell, R.A., Rakic, P. and Town, T. (2008). *Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signaling.* Proc. Natl. Acad. Sci. U.S.A. 105, 13127-13132.

Bruel-Jungerman, E., Lucassen, P.L. and Francis, F. (2011). *Cholinergic influences on cortical development and adult neurogenesis.* Behav. Brain Res. 221, 379-388.

Burgos-Ramos, E., Hervás-Aguilar, A., Aguado-Liera, D., Puebla-Jiménez, L., Hernández-Pinto, A.M., Barrios, V. and Arilla-Ferreiro, E. (2008). *Somatostatin and Alzheimer's disease*. Mol. Cell Endocrinol. 286, 104-111.

Canto, C.B., Wouterlood, F.G. and Witter, M.P. (2008). *What does the anatomical organization of the entorhinal cortex tell us?* Neural. Plast. Article ID 381243,18 pages.

Chakravarthy, B, Gaudet, C., Ménard M., Atkinson, T., Chiarini, A. Dal Prà, I., and Whitfield, J. (2010). *The p75 neurotrophin receptor is localized to primary cilia in adult mouse hippocampal dentate gyrus granule cells*. Biochem. Biophys. Res. Commun. 401, 458-462.

Chiarini, A., Dal Prà, I., Whitfield, J.F. and Armato, U. (2006). *The killing of neurons by beta-amyloid peptides, prions, and proinflammatory cytokines.* Ital. J. Anat. Embryol. 111, 221-246.

Choi, S.S., Syn, W.K., Karaca, G.F., Omenetti, A., Moylan, C.A., Witek, R.P., Agboola, K.M., Jung, Y., Michelotti, G.A. and Diehl, A.M. (2010). *Leptin promotes the myofibroblastic phenotype in hepatic stellate cells by activating the hedgehog pathway.* J. Biol. Chem. 285, 36551-36560.

Colditz, M.J., Catts, V.S., Al-menhali, N., Osborne, G.W., Bartlett, P.F. and Coulson, E.J. (2010). *p75 neurotrophin receptor regulates basal and fluoxetine-stimulated hippocampal neurogenesis*. Exp. Brain Res. 200, 161-167.

Corbit, K.C., Aanstad, P., Singla, V., Norman, A.R., Stainier, D.Y. and Reiter, J.F. (2005). *Vertebrate smoothened functions at the primary cilium*. Nature 437, 1018-1031.

Dal Prà, I., Whitfield, J.F., Pacchiana, R., Bonafini, C., Talacchi, A., Chakravarthy, B., Armato, U. and Chiarini, A. (2011). *The amyloid-β_{42} proxy, amyloid β(25-35), induces normal human cerebral astrocytes to produce amyloid-β_{42}.* J. Alzheimer's Dis. 24, 335-347.

Davenport, J.M., Watts, A.J., Roper, V.C., Croyle, M.J., van Groen, T., Wyss, J.M., Nagy, T.R., Kesterson, R.A. and Yoder, B.K. (2007*). Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease.* Curr. Biol. 17, 1586-1594.

Deng, W., Aimone, J.B. and Gage, F.H. (2010). *New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?* Nat. Rev. Neurosci. 11, 339-350.

Dobrowsky, R.T. and Carter, B.D. (1998). Coupling of the p75 neurotrophin receptor to sphingolipid signaling. Ann. N.Y. Acad. Sci. 845: 32-45.

Drapeau, E. and Nora Abrous, D. (2008). Stem cell review series: role of neurogenesis in age-related memory disorders. Aging Cell 7, 569-589.

Dumas, T.C., Powers, E.C., Tarapore, P.E. and Sapolski, R.M. (2004). *Overexpression of calbindin D (28k) in dentate gyrus granule cells alters mossy fiber presynaptic function and impairs hippocampal-dependent memory.* Hippocampus 14, 701-709.

Duvernoy, H.M. *The Human Hippocampus*. Munich: J.F. Bergmann Verlag; 1988.

Eichenbaum, H. *The Cognitive Neuroscience of Memory*. Oxford, New York: Oxford University Press; 2012.

Einstein, E.B., Patterson, C.A., Hon, B.J., Regan, K.A., Reddi, J., Melnikoff, D.E., Mateer, M.J., Schultz, S., Johnson, B.N. and Tallent, M.K. (2010). *Somatostatin signaling in neuronal cilia is critical for object recognition memory.* J. Neurosci. 30, 4306-4314.

Funahashi, H., Yada, T., Suzuki, R. and Shioda, S. (2003). *Distribution, function, and properties of leptin receptors in the brain.* Int. Rev. Cytol. 224, 1-27.

Garza, J.C., Guo, M., Zhang, W. and Lu, X.-Y. (2008). *Leptin increases adult hippocampal neurogenesis in vitro and in vivo.* J. Biol. Chem. 283, 18238-18247.

Goldman, S.A. and Chen, Z. (2011). *Perivascular instruction of cell genesis and fate in the adult brain.* Nat. Neurosci. 14, 1382-1389.

Goetz, S.C. and Anderson, K.V. (2010). *The primary cilium: a signalling centre during vertebrate development.* Nat. Rev. Genet. 11, 331-344.

Goetz, S.C., Ocbina, P.J.R. and Anderson, K.V. (2009). *The primary cilium as a hedgehog signal transduction machine.* Methods Cell Biol. 94,199-222.

Gould, E. and Cameron, H.A. (1996). *Regulation of neuronal birth, migration and death in the rat dentate gyrus.* Dev. Neurosci. 18, 22-35.

Guadavaccaro, D., Corrente, G., Covone, F., Micheli, L., D'Agnano, I., Starace, G., Caruso, M. and Tirone, F. (2001). *Arrest of G_1-S progression by the p53-inducble gene PC3 is Rb-dependent and relies on the inhibition of cyclin D1 transcription.* Mol. Cell. Biol. 20, 1797-1815.

Guo, D.-F. and Rahmouni, K. (2011). *Molecular basis of the obesity associated with Bardet-Biedl syndrome.* Trends Endocrinol. Metab. 22, 286-293.

Gupta, P.S., Prodromu, N.V. and Chapple, J.P. (2009). *Can faulty antennae increase adiposity? The link between cilia proteins and obesity*. J. Endocrinol. 293, 327-336.

Han, Y.G., Spassky, N., Romaguera-Ros, M., Garcia-Verdugo, J.M., Aguilar, A., Schneider-Maunoury, S. and Alvarez-Buylla, A. (2008). *Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells.* Nat. Neurosci. 11, 277-284.

Händel, M., Schultz, S., Stanarius, A., Schreff, M., Erdtmnn-Vouriliotis, M., Schmidt, H., Wolf, G. and Höllt, V. (1999). *Selective targeting of somatostatin receptor 3 to neuronal cilia.* Neuroscience 89, 909-926.

Kempermann, G. *Adult Neurogenesis 2*. Oxford, New York: Oxford University Press; 2011.

Kenney, A.M. and Rowitch, D.H. (2000). *Sonic hedgehog promotes G_1 cyclin expression and sustained cell cycle progression in mammalian neuronal precursors.* Mol. Cell. Biol. 20, 9055-9067.

Küchler, J., Hartmann, W., Waha, A., Koch, A., Endl, E., Wurst, P., Kindler, D., Mikeska, T., Waha, A., Goodyer, C.G., Büttner, R., Schilling, K. and Pietsch, T. (2011). *p75(NTR) induces apoptosis in medulloblastoma cells*. Int. J. Cancer 128, 1804-1812.

Kumamoto, N., Gu, Y., Wang, J., Janoschka, S., Takemaru, K.-I., Levine, J. and Ge, S. (2012). *A role for primary cilia in glutamatergic synaptic integration of adult-born neurons*. Nat. Neurosci.,doi:10.1038/nn.3042.

Levin, B.E., Magnan, C., Dunn-Meynell, A. and Le Foll, C. (2011). *Metabolic sensing in the brain: who, what, where, and how?* Endocrinology 152, 2552-2557.

Li, M.-D. (2011*). Leptin and beyond: an odyssey to the central control of body weight*. Yale J. Biol. Med. 84, 1-7.

Makuch, R., Baratta, J., Karaelias, L.D., Lauterborn, J.C., Gall, C.M., Yu, J. and Robertson, R.T. (2001). *Arrival of afferents and the differentiation of target neurons: studies of developing cholinergic projections to the dentate gyrus.* Neuroscience 104, 81-91.

Mejia-Gervacio, S., Murray, K. and Lledo, P.-M. (2011). *NKCC1 controls GABAergic signaling and neuroblast migration in the postnatal forebrain.* Neural Dev. 2011 Feb 1:6:4

Mok, C.A., Héon, E. and Zhen, M. (2010). *Ciliary dysfunction and obesity*, Clin. Genet. 77, 18-27.

Morrens, J., Van den Broeck, W. and Kempermann, G. (2012). *Glial cells in adult neurogenesis.* Glia 60, 159-174.

Moscovitch, M., Rosenbaum, R.S., Gilboa, A., Addis, D.R., Westmacott, R., Grady, C., McAndrews, M.P., Levine, B., Black, S., M., Winocur, G. and Navel, L. (2005). *Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account*. J. Anat. 207, 35-66.

Mu, Y., Lee, S.W. and Gage, F.H. (2010). *Signaling in adult neurogenesis*. Curr. Opin. Neurobiol. 20, 416-423.

Nacher, J., Rosell, D.R., Alonso-Liosa, G. and McEwen, B.S. (2001). *NMDA receptor antagonist treatment induces a long-lasting increase in the number of proliferating cells, PSA-NCAM-immunoreactive granule neurons and radial glia in the adult rat dentate gyrus.* Eur. J. Neurosci.13, 512 520.

Nachury, M.V., Seeley, E.S. and Jin, H. (2010). *Trafficking to the ciliary membrane: how to get across the pericilliary diffusion barrier?* Annu. Rev. Cell Dev. Biol. 26, 59-87.

Nieuwenhuys, R. (1994). *The neocortex. An overview of its evolutionary development, structural organization and synaptology.* Anat. Embryol. 190, 307-337.

Nottebohm, F. (1980). *Testosterone triggers growth of brain vocal control nuclei in adult femalecanaries*. Brain Res. 189, 429-436.

Nottebohm, F. (1985). *Neuronal replacement in adulthood*. Ann. N.Y. Acad. Sci. 457, 143-161.

Paz-Filho, G., Wong, M.-L. and Licinio, J. (2011). *Ten years of leptin replacement therap*y. Obesity Rev. 12, e315-e323.

Penfield, W. and Perot, P. (1963). *The brain.s record of auditory and visual experience.* Brain 86, 595-696.

Rohtagi, R. and Snell, W.J. (2010). *The ciliary membrane*. Curr. Opin. Cell Biol. 22, 541-546.

Rugg, M.D., Johnson, J.D., Park, H. and Uncapher, M.R. (2008). *Encoding-retrieval overlap in human episodic memory: a functional neuroimaging perspective.* Prog. Brain Res. 169, 339-352.

Sandeman, D.C., Bazin, F. and Beltz, B.S. (2011). *Adult neurogenesis: examples from the decapod crustaceans and comparison with mammals*. Arthropod Struct. Devel. 40, 258-275.

Satir, P. (2007). *Cilia biology; stop overeating now!* Curr. Biol. 17, R963-R965.

Satir, P.and Christensen, S.T. (2008). *Structure and function of mammalian cilia*. Histochem. Cell Biol. 129, 687-693.

Scholey, J.M. (2008). *Intraflagellar transport motors in cilia: moving along the cell's antenna.* J.Cell Biol. 180, 23-29.

Schwarz, A. and Futerman, A.H. (1998). *Inhibition of sphingolipid synthesis, but not degradation, alters the rate of dendrite growth in cultured hippocampal neurons.* Brain Res. Dev. Brain Res. 108, 125-130.

Selkoe, D.J., Mandelkow, E. and Holtzman, D.M., Editors (2012). *The Biology of Alzheimer Disease*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press; 1-511.

Seo, P., Guo, D.-F., Bugge, K., Morgan, D.A., Rahmkouni, K. and Sheffield, V.V. (2009), *Requirement of Bardet-Biedl syndrome for leptin receptor signaling.* Hum. Mol. Genet. 18, 1323-133.

Shepherd, G.M. (2011). *The microcircuit concept applied to cortical evolution: from three-layer to six-layer cortex. Front*. Neuroanat. doi10.3389/fnana.2011.00030.

Shruster, A., Melamed, E. and Offen, D. (2010). *Neurogenesis in the aged and neurodegenerative brain.* Apoptosis 15, 1415-1421.

Small, S.A., Schobel, S.A., Buxton, R.B., Witter, M.P. and Barnes, C.A. (2011). *A pathophysiological framework of hippocampal dysfunction in ageing and disease.* Nat. Rev. Neurosci. 12, 585-601.

Sotthibundhu, A., Li, Q.X., Thangnipon, W. and Coulson, E.J. (2009). *Abeta(1-42) stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor.* Neurobiol. Aging 30, 1975-1985.

Stratigopoulos, G., LeDuc, C.A., Cremona, M.K., Ghung, W.K. and Liebel, R.L. (2011). *CUT-like homeobox (CUX) regulates expression of the fat mass and obesity-associated and retinitis pigmentosa GTPase regulator-interacting protein-1-like (RPGRIP1L) genes coordinates leptin receptor signaling*. J. Biol. Chem. 286, 2155-2170.

Strausfeld, N.J. *Arthropod Brains*. Cambridge MA: Belnap Press of Harvard University Press; 2012.

Suh, H., Deng, W. and Gage, F.H. (2009). *Signaling in adult neurogenesis*. Annu. Rev. Cell. Devel. Biol. 25, 253-275.

Suthana, N., Haneef, Z., Stern, J., Mukamel, R., Behnke, E., Knowlton, B. and Fried, J. (2012). *Memory enhancement and deep brain stimulation of the entorhinal area.* N. Engl. J. Med. 366, 502-510.

Toni, N. and Sultan, S. (2011). *Synapse formation on adult-born hippocampal neurons*. Eur. J. Neurosci. 33, 1062-1068.

Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H. and Gagae, F.H. (2007). *Synapse formation on neurons born in the adult hippocampus.* Nat. Neurosci. 10, 727-734.

Treves, A. (2009). Spatial cognition, memory capacity, and the evolution of mammalian hippocampal networks. In: Tommasi, L., Peterson, M.A. and Nadel, L. (Editors). *Cognitive Biology: Evolutionary and Developmental Perspectives on Mind, Brain, and Behavior*. Cambridge MA: MIT Press; 2009; 41-59.

Treves, A., Tashiro, A., Witter, M.E. and Moser, E.I. (2008). *What is the dentate gyrus good for?* Neuroscience 154, 1155-1172.

Van der Borght, K., Mulder, J., Keijser, J, N., Eggen, B.J., Luiten, P.G. and Vand der Zee, E.A. (2005). *Input from the medial septum regulates adult hippocampal neurogenesis*. Brain Res. Bull. 67, 117-125.

Verhey, K.J., Dishinger, J. and Kee, H.L. (2011). *Kinesin motors and primary cilia*. Biochem. Soc. Trans.39, 1120-1125.

Waldau, R. and Shetty, A.K. (2008). *Behavior of neural stem cells in the Alzheimer* brain. Cell Mol Life Sci. 65, 2372-2384.

Wang, G., Krishnamurthy, K. and Bieberich, E. (2009). *Regulation of primary cilia formation by ceramide.* J. Lipid Res. Res. 50, 2103-2110.

Wang, Z., Li, Y., Chan, G.C., Phan, T., Nudelman, A.S., Xia, Z. and Storm, D.R. (2009). *Adult type 3 adenylyl cyclase-deficient mice are obese*. PLoS 4, e6979.

Witter, M.P. (2007). *Intrinsic and extrinsic wiring of CA3: indications for connectional heterogeneity*. Learn. Mem. 14, 705-713.

Woo, N.H., Teng, H.K., Siao, C.J., Chiaruttini, C., Pang, P.T., Milner, T.A., Hempstead, B.L. and Lu, B. (2005). *Activation of p75NTR by proBDNF facilitates hippocampal long-term depression*. Nat. Neurosci. 8, 1069-1077.

Young, K.M., Merson, T.D., Sotthibundhu, A., Coulson, E.J. and Bartlett, P.F. (2007). *p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells*. J. Neurosci. 27, 5146-5155.

Yu, Y., He, J. Zhang, Y., Luo, H., Zhu, S., Yang, Y., Zhao, T., Wu, J., Huang, Y., Kong, J., Tan, Q. and Li, X.M. (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.