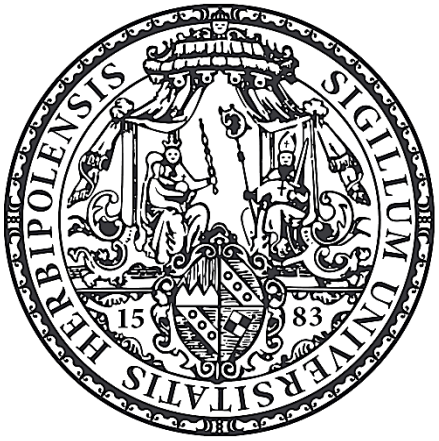


Alzheimer`s disease and brain insulin resistance: The diabetes inducing drug streptozotocin diminishes adult neurogenesis in the rat hippocampus – an *in vivo* and *in vitro* study

Alzheimer-Krankheit und Insulinresistenz im Gehirn: Streptozotocin, das Änderungen im Insulinstoffwechsel hervorruft, reduziert die Neubildung von Neuronen im Hippocampus von adulten Ratten - *In vivo*- und *In vitro*-Untersuchungen

Doctoral thesis for a doctoral degree
at the Graduate School of Life Sciences,
Julius-Maximilians-Universität Würzburg,
Section Neuroscience

submitted by

Ping Sun

from

Zhejiang, China

Würzburg, 2015

Submitted on: ……..

Office stamp

Members of the *Promotionskomitee*:

Chairperson: Prof. Dr. Michael Sendtner

Primary Supervisor: PD Dr. Angelika Schmitt

Supervisor (Second): Prof. Dr. Wolfgang Rössler

Supervisor (Third): Prof. Dr. Jürgen Deckert

Supervisor (Fourth): Dr. Camelia-Maria Monoranu

Date of Public Defence: ……..……

Date of Receipt of Certificates: ……..

Table of Content

Summary

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease of the brain, which is characterized by a progressive loss of memory and spatial orientation. Only less than 5-10% of AD sufferers are familial cases due to genetic mutations in the *amyloid precursor protein* (*APP*) gene or *presenilin* (*PS*) 1 and 2 genes. The cause of sporadic AD (sAD) which covers > 95% of AD patients is still unknown. Current research found interactions between aging, diabetes and cognitive decline including dementia in general and in AD in particular. Disturbances of brain glucose uptake, glucose tolerance and utilization and impairment of the insulin/insulin receptor (IR) signaling cascade are thought to be key targets for the development of sAD.

In the brain of AD patients, neural plasticity is impaired indicated by synaptic and neuronal loss. Adult neurogenesis (AN), the generation of functional neurons in the adult brain, may be able to restore neurological function deficits through the integration of newborn neurons into existing neural networks. The dentate gyrus of the hippocampus is one out of few brain regions where life-long AN exists. However, there is a big controversy in literature regarding the involvement of AN in AD pathology. Most animal studies used transgenic mice based on the Amyloid ß (Aß) hypothesis which primarily act as models for the familial form of AD. Findings from human post mortem AN studies were also inconstistent. In this thesis, we focused on the possible involvement of AN in the pathogenesis of the sporadic form of AD. Streptozotocin intracerebroventricularily (STZ icv) treated rats, which develop an insulin-resistant brain state and learning and memory deficits preceding Aß pathology act as an appropriate animal model for sAD. We used STZ treatment for both parts of my work, for the *in vivo* and *in vitro* study.

In the first part of my thesis, my coworkers and I investigated STZ icv treatment effects on different stages of AN in an *in vivo* approach. Even if STZ icv treatment does not seem to considerably influence stem cell proliferation over a short-term (1

month after STZ icv treatment) as well as in a long-term (3 months after STZ icv treatment) period, it results in significantly less immature and newborn mature neurons 3 months after STZ icv treatment. This reduction detected after 3 months was specific for the septal hippocampus, discussed to be important for spatial learning. Subsequently we performed co-localization studies with antibodies detecting BrdU (applied appr. 27 days before sacrifice) and cell-type specific markers such as NeuN, and GFAP, we found that STZ treatment does not affect the differentiation fate of newly generated cells. Phenotype analysis of BrdU-positive cells in the hilus and molecular layer revealed that some of the BrdU-positive cells are newborn oligodendrocytes but not newborn microglia.

In the second part of my thesis I worked with cultured neural stem cells (NSCs) isolated from the adult rat hippocampus to reveal STZ effects on the proliferation of of NSCs, and on the survival and differentiation of their progeny. Furthermore, this *in vitro* approach enabled me to study cellular mechanisms underlying the observed impaired neurogenesis in the hippocampus of STZ-treated rats. In contrast to our findings of the STZ icv *in vivo* study we revealed that STZ supplied with the cell culture medium inhibits the proliferation of NSCs in a dose-dependent and time-dependent manner. Moreover, performing immunofluorescence studies with antibodies detecting cell-type specific markers after triggering NSCs to differentiate, we could show that STZ treatment affects the number of newly generated neurons but not of astrocytes. Analyzing newborn cells starting to differentiate and migrate I was able to demonstrate that STZ has no effect on the migration of newborn cells. Trying to reveal cellular mechanisms underlying the negative influence of STZ on hippocampal AN, we performed qRT-PCR and immunofluorescence staining and thus could show that in NSCs the expression of glucose transporter (GLUT)3 mRNA as well as IR and GLUT3 protein levels are reduced after STZ treatment. Therefore, the inhibition of the proliferation of NSCs may be (at least partially) caused by these two molecules. Interestingly, the effect of STZ on differentiating cells was shown to be different, as IR protein expression was not

significantly changed but GLUT3 protein levels were decreased in consequence of STZ treatment.

In summary, this project delivered further insights into the interrelation between AN the sporadic form of sAD and thus provides a basis of new therapeutic approaches in sAD treatment through intervening AN. Discrepancies between the results of the two parts of my thesis, the *in vivo* and *in vitro* part, were certainly caused to a certain extent by the missing microenvironment in the *in vitro* approach with cultured NSCs. Future studies e.g. using co-culture systems could at least minimize the effect of a missing natural microenvironment of cultured NSCs, so that the use of an *in vitro* approach for the investigation of STZ treatment underlying cellular mechanisms can be improved.

Zusammenfassung

Die Alzheimer-Krankheit (AK) ist die häufigste neurodegenerative Erkrankung weltweit. Nur etwa 5 bis 10% der Betroffenen leiden an der familiären Form, die auf bestimmten Mutationen in einzelnen Genen, wie z.B. dem *Amyloid precursor protein (APP)*-Gen, zurückzuführen ist. Die Ursache der sporadischen Form der AK (sAK), die mehr als 95% der Betroffenen ausmacht, ist hingegen noch weitgehend unbekannt. Jüngste Erkenntnisse weisen auf eine Wechselwirkung von hohem Alter, Stoffwechselkrankheiten wie z.B. Diabetes, und kognitiven Defiziten, welche eine Demenz im Allgemeinen und die Alzheimer-Krankheit im Besonderen kennzeichnen, hin. Deshalb werden Störungen in der Glukoseaufnahme, in der Glukosetoleranz, und in der Funktion des Insulin/Insulinrezeptorsignalweges als Schlüsselelemente für die Entstehung einer sAK angesehen.

Die neuronale Plastizität der Gehirne von AK-Patienten ist stark eingeschränkt, was sich vor allem durch den Verlust von Synapsen als auch durch den Verlust ganzer Nervenzellen zeigt. Die adulte Neurogenese (AN), die Neubildung von Neuronen im Gehirn von erwachsenen Individuen, könnte durch den Einbau neu gebildeter Neurone in existierende neuronale Netzwerke eine wichtige Rolle bei der Regenerierung neurologischer Defizite spielen. Der Gyrus dentatus im Hippocampus ist eine der wenigen Gehirnregionen, in welcher lebenslang AN stattfindet. Jedoch ist noch unklar, ob eine veränderte AN an der Pathogenese der AK beteiligt ist. Es wurden bereits viele Untersuchungen zur AN in Tiermodellen durchgeführt, wobei die überwiegende Anzahl von bisher verwendeten Tiermodellen auf der Amyloid ß-(Aß) Hypothese basieren, und somit primär Modelle für die familiäre AK darstellen. Studien mit humanem *post mortem*-Gewebe gaben bisher jedoch auch noch keine klaren Hinweise auf die mögliche Bedeutung einer veränderten AN für die AK.

In dieser Thesis sollte die Rolle der AN für die Pathogenese der sAD untersucht

werden. Dafür wurden Ratten mit Streptozotocin intracerebroventrikulär (STZ icv) behandelt. Diese so behandelten Ratten gelten als Tiermodell für die sAK, da sie bereits kurze Zeit nach ihrer STZ icv-Behandlung kognitive Defizite zeigen, ihr Gehirn eine Insulin-Resistenz entwickelt, und etwas später dann auch erste Anzeichen einer Aß-Pathologie nachweisbar sind.

Im ersten Teil dieser Arbeit wurde in einem *in vivo*-Ansatz der mögliche Einfluss einer STZ icv-Behandlung auf die verschiedenen Stadien der AN untersucht. Wir konnten zeigen, dass 1 Monat nach STZ icv-Behandlung weder die Proliferation neuraler Stammzellen (neural stem cells, NSCs) noch die Bildung junger Neurone verändert war, dass aber nach 3 Monaten signifikant weniger junge unreife und auch reife Neurone entstanden sind. Diese reduzierte Anzahl neu gebildeter Neurone konnte nur im septalen Teil des Hippocampus, dem eine bedeutende Rolle beim räumlichen Lernen zugesprochen wird, nachgewiesen werden. Durch eine quantitativ ausgewertete Ko-Lokalisationsstudie mit Antikörpern gegen Bromodesoxyuridin (BrdU) (mehrmalige i.p.-Gabe 27 Tage vor Gewebeentnahme) und zelltyp-spezifischen Markern wie dem Neuronenmarker NeuN und dem Marker für Astrozypen GFAP konnten wir zeigen, dass die STZ icv-Gabe nur die Anzahl der neu gebildeten Neuronen, aber nicht die Differenzierungsrichtung der neu gebildeten Zellen verändert. Eine qualitative Phänotypanalyse BrdU-positiver Zellen ergab außerdem, dass im Hilus und in der Molekularschicht des Gyrus dentatus lokalisierte BrdU-positive Zellen neu gebildeten Oligodendrozyten, aber nicht neu gebildeten Mikrogliazellen, zugeordnet werden konnten.

Im zweiten Teil meiner Arbeit habe ich NSCs aus dem adulten Hippocampus isoliert und kultiviert, um auch auf diese Art und Weise mögliche Effekte von STZ auf die Proliferation von NSCs als auch auf das Überleben und die Differenzierung von neu geborenen Zellen zu untersuchen. Ziel dieser *in vitro*-Studie war eine genauere Analyse der durch STZ-Gabe ausgelösten grundlegenden zellulären Mechanismen. Im Widerspruch zu den Ergebnissen der *in vivo*-Studie konnte ich einen Dosis- und

Zeit-abhängigen negativen Effekt von STZ auf die Proliferation der NSCs zeigen. Darüber hinaus führte die Zugabe von STZ zum Medium letztendlich zu einer verringerten Bildung von Neuronen, die Neubildung von Astrozyten zeigte sich jedoch unverändert. In einem Test zur Untersuchung der Migration neu gebildeter Zellen konnte ich keinen Einfluss von STZ auf die Migration nachweisen. Weitere Analysen ergaben, dass die verringerte Proliferation der NSCs im Zusammenhang mit einer reduzierten mRNA- als auch Protein-Expression des Glukosetransporters(GLUT)3 und mit reduzierten Insulinrezeptorkonzentrationen stehen könnte. In sich differenzierenden Zellen jedoch wurde neben einer ebenfalls reduzierten GLUT3- Proteinexpression keine veränderte Insulinrezeptorenausstattung detektiert.

Zusammenfassend gibt die vorliegende Arbeit mithilfe des *in vivo*- als auch *in vitro*-Ansatzes Hinweise auf eine Bedeutung der hippocampalen AN für die Entstehung der sAK und bietet dadurch Ansatzpunkte für neue therapeutische Ansätze. Die im *in vivo*- und *in vitro*-Ansatz erzielten unterschiedlichen Resultate, die sicherlich zum Teil durch die fehlende Mikroumgebung der NSCs und sich differenzierenden Zellen im *in vitro*-Ansatz verursacht wurden, können in Zukunft z.B. durch Ko-Kulturen zumindest verringert werden, so dass mithilfe von *in vitro*-Ansätzen grundlegende zelluläre Mechanismen einer STZ-Effekts in Zukunft besser untersucht werden können.

Abbreviations

5-HT	5-hydroxytryptamine
Aβ	Amyloid-β
ABC	Avidin-biotin complex
AD	Alzheimer's disease
AICD	APP intracellular domain
AN	Adult neurogenesis
ANOVA	One way analysis of variance
APC	Adenomatous polyposis coli
APP	Amyloid precursor protein
APOE	Apolipoprotein E
asf	Area sampling fraction
ATP	Adenosine triphosphate
BACE	β-secretase
BDNF	Brain-derived neurotrophic factor
bHLH	Basic helix-loop-helix
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
CA	Cornu Ammonis
cAMP	Cyclic adenosine monophosphate
ChAT	Choline acetyltransferase

CREB	cAMP response element-binding protein
DAB	3,3’-Diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DCX	Doublecortin
DG	Dentate gyrus
DMF	Dimethylformamide
D-PBS	Dulbecco's Phosphate-Buffered Saline
EGF	Epidermal growth factor
fAD	Familial Alzheimer’s disease
FGF-2	Fibroblast growth factor-2
GABA	Gamma-Aminobutyric acid
GCL	Granule cell layer
GFAP	Glial fibrillary acidic protein
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
Iba-1	Ionized calcium-binding adapter molecule 1
icv	Intracerebroventricular
IDE	Insulin degrading enzyme
IGF-1	Insulin-like growth factor-1
ip	Intraperitoneal
IR	Insulin receptor
IRS	Insulin receptor substrate

iv	Intravenous
Ki67	Ki-Kiel67
MCM2	Mini-chromosome maintenance protein 2
ML	Molecular layer
MRI	Magnetic resonance imaging
NSCs	Neural stem cells
NeuN	Neuronal Nuclei
NeuroD	Neurogenic differentiation
NMDA receptor	N-methyl-D-aspartate receptor
NFT	Neurofibrillary tangles
NO	Nitric oxide
NT-3	Neurotrophin-3
PARP	PolyADP-ribosepolymerase
PB	Phosphate buffer
PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PFA	Paraformaldehyde
PKB	Protein kinase B
PI3-K	Phosphatydilinoditol 3 kinase
PPD	Papain-Protease-DNase
PS	Presenilin
PSA-NCAM	Polysialylated-neural cell adhesion molecule

ROS	Reactive oxygen species
RT	Room temperature
sAD	Sporadic Alzheimer's disease
sAPPα	Soluble fragment of APP
SD	Standard deviation
SEM	Standard error of the mean
SGZ	Subgranular zone
SODs	Superoxide dismutases
SSC	Saline sodium citrate
ssf	Section sampling fraction
STZ	Streptozotocin
SVZ	Subventricular zone
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBS	Tris buffered saline
tsf	Thickness sampling fraction
VEGF	Vascular endothelial growth factor
WT	Wild type

1. Introduction

1.1 Alzheimer's disease

1.1.1 Alzheimer`s disease, its classification and prevelance

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease of the brain which affects people in several ways, including memory (Ballard et al., 2011), judgment (Marson et al., 2000), language (Murdoch et al., 1987), problem-solving (Willis et al., 1998), executive ability (Binetti et al., 1996) as well as personality (Bozzola et al., 1992). AD typically develops slowly and gradually gets worse over the course of several years. In early stages, symptoms can be observed by friends, family or colleagues including greater difficulty performing tasks in social interaction, increasing trouble with planning or organizing and hard learning new skills. Many people in the early stage also change mood, like experiencing increased anxiety. Then cognitive impairment becomes evident with the disease development. With the symptoms continue to worsen, the ability to perform basic activities of daily life is decreased, major personality and behavioral changes were also found. AD is fatal and normally leads to death (Reisberg et al., 1982).

Only less than 5-10% of AD sufferers are familial cases due to genetic mutations in the *amyloid precursor protein* (*APP*) gene or *presenilin* (*PS*) 1 and 2 genes, and inheriting any of these genetic mutations ensures the development of AD (Correia et al., 2011). Mutations in the *PS1* and *PS2* genes account for as much as 50% of all familial AD (fAD) cases (Tandon and Fraser, 2002). More than 30 mutations in the *APP* gene and more than 180 mutations in *PS1* and *PS2* genes have been identified so far (Bi, 2010). All of these mutations cause overproduction of Aβ (Lemere et al., 1996, Eckman et al., 1997, Takeda et al., 2004). The disease symptoms with familial AD patients tend to develop before age 65, sometimes as early as age 30 (Reitz et al.,

2011). The cause of the late-onset sporadic AD (sAD) which affects > 95% of AD patients is still unknown. The symptoms of most of the sAD cases begin after 65 years of age (2014 Alzheimer's Disease Facts and Figures).

Worldwide, nearly 44 million people have AD or related dementia (Alzheimer's Disease International) and places a considerable burden on society (Qiu et al., 2009). North America and Western Europe exhibit the highest prevalence and incidence rate compared to other developed and developing countries (Alzheimer's Disease International). In the US, estimated 5.2 million Americans of all ages had AD in 2014 (2014 Alzheimer's Disease Facts and Figures).

1.1.2 Pathological features of AD

The two most prominent neuropathological hallmarks of AD are extracellular senile plaques which mainly consist of the amyloid-β (Aβ) and intracellular accumulation of tau protein (Braak and Braak, 1991). Aβ, peptides of 36-43 amino acids, is derived from the sequential cleavage of the APP by the β- and γ-secretases which form a multi-protein complex comprising PS, presenilin enhancer 2 (PEN-2), anterior pharynx-defective 1 (APH-1) and nicastrin (Nunan and Small, 2000). Depend on the different cleavage site for γ-secretases, varying lengths of Aβ peptides have been observed, such as Aβ40 and Aβ42 (Fortini, 2002). Aβ40 is relatively benign, but Aβ42 aggregates more rapidly into neurotoxic oligomer with a β sheet structure and then form to plaques (Burdick et al., 1992, Bloom, 2014). Senile plaques consist of degenerating neuronal processes with tau paired helical filaments surrounding with deposits of Aβ (Davies et al., 1988, Cras et al., 1991). Intracellular aggregates of the hyperphosphorylated microtubule-associated protein tau form insoluble tangles, and are thought to be causally related to neuronal apoptosis (Goedert et al., 1989). Even if the amyloid cascade hypothesis is considered to be most popular hypotheses in AD research, cognition and memory decline in AD is not correlated with plaques (Nelson

et al., 2012). The NFTs of AD have a relatively strong correlation with cognitive function than amyloid plaques, but it is not unusual to see tangles in the brain of healthy, even as young as thirty years of age (Braak and Del Tredici, 2011).

Recent evidence indicates that AD neuropathology begins in brainstem nuclei, that send diffuse projections to a variety of brain regions (Braak and Del Tredici, 2012). As shown by Braak and Del Tredicie proximal axons of some coeruleus neurons in the brain of remarkably young individuals already exhibit nonfibrillar abnormal tau (Braak and Del Tredici, 2011). Moreover, according to the previously proposed *seeded polymerization theory* intracellular oligomeric Aβ in brainstem neurons possibly acts as the initiator of plaque formation in other brain regions such as the cerebral cortex and hippocampus and thus initiates the production of plaques in AD brains (Muresan and Muresan, 2008). This hypothesis of is supported by the *in vitro* study of Muresan and Muresan (2008) who found that brainstem-derived neuronal cells – but not cortical or hippocampal neurons – show large amounts of Aß accumulated at the terminals of their processes (Muresan and Muresan, 2008). The earliest stage of cortical neurofibrillary pathology emerges in the superficial entorhinal layer pre-α of the trans-entorhinal region (Braak and Braak, 1985). With the disease becomes worsen, the neurofibrillary pathology can be seen in all areas of the isocortex including sensory and motor cortex fields (Braak and Braak, 1991).

However, he level of synapse loss in AD brains demonstrates a higher correlation with decreased cognitive function than plaque and tangles development (Terry et al., 1991), suggesting disappearance of synapses is a key event in early cognitive decline (Gylys et al., 2004). Neurons in several brain regions are affected in AD, such as in the cerebral cortex which is involved in conscious thought and language, and hippocampus that is essential for the consolidation of information from short-term memory to long-term memory and spatial navigation. (Davies et al., 1980). The biochemical and molecular abnormalities which have been found in the AD patient's brain include increased activation of apoptotic genes and signaling pathways (Sajan

et al., 2007), impaired glucose utilization and energy metabolism (Fukuyama et al., 1994), mitochondrial dysfunction (Hirai et al., 2001), increased oxidative stress (Gibson et al., 1999), acetylcholine transmission dysfunction (Perry et al., 1978), altered Insulin/Insulin receptor (IR) signaling cascade (Ronnemaa et al., 2008), and DNA damage (Anderson et al., 1996).

1.1.3 Risk factors

Many factors contribute to the likelihood of developing AD and the greatest known risk factor is advancing age. The risk of developing this disease doubles every 5 years (Brentjens and Saltz, 2001) after the age of 65, and about one-third of people aged 85 and older have AD (2014 Alzheimer's Disease Facts and Figures). When AD runs in families, heredity, shared environmental and lifestyles factors may play a role (Donix et al., 2012).

Growing evidence suggests that the health of the brain is closely related to the overall health of heart and blood vessels. Many factors that increase the risk of cardiovascular disease are also associated with a higher risk of developing AD or other types of dementia (Newman et al., 2005) such as smoking (Merchant et al., 1999), alcohol consumption (Graves et al., 1991), obesity (Kivipelto et al., 2005), diabetes (Biessels and Kappelle, 2005), high cholesterol and hypertension in midlife (Kivipelto et al., 2002). The gene for Apolipoprotein E (APOE) protein, which mediates cholesterol metabolism (van den Elzen et al., 2005), but is also critically involved in the *vivo* conversion of “normal” Aβ into Aß which contains high β-sheet content and associated with cellular toxicity (Holtzman, 2001) is an interesting candidate for AD research.

Already in 1993 Strittmatter et al showed that individuals which carry the ε4 form of the Apolipoprotein E (*APOE)* gene have a higher risk for developing AD than carriers

of the ε2 and 3 alleles (Saunders et al., 1993, Strittmatter et al., 1993, Breitner et al., 1999). Researchers estimate that between 40 and 65 percent of people diagnosed with AD have one or two copies of the *APOE ε4* gene variant (Hauser and Ryan, 2013). Interestingly, women with an *APOE ε4/ε4* genotype have a 45% probability of developing AD by the age of 73, whereas men have only a 25% risk (Breitner et al., 1999). Up to now the role of *APOE* ε4 in AD pathogenesis is not fully elucidated.

In recent years it has been more and more accepted that disturbances of brain glucose uptake, glucose tolerance, glucose utilization and impairment of the insulin/IR signaling cascade are key targets for the neuropathology of sAD (Grunblatt et al., 2007, de la Monte, 2009, Salkovic-Petrisic et al., 2009, Correia et al., 2011, Talbot et al., 2012). As key energy source in the body, glucose is even more important in the brain and it acts as the primary source of energy for brain cells (Pardridge, 1983). It is taken up into cells by facilitated diffusion mediated by a series of glucose transporter family (Duelli and Kuschinsky, 2001). Glucose transport across the blood-brain barrier (BBB) into brain is fascilitated by GLUT1 (Leybaert et al., 2007). Two different GLUT1 isoform, one with a molecular weight of 45 kDa and the other one with 55 kDa, exist in the brain (Cunnane et al., 2011). The 55 kDa GLUT1 isoform is located at the luminal and the abluminal membranes of the brain endothelium cells and is responsible for the glucose transport across the BBB (Gerhart et al., 1989). Then, Glucose is taken up by astrocytes, oligodendroglia, microglia as well as neurons. It is transported into astrocytes via the 45 kDa GLUT1 isoform and exhibits highest concentration in astrocytic end feet and astrocytic processes surrounding synapses (McCall et al., 1996). The 45 kDa form of GLUT1 is also localized in oligodendroglia but not microglia (Yu and Ding, 1998). On the other hand, GLUT3, another major important glucose transporter in the brain which displays higher transport rates than GLUT1 (Dienel, 2012), facilitates neuronal glucose uptake (Simpson et al., 2007). Astrocytes, oligodendrocytes and endothelial cells have not been found to express GLUT3 (Haber et al., 1993). GLUT5 is expressed in microglial cells (Payne et al., 1997). GLUT1, 3 and 5 are all insulin-independent glucose transporters (Banks et al., 2012).

GLUT4, on the other hand, is an insulin-regulated glucose transporter which is found to be expressed in some specific neuronal cells in the cerebellum, hypothalamus and hippocampus (Grillo et al., 2009). Insulin stimulates the translocation of GLUT4 to neuron membranes and increases in glucose uptake (Grillo et al., 2009). Radioactive in situ hybridization for GLUT3 combined with immunocytochemistry for GLUT4 demonstrates the presence of GLUT3 mRNA in GLUT4-positive cells in the cerebral cortex, hippocampus, medial septum and cerebellum (Apelt et al., 1999). It is believed that GLUT4 plays a role in the brain by rapidly providing additional glucose to neurons under conditions of high-energy demand (El Messari et al., 1998).

Numerous studies have found that individuals with diabetes, both type 1 diabetes mellitus (T1DM) and T2DM, have a lower level of cognitive function and are at higher risk for dementia than individuals without diabetes (Ott et al., 1999, J et al., 2009). The possible physiological and molecular mechanisms are linked to glycemia, insulin resistance, oxidative stress, advanced glycation endproducts, inflammatory cytokines, as well as macrovascular and microvascular disease (Whitmer, 2007). In the brain of T2DM patients, more hippocampal and amygdalar atrophy was shown compared to elderly subjects of the same age without T2DM using magnetic resonance imaging (MRI) (den Heijer et al., 2003).

Lots of studies suggest Alzheimer-like changes found in different animal models for diabetes such as the streptozotocin (STZ) ip injection rat model (Biessels et al., 1998), T1DM BioBreeding/Worcester (BB/W) rat model (Li et al., 2002), T2DM BBZDE/Wor rats model (Li et al., 2007), and high-fat diet feeding mouse model (Moroz et al., 2008). High-fat diet feeding T2DM mouse model shows AD-like neurodegeneration with histopathological, molecular and biochemical brain abnormalities like increased levels of tau and Aβ, brain atrophy, insulin resistance, oxidative stress (Moroz et al., 2008). Spontaneously type 1 diabetic BB/W rats show progressively impaired cognitive function associated with suppressed insulin/ insulin-like growth factor-1 (IGF-1) expression and increased neural apoptosis in hippocampus which can be

prevented by treating with insulinomimetic C peptide(Li et al., 2002). Neural loss also found in T2DM BBZDE/Wor rats is associated with increased dystrophic neuritis, APP, β-secretase, Aβ and decreased insulin and IGF-1 receptor expression (Li et al., 2007). In diabetic rats induced/produced by STZ ip injections impaired hippocampal plasticity as well as impaired cognitive performances has been found (Biessels et al., 1998).

Postmortem studies suggest disturbances of cerebral glucose metabolism and insulin receptor (IR) signal transduction in AD (Hoyer, 2004) which are pathophysiological features of T2DM (Goldstein, 2002). IR signaling impairment triggers a chain reaction, including the decreased level of insulin receptor substrate (IRS) and altered downstream signaling pathways (Salkovic-Petrisic et al., 2009). This altered IR signaling pathway could result in impairment of glucose/energy metabolism, phosphorylation of tau protein as well as accumulation of intraneuronal Aβ (see Fig. 1) (Salkovic-Petrisic et al., 2009). In brain, advanced AD is associated with strikingly reduced levels of insulin and IGF-1 polypeptide and receptor genes (Steen et al., 2005). The signaling pathways which mediate insulin/IGF-1-stimulated neuronal survival, energy metabolism and mitochondrial functions were disturbed in AD (Fig.1) (Steen et al., 2005). Peripheral insulin resistance without T2DM is a risk factors for AD within 3 years and after 3 years the risk is no longer increased (Schrijvers et al., 2010). In the brain of AD patients, glucose utilization abnormalities happen both, in fAD (Hoyer and Nitsch, 1989) and sAD cases (Hoyer et al., 1991). So, insulin resistance defined as reduced cellular responsiveness to insulin which is key feature of T2DM (Goldstein, 2002) also occurs in the AD brain. Abnormalities in insulin/IGF-1 pathways in AD were quite similar to the effects of diabetes. Besides, AD neuropathology is shown to be stronger in AD patients with diabetes compared to AD patients without diabetes, including larger plaques and more tau positive cells (Valente et al., 2010). All these findings suggest AD may represent a neuro-endocrine disorder and some researchers even name it “Type 3 diabetes” (Steen et al., 2005, de la Monte and Wands, 2008). According to this hypothesis, using antidiabetes drugs

may reduce or even prevent AD-type neurodegeneration. Some antidiabetes drugs are based on glucagon-like peptide-1 (GLP-1) that is an incretin hormone which can facilitate insulin signaling (Drucker et al., 2010). Liraglutide, a long-acting GLP-1 agonist for treatment of T2DM, can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a transgenic model of Alzheimer's disease (McClean and Holscher, 2014). Another GLP-1 analogue (Val8)GLP-1 is shown to enhance neuronal stem cells proliferation, whereas the GLP-1 receptor antagonist exendin (9-39) impairs spatial learning (McGovern et al., 2012).

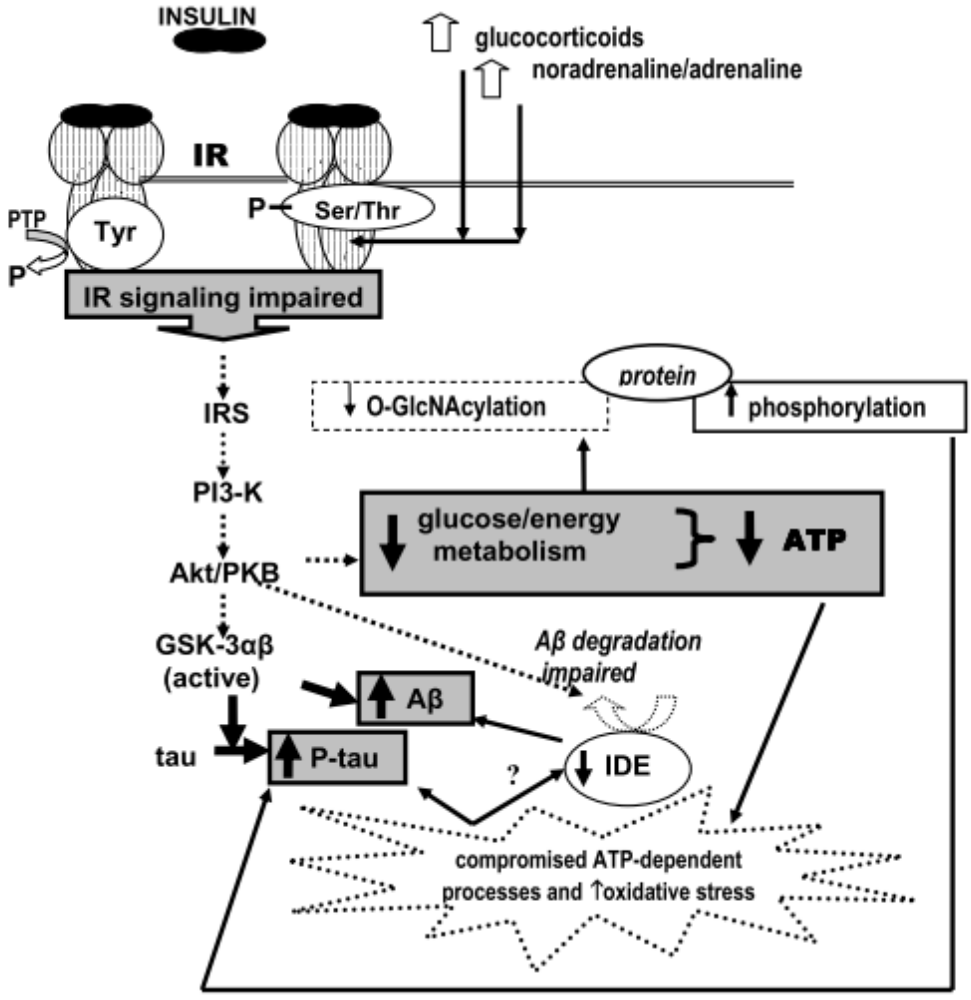


Fig.1. Proposed mechanism of the neuronal insulin receptor signaling cascade impairment (Salkovic-Petrisic et al., 2009).

Insulin receptor (IR) signaling impairment triggers a chain reaction, including the level of insulin receptor substrate (IRS) and downstream signaling pathway, like phosphatydilinoditol 3 kinase (PI3-K) pathway. IR signaling pathway impairment induced PI3-K dysfunction leads to reduced

protein kinase B (Akt/PKB) which involved in glucose/energy metabolism regulation, insulin degrading enzyme (IDE) activity regulation as well as phosphorylation and inactivation of glycogen synthase kinase 3α/β(GSK-3 α/β). Dashed lines/arrows represent reduction or inhibition of the processes while solid lines/arrows represent stimulation of the processes.

1.2 Animal models for Alzheimer's disease

1.2.1 Transgenic mouse models

As described in 1.1.2, AD is characterized by the accumulation of Aβ plaques and NFTs. As the amyloid cascade hypothesis and the tau protein hypothesis are considered to be most popular hypotheses for AD most animal models are based on them.

After the discovery of certain mutations in the *APP* gene of fAD patients, researchers tried to produce transgenic mouse models based on the overexpression of APP containing fAD mutations. Game et al. first reported a successful transgenic AD model using a platelet derived growth factor-β (PDGF) promoter driven human *APP* gene containing an FAD associated mutation (V717F) (Games et al., 1995). These PDAPP mice exhibit high human APP expression and exhibit extracellular Aβ deposition, dystrophic neuritis, reactive astrocytes, activated microglia, as well as loss of synaptic and dendritic density (Games et al., 1995). PDAPP mice showed cognitive deficits at a young age, including spatial working memory deficits at four months of age assessed by the Morris water maze (Hartman et al., 2005) and recognition memory deficit at six months of age assessed by novel object recognition (Dodart et al., 1999). Hsiao et al. overexpressed a human APP transgene containing the Swedish fAD mutation (K670N/M671L) driven by a hamster prion promoter (termed Tg2576 mice) (Hsiao et al., 1996). These mice develop numerous Aβ plaques by 11-13 months of age, and oxidative damage and synaptic plasticity changes could be found (Hsiao et al., 1996). Impaired learning and memory in Tg2576 mice have

been reported at less than six months (Hsiao et al., 1996). Subsequently, many other transgenic lines were developed with similar methods as used for the generation of PDAPP and Tg2576 mice such as TgCRND8 mice [carrying the human APP 695 (hAPP) including the “Swedish” and “Indiana” mutations (hAPPSw,Ind mice) controlled by the Syrian hamster prion promoter] (Chishti et al., 2001), APP 23 mice [carrying hAPP with Swedish mutation] (Sturchler-Pierrat et al., 1997). PS1 and PS2 fAD mutant transgenic lines were also developed, but single transgenic PS1 or PS2 mice do not develop Aß plaques (Holcomb et al., 1998). PS1/APP double transgenic mice, generated by crossing human PS1 with A246E mutation and APP with Swedish mutation (APPSwe), show elevated Aβ 42 peptide and numerous plaques by nine months of age (Borchelt et al., 1997) earlier than PDAPP and Tg2576 mice. All these mice generate Aβ amyloidosis and show a variety of neuropathological and behavioral defects including neural and synaptic loss, activated astrocytes and microglia as well as learning and memory deficits. However, in these mice model, neurofibrillary tangles are not present, which is another hallmark of AD.

Besides the transgenic mice with fAD mutations in APP and PS1/2, AD models were also produced which are based on the tau protein hypothesis. The first tau transgenic mouse model expressed the longest human wild-type (WT) tau isoform in neurons where pre-tangle formations and hyperphosphorylation of tau was observed (Gotz et al., 1995). Then, other Tau models were produced by the expression of human mutant P301L and P301S Tau in mice which then develop NFTs, neural loss, brain atrophy, impaired spatial learning and memory (Lewis et al., 2000, Yoshiyama et al., 2007, Takeuchi et al., 2011). These tau transgenic models are valuable tools in AD research and their pathology such as synapse and neuronal loss, NFTs formation, impaired learning and memory or inflammation are features of AD. However, Aβ amyloidosis is not successfully present in Tau model.

In order to develop a mouse dealing with both AD hallmarks, amyloid plaques and NFTs, triple-transgenic mice were generated in 2003 harboring $PS1_{M146V}$, APP_{Swe} and

Tau_{P301L}(Oddo et al., 2003). Age-related, progressive neuropathology including amyloid plaques and tangles are presented successfully in these mice (Oddo et al., 2003, Billings et al., 2005). In triple-transgenic mice, synaptic dysfunction and cognitive impairment precedes the accumulation of pathological lesions (Oddo et al., 2003, Billings et al., 2005). However, triple-transgenic model are still based on fAD mutations which had been found in only less than 5% of AD sufferers. Most AD patients do not have any mutations in their *APP*, *PS1* and *PS2* genes.

1.2.2 The Streptozotocin intracerebroventricular rat model

As already discussed in 1.1.3, diabetes is a risk factor for AD and diabetes as well as AD share lot of similarities in molecular, biochemical and mechanistic abnormalities (Hoyer, 2004, Nicolls, 2004, Steen et al., 2005). Based on these facts, researchers developed an experimental animal model by intracerebroventricular (icv) application of streptozotocin (STZ) in order to mirror an insulin resistant brain state (Fig.2) (Lannert and Hoyer, 1998, Grunblatt et al., 2007, Salkovic-Petrisic et al., 2009). STZ [2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose] is a naturally occurring compound produced by soil bacterium *Streptomyces achromogen*s which is particularly toxic to the insulin-producing β cells of the pancreas in mammals (Eileen Dolan, 1997). It is used in medicine for treating certain cancers of the Islets of Langerhans (Murray-Lyon et al., 1968, Brentjens and Saltz, 2001) and is used in research to produce animal models for T1DM applying high doses of STZ via i.p. injections (Like and Rossini, 1976) and T2DM with i.p. injection(s) of low STZ doses (Reaven and Ho, 1991, Wang and Gleichmann, 1998, Reed et al., 2000). High dose STZ treatment destroys β cells through methylating DNA, activating polyADP-ribosepolymerase (PARP) and then depleting intracellular NAD^+ (Szkudelski, 2001). Multiple low doses of STZ could reduce the expression of the glucose transport protein 2 (GLUT2) and this reduction results in the failure of β cells to respond to glucose, and causes peripheral insulin resistance by damage of IR

signaling (Kadowaki et al., 1984, Blondel and Portha, 1989, Wang and Gleichmann, 1998).

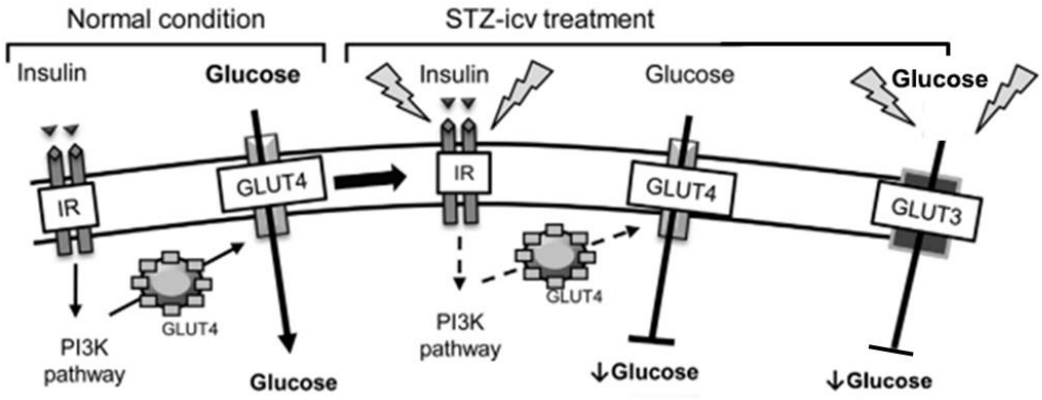


Fig.2. Insulin resistance in the brain after STZ icv treatment (modified from (Salkovic-Petrisic et al., 2013b)

Under the physiological condition, the uptake of glucose via glucose transporter GLUT4 is regulated by the insulin receptor (IR) signaling downstream the phosphatidyl inositol-3 kinase (PI-3K) pathway. IR signaling as well as GLUT3 has been found damaged by intracerebroventricular administration of streptozotocin (STZ icv).

Regarding the close relationship between diabetes and AD as well as insulin resistance in the brain after STZ icv treatment (Fig.2), STZ icv treated rats are proposed to be an animal model for sAD (Lannert and Hoyer, 1998, Grunblatt et al., 2007, Salkovic-Petrisic et al., 2009). STZ icv treatment is shown to cause deficits in learning and memory already 2-4 weeks after STZ icv injections (Mayer et al., 1990, Lannert and Hoyer, 1998, Ishrat et al., 2006, Salkovic-Petrisic et al., 2006, Khan et al., 2012, Salkovic-Petrisic et al., 2014). Intracellular Aβ42 accumulations and vascular Aβ deposits have been found in the parietal cortex 3 months after STZ icv treatment (Salkovic-Petrisic et al., 2011, Knezovic et al., 2015). Starting from 6 months up to 9 months, both intra- and extracellular primitive plaque-like Aβ42 accumulations can be detected in the parietal cortex and subhippocampal thalamic region and to a lesser extent in the hippocampus (Knezovic et al., 2015). For hyperphosphorylated tau protein, AT8 immunoreactivity (tau protein phosphorylated at Ser202/Thr205 sites) was increased in a time-dependent manner, appearing first in neocortical regions 1 month after STZ icv treatment and

spreading to the hippocampal areas (Knezovic et al., 2015). After STZ icv administration, brain glucose/energy metabolism abnormalities have been found in all hippocampal subfields, such as decreased glucose utilization (Duelli et al., 1994) and reduced glycolytic key enzymes activity (Plaschke and Hoyer, 1993), diminished adenosine triphosphate (ATP) and creatine phosphate (Lannert and Hoyer, 1998) as well as decreased IR expression (Barilar et al., 2015).. Choline acetyltransferase (ChAT) activity in the hippocampus is shown to be decreased already 1 week after STZ icv treatment, which lasts at least until 3 weeks post-injections (Blokland and Jolles, 1993). Also these alterations of the cholinergic system in the STZ icv rat model resemble biochemical changes observed in Alzheimer's disease (Sharma and Gupta, 2001, Saxena et al., 2011). Most of Alzheimer-like alterations typical for AD patients were also shown to happen in the STZ icv treated rats as summarized in table 1. Moreover, in Fig.3 a timetable with brain metabolism changes as well as AD-like pathological characteristics observed in STZ icv rats are displayed.

Table 1. Summany of the similarities between human sAD and the STZ icv rat model

	STZ icv RAT MODEL	HUMAN sAD
Behavioral		
Cognitive deficits	learning & memory ↓	dementia
Biochemical		
Glucose/energy	metabolism ↓	metabolism ↓
Acetylcholine transmission	↓	↓
Oxidative stress	↑	↑
Mitochondrial	dysfunction	dysfunction
Insulin	altered I/IR signalling cascade	altered I/IR signalling cascade
Structural		
Tau protein	tau hyperphosphorylation	neurofibrillar tangles
Aß	Aß accumulation	amyloid plaques
Synaptic dysfunction	?	↑
Brain atrophy	?	↑
Neuronal plasticity		
e.g. Adult neurogenesis	?	≈

Note: Summarized from (Salkovic-Petrisic et al., 2006, Grunblatt et al., 2007, Salkovic-Petrisic et al., 2009, Salkovic-Petrisic et al., 2011, Salkovic-Petrisic et al., 2013a).

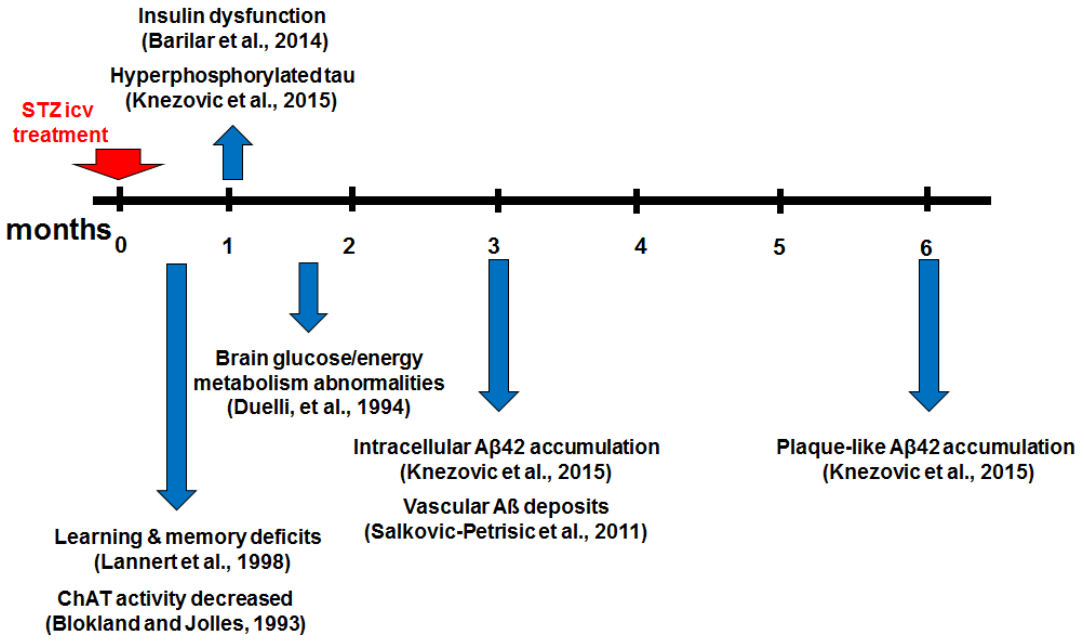


Fig.3. Timetable of pathological characteristics in STZ icv rats

In summary, the STZ rat model represents a feasible experimental approach to explore the underlying cellular and molecular mechanisms involved in the initial and late stages of sAD pathology.

1.3 Adult neurogenesis

The phenomenon of adult neurogenesis (AN), the generation of functional neurons in the adult brain, has been shown to be active not only in most of the animals tested, but also in humans (Altman and Das, 1965, Eriksson et al., 1998, Wiskott et al., 2006). Neural stem cells (NCSs) are responsible for the generation of new neurons and reside in two main locations in the adult mammalian brain: the subventricular zone (SVZ) of the lateral ventricle, and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus (Fig.4) (Ming and Song, 2005, von Bohlen und Halbach, 2011). In other brain regions than the SVZ and the SGZ the birth of new neurons appears to be extremely limited in the adult mammalian. Only after pathological stimulation like brain insults, AN appears to occur in these other regions (Ming and Song, 2005). But even if we are always mentioning the generation of new neurons, it cannot be denied that the proliferation of NSCs also results in the production of a small number of astrocytes [1-13% ; (Malberg et al., 2000, van Praag et al., 2002, Schmitt et al., 2007)] and oligodendrocytes (Bribian et al., 2012).

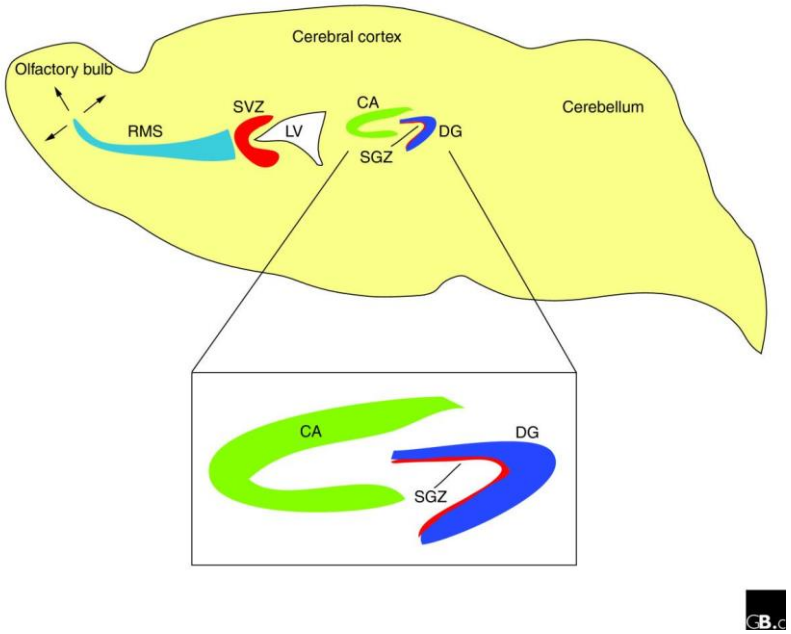


Fig.4. Neurogenic zones in the adult mouse brain (Pozniak and Pleasure, 2006).
Newborn cells are continually born throughout whole life in predominantly two regions of the brain: the subventricular zone (SVZ) of the lateral ventricle (LV), where newborn neurons migrate to the olfactory bulb via the rostral migratory stream (RMS); and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus, where most of these newborn cells passing through several differentiation stage and finally become integrated into the local neuronal network as granule cells. Newly generated granule cells receive synaptic input from entorhinal cortex and extend axons along the mossy fibers tract that pass through the hilus and form synapses with pyramidal neurons from cornu ammonis (CA) section 3, another part of hippocampus.

From a functional point of view, hippocampal AN plays an important role in several brain functions such as learning and memory processes (Aimone et al., 2010) and stress responses (Snyder et al., 2011). Some mutant mice with decreased SGZ neurogenesis have impaired performance on hippocampus-dependent learning tasks, such as methyl-CpG binding protein 1 ($mbd1^{-/-}$) mice (Zhao et al., 2003), fibroblast growth factor receptor 1 ($fgfr1^{flox/-}$) mice (Zhao et al., 2007), neurotrophin-3 ($NT\text{-}3^{flox/-}$) mice (Shimazu et al., 2006). Using different models in which AN was chemically [systemic treatment with antimitotic drugs like methylazoxymethanol acetate (MAM)] (Shors et al., 2001), physically (x-ray irradiation) (Saxe et al., 2006) or genetically [ablation of glial fibrillary acidic protein (GFAP)-positive neural progenitor cells] (Saxe et al., 2006) inhibited, impaired hippocampus-dependent learning and memory ability has been found. Besides other possible influcences different performances in

the behavioral task could be related to the different age of mice analysed. For example, Raber and colleagues found that 2 months old mice with x-ray radiation in the hippocampus exhibited impaired spatial learning and memory in the Barnes maze but not in the Morris water maze (Raber et al., 2004). However, the study from Rola and colleague showed that 21-day-old mice which received x-ray radiation showed impaired spatial learning and memory performance in the Morris water maze but not in the Barnes maze (Rola et al., 2004). Additionally, the maturation stage of AN on learning and memory is revealed by Deng and coworkers that mice with a reduced population of adult-born DG neurons at the immature stage were deficient in forming robust, long-term spatial memory and displayed impaired performance in extinction tasks (Deng et al., 2009). The cascade of events linking the birth of new neurons to learning processes is unclear, as is the way in which new neurons are integrated into and/or removed from behaviorally relevant hippocampal neural networks. First insights into these issues were gained from a study of Dupret and co-workers on the effect of spatial learning in the Morris water maze on cell birth and death in the rodent hippocampus. This work revealed that spatial learning is associated with three different events: learning promoted survival of relatively mature neurons, apoptosis of more immature cells, and finally, proliferation of neural precursors (Dupret et al., 2007).

Recently, more and more studies of AN in memory formation have been focused on a specific function - pattern separation, which is a process of making similar representations more distinct (Snyder and Cameron, 2012). Both, mice with ablated neurogenesis due to focal x-ray radiation and mice with targeted lentiviral expression of dnWnt show impaired spatial discrimination for similar but not distinct spatial locations (Clelland et al., 2009). Until now, we only know little about the function of AN, so that future studies should highlight the precise functional relevance of newly born neurons.

1.3.1 Different stages of AN

AN is a complex multi-stage process during which proliferating stem cells as well as the differentiation and migration of their newborn progeny happens (Ming and Song, 2005). From which cells NSCs originate is still on debate and several cell types have been suggested to be the real NSCs including migratory neuroblasts with large nucleus co-expressing β–tubulin (Type A) (Bonfanti and Theodosis, 1994, Lois et al., 1996), astrocytes expressing GFAP (Type B) (Doetsch et al., 1999), transit amplifying cells (Type C), and multiciliated ependymal cells (Type D) (Johansson et al., 1999). The most popular view is that astrocyte-like cells which co-express GFAP are the real neural stem cells (Alvarez-Buylla and Lim, 2004). In the SGZ, several researchers suggested to divide AN in five stages which begin with a subset GFAP positive cells (Fig.5) (Seri et al., 2001). These cells, their cell bodies locate in the SGZ and have radial processes going through the granule cell layer (GCL) and short processes extend along the border of the GCL and hilus (Ming and Song, 2005). In the end of this stage these cells give rise to transit amplifying cells, and then get into stage 2 when transit amplifying cells differentiate into immature neurons during about 4 days (Ming and Song, 2005). Newly generated neurons migrate only a short distance to the inner GCL to become granule neurons (Ming and Song, 2005). In stage 4, immature neurons extend their axonal projections along mossy fiber pathways to the CA3 pyramidal cell layer and their dendrites toward the molecular layer (ML) (Ming and Song, 2005). Then the immature neurons receive inputs e.g. from the entorhinal cortex and send output to the CA3 region and form connections with CA3 pyramidal cells (Ming and Song, 2005). It takes about 2 months for the maturation of newborn cells in the hippocampus (Piatti et al., 2011).

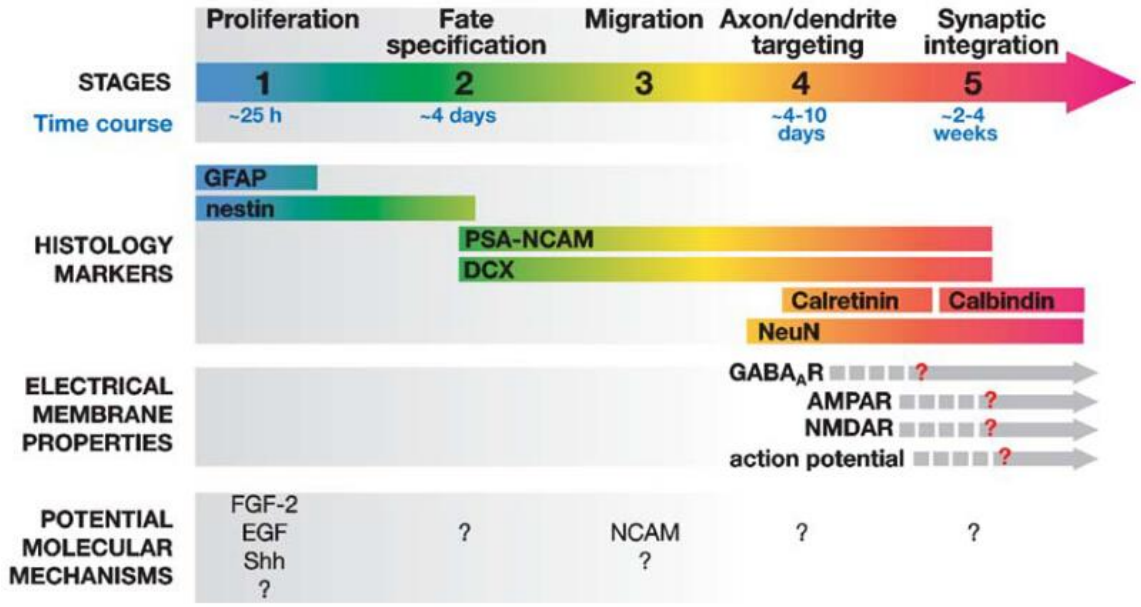


Fig.5. Different stages of AN in dentate gyrus (Ming and Song, 2005)
Adult neurogenesis in dentate gyrus is suggested to be divided into five stages. Stage 1 (blue). Proliferation: the cell bodies of neural stem cells (NSCs) locate in the SGZ and have radial processes going through the granule cell layer (GCL) and short processes extend along the border of the GCL and hilus. In this stage, these cells could be stained with GFAP antibody and nestin antibody and regulated by basic fibroblast growth factor (FGF-2), epidermal growth factor (EGF), sonic hedgehog (Shh). Stage 1 lasts about 25h and in the end of stage 1 NSCs give rise to transit amplifying cells. Stage 2 (green). Fate specification: transit amplifying cells differentiate into immature neurons during about 4 days. In this stage, newborn cells start to express markers like polysialylated-neural cell adhesion molecule (PSA-NCAM) and doublecortin (DCX). Stage 3 (yellow). Migration: Immature neurons migrate a short distance into the inner granule cell layer. Stage 4 (orange). Axon/dendrite targeting: immature neurons extend their axonal projections along mossy fiber pathways to pyramidal cells of the cornu ammonis region 3 (CA3) and their dendrites toward the molecular layer (ML). In this stage, cells express calretinin for a short time and start to express the neuron marker neuronal nuclei (NeuN). This stage takes about 4-10 days. Stage 5 (purple). Synaptic integration: newborn cells receive inputs e.g. from the entorhinal cortex and send output to the CA3 region and form connections with CA3 pyramidal cells. In this stage, the cells start to express calbindin and form action potentials.

1.3.2 The phenomenon of adult neurogenesis is tightly regulated

AN in the hippocampus is a dynamic process that could be regulated at different stages by both, intrinsic and extrinsic factors (Fig.5) (Ming and Song, 2005). Aging is the most important factor influencing AN, as it declines rapidly with aging (Kuhn et al., 1996). Chronic stress elicits a negative regulation of AN (Gould et al., 1997) while

acute stress enhances hippocampal AN (Kirby et al., 2013). On the other hand, increased environmental complexity (Kempermann et al., 1997) and physical activity (Gould et al., 1999, van Praag et al., 1999) leads to an increase of AN. Most brain injuries lead to increased NSC proliferation in the SGZ and induce newborn cells to migrate to the injury site, such as ischemic brain injury (Arvidsson et al., 2002), mechanical lesions (Gould and Tanapat, 1997) and prolonged seizures (Parent and Lowenstein, 1997).

Intrinsic hormones, for example, adrenal steroids may contribute to the aging-associated AN decline (Cameron and Gould, 1994). Other hormones, including estrogen (Tanapat et al., 1999), thyroid hormone (Desouza et al., 2005), and progesterone (Shingo et al., 2003) also regulate AN. Beside hormones, many neurotransmitters were also found to influence AN, including neurotransmitters like dopamine, serotonin (5-hydroxytryptamine, 5-HT), acetylcholine, gamma-Aminobutyric acid (GABA), glutamate, and the gaseous neurotransmitter nitric oxide (NO) (Ming and Song, 2005). Experimental depletion of dopamine in rodents decreased the cell proliferation and survival of NSCs in SGZ (Hoglinger et al., 2004) and a *postmortem* study showed that number of NSCs was reduced in SGZ in Parkinson disease patients (Borta and Hoglinger, 2007). An *in vitro* study found that dopamine increased the number of NSCs via D1-like but not D2-like receptor as D1-like receptor antagonist R-SCH23390 but not D2-like receptor antagonist sulpiride could attenuate dopamine induced increase of NSCs (Takamura et al., 2014). Interestingly, another dopamine receptor antagonist haloperidol could increase cell proliferation in a dopamine D2 receptor-dependent manner (Kippin et al., 2005). 5-HT is shown to have a stimulating effect on AN in the DG (Brezun and Daszuta, 2000) and mice lacking the 5-HT transporter which exhibit higher 5-HT levels in the synaptic cleft also have higher levels of hippocampal AN (Schmitt et al., 2007, Karabeg et al., 2013). Acetylcholine positively influences stem cell proliferation in the SGZ, whereas cholinergic pathology *per se* may have a detrimental influence on neurogenesis (Perry et al., 2012). In SVZ, ChAT expressing neurons have been

found to exhibit an activity-dependent control of NSCs` proliferation (Paez-Gonzalez et al., 2014). Glutamate promotes proliferation of SVZ-derived progenitor cells *in vitro* (Brazel et al., 2005), whereas in the DG activation of N-methyl-D-aspartate receptor (NMDA receptor), a glutamate receptor, results in diminished hippocampal AN and treatment with NMDA receptor antagonists increases the birth of neurons (Cameron et al., 1995). GABA released by parvalbumin expressing interneurons depolarizes progenitor cells and inhibits proliferation via GABA-receptor activation (Song et al., 2012). Under physiological conditions, NO inhibits stem cell proliferation (Packer et al., 2003), but after focal cerebral ischemia the expression of inducible nitric oxide synthase (iNOS) which catalyzes the production of NO could stimulate AN (Zhu et al., 2003).

Growth factors also play an important role in the regulation of AN, such as fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF) (Kuhn et al., 1997), brain-derived neurotrophic factor (BDNF) (Waterhouse et al., 2012), vascular endothelial growth factor (VEGF) (Cao et al., 2004), insulin (Arsenijevic et al., 2001) and IGF-1 (Aberg et al., 2000). Several drugs regulate AN including antidepressants (Malberg et al., 2000) and Lithium (Chen et al., 2000) (up-regulation), opiates (Eisch et al., 2000) and methamphetamine (Teuchert-Noodt et al., 2000) (down-regulation).

1.3.3 Energy metabolism influence proliferation and differentiation of neural stem cells

That energy metabolism, especially glucose metabolism, could have an influence on AN is substantiated by microarray studies performed by Geschwind et al. (Geschwind et al., 2001) and Ivanova et al. (Ivanova et al., 2002). They showed that proliferation of NSCs and the transition from a newborn undifferentiated cell to a differentiated neuron, astrocyte, or oligodendrocyte is accompanied by many changes of the expression of metabolism related genes such as *IGF binding protein 3*, *cytochome c*

oxidase (Geschwind et al., 2001), *enolase 1*, *acetyl-coenzyme A synthetase 1* and *pyruvate dehydrogenase E1 alpha subunit* (Karsten et al., 2003).

The Insulin/IGF-1 signaling pathway is central in coordinating energy taking and expenditure (Rafalski and Brunet, 2011). Although IGF-1 mRNA was detected in all regions of the neonatal brain, there is considerable regional variation in the level of expression with highest expression levels in regions where neurogenesis persists after birth such as the hippocampus and the subventricular zone (Bartlett et al., 1991). The association of AN and insulin signaling pathways is substantiated by the fact that adult NSCs express the IGF-1 receptor (Aberg et al., 2003). Both, insulin and IGF-1, have mitogenic effects (Hofmann et al., 1989). For insulin, it is shown that it promotes the function of FGF 2 and activates stem cell proliferation (Arsenijevic et al., 2001). Han and colleagues found that high insulin concentration could induce NSCs differentiation into neurons (Han et al., 2008). For IGF-1, Aberg and coworkers found that high doses could promote NSCs proliferation and a low IGF-1 dose gives an instructive signal to cultured NSCs to become neurons (Aberg et al., 2003). That is why it is not surprising that the size of the brain in mice overexpressing IGF-1 are 55% larger than those of controls (Carson et al., 1993). Interestingly, IGF-1 could stimulate NSCs differentiation into oligodendrocytes in an animal model (Ye et al., 1995) and in cell culture (Hsieh et al., 2004).

Several signaling pathways downstream of the insulin/IGF-1 receptor may be involved in the proliferation of NSCs and the differentiation of newborn cells (Fig.6). IGF-1 is known to promote cell proliferation by activating the phosphoinositide 3-kinase/Protein kinase B (PKB, also called Akt) (PI3K/Akt) pathway (Blakesley et al., 1996). IGF-1 enhances the phosphorylation of Akt in NSCs and using 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one, an inhibitor for PI3K, could inhibit the IGF-I-induced survival of NSCs (Kalluri et al., 2007). Mice with brain-specific deletion of the *pten* gene, a negative regulator for PI-3K/Akt signaling, exhibit an increased number of embryonic NSCs (Groszer et al., 2001). Furthermore,

PI3K/Akt signaling also is shown to regulate the differentiation of neuroal precursor cells by IGF-1 (Otaegi et al., 2006). The PI3K-Akt-FoxO (Forkhead box protein O) signaling pathway, a branch of PI3K/Akt signaling, plays a prominent role in NSC proliferation and renewal. FoxO-deficient mice show initial increased brain size and proliferation of neural progenitor cells during early postnatal life, followed by precocious significant decline in the NSC pool and accompanying neurogenesis in adult brains (Paik et al., 2009). PI3K-Akt-mTOR signaling, another branch of PI3K/Akt pathway, may also play a role in the regulation of NSCs as inhibition of mTOR (mammalian target of rapamycin)-containing complexes by rapamycin reverses insulin-dependent enhancement of neurogenesis (Han et al., 2008).

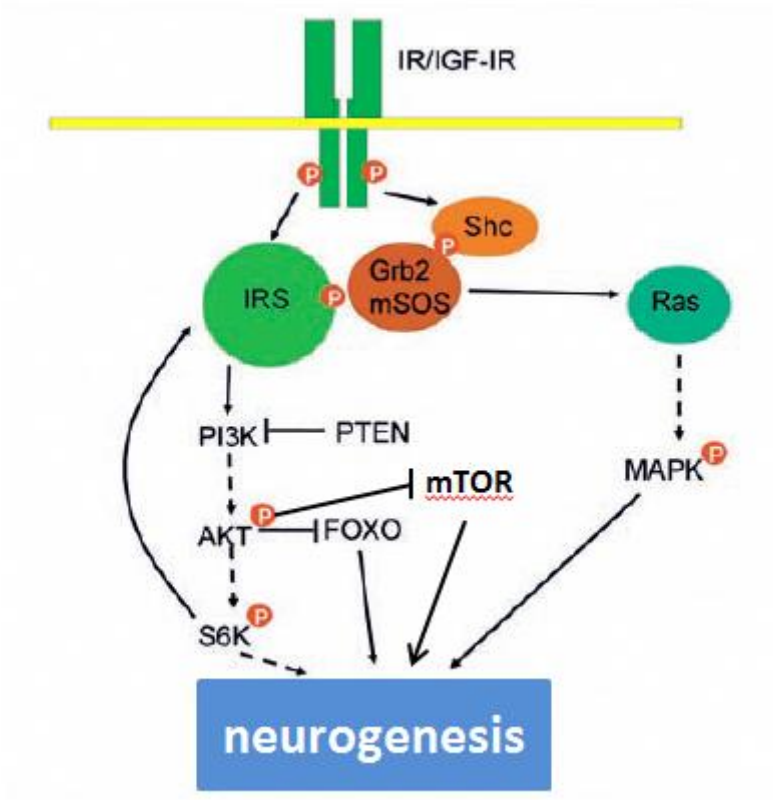


Fig.6. Potential pathways by which insulin/IGF signalling can regulate neurogenesis [modified from (Bateman and McNeill, 2006)]

Abbreviation: AKT: also called PKB (Protein kinase B); FOXO: Forkhead box protein O; Grb2: Growth factor receptor-bound protein 2; IR: Insulin receptor; IGF-1R: Insulin-like growth factor-1 receptor; IRS: Insulin receptor substrate; mSOS: mammalian homologues of son of sevenless; MAPK: Mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3-kinase; PTEN: phosphatase and tension homologue; Ras: Rat sarcoma; shc: Src homology 2 domain containing transforming protein.

1.3.4 Different methods to analyze adult neurogenesis

In vivo studies commonly use thymidine analogs which could incorporate DNA of dividing cells during the S-phase of the cell cycle to monitor cell proliferation (Taupin, 2007). Decades ago, [3H]-thymidine autoradiography had been used for AN studies (Sidman et al., 1959, Kaplan and Hinds, 1977), but now BrdU (5-bromo-2'-deoxyuridine) has replaced it because of many advantages as it can be quickly detected via immunohistochemistry and combined with other antibodies it can be used to perform immunofluorescence double stainings (Gratzner et al., 1975, Gratzner, 1982, Nowakowski et al., 1989, Magavi and Macklis, 2008). Since BrdU can cross the blood-brain barrier (BBB), it may be delivered to the brain by intracerebro-ventricular (icv), intraperitoneal (ip), intravenous (iv) injection, or even orally (Taupin, 2007). Different BrdU labeling paradigms are used to determine cell proliferation, migration, maturation as well as the fate of newborn cells by varying the period between the BrdU injection(s) and the sacrifice of the animals (Miller and Nowakowski, 1988, Taupin, 2007). A short-term survival of animals (between 1 and 3 hours) after BrdU application could be used for the detection of NSCs proliferation (Palmer et al., 2000). The fate of newly generated neuronal cells and maturation stages can be determined with a somehow long-term survival of the animals after BrdU application up to ca. 4 weeks (Kuhn et al., 1996, Duman et al., 2001, Taupin, 2007). Via immunofluorescence double stainings and the use of antibodies detecting BrdU and other cell-type specific marker [such as neuronal nucleus (NeuN), adenomatous polyposis coli (APC) as well as GFAP] the cellular fate of a newborn cell can be verified.

Besides BrdU incorporation, staining with different markers has been proposed to assay different stages of AN. Proliferating NSCs express proteins such as Ki-Kiel67 (Ki67), mini-chromosome maintenance proteins 2 (MCM2). MCM2 is one of the highly conserved mini-chromosome maintenance proteins that are involved in the initiation of eukaryotic genome replication and is commonly used as a marker for

proliferating cells (Kneissl et al., 2003). Newborn cells with a neuronal fate express markers such as neurogenic differentiation (NeuroD), doublecortin (DCX) and neuronal nucleus (NeuN). NeuroD, a basic helix-loop-helix (bHLH) transcription factor, is excusively expressed in mitotic neuroblasts (Miyata et al., 1999). The microtubule binding protein DCX labels mitotic active neuroblasts as well as postmitotic immature neurons, which extend their dendrites into the molecular layer of the DG (Brown et al., 2003). NeuN is a neuronal nuclear antigen that is commonly used as a marker for mature neurons (Mullen et al., 1992).

Living organisms are extremely complex systems and it is hard to explore their basic biological functions. In order to simplify research work, *in vitro* methods have been established for studying proliferation and differentiation signaling pathways in AN. Numerous investigators have isolated NSCs from the mouse and rat neurogenic brain regions and used a variety of mitogenic growth factors to stimulate cell proliferation, such as EGF and FGF-2 (Gage et al., 1995, Gritti et al., 1996, Svendsen et al., 1997, Gritti et al., 1999). Under different conditions, NSCs grow as monolayers or as so-called neurospheres. When mouse CNS-derived NSCs were plated on uncoated plates and cultured in medium containing EGF, FGF-2 and heparin, the cells form a monolayer of cells (Ray and Gage, 2006). However, if the NSCs were exposed to EGF or FGF-2 alone or EGF+FGF-2 (together) without heparin, they form neurospheres (Ray and Gage, 2006). For rats, in the presence of FGF-2, EGF and heparin, cells proliferate within neurospheres, but if these cells were plated on uncoated plates in serum and then let them grow in N2+FGF-2 medium, they will form a monolayer (Ray and Gage, 2006). NSCs proliferation can be detected by counting the number of neurospheres, measuring the size of neurospheres or analyzing the absolute number of proliferating cells by the incorporation of BrdU with subsequent BrdU-Immunohistochemistry (Stafford et al., 2007, Karkkainen et al., 2014). Different markers could be used like in *in in vitro* studies to detect the fate of NSCs, such as neuron-specific class III beta-tubulin (Tuj-1) and GFAP (Karkkainen et al., 2014).

1.4 Adult neurogenesis in Alzheimer's disease

There is a big controversy in literature regarding the possible involvement of AN in AD etiopathology (Table 2). The study of Jin and coworkers demonstrated increased AN (marked by DCX) in hippocampus of AD patients (Jin et al., 2004). However, Boekhorn and coworkers couldn`t detect significant changes in cell proliferation (marked by Ki67) in the human DG of AD brains compared to controls (Boekhoorn et al., 2006).

Table 2. Neurogenesis in human *postmortem* tissues and in animal models of AD

	Neurogenesis assessment		References
post mortem brain			
	↓	in the SVZ	Ziabreva et al. 2006
	↑	in the DG	Jin et al. 2004a
	→	in the DG	Boekhoorn et al., 2006
transgenic animals			
APP23 mice	↑	in the DG	Ermini et al. 2008
TgCRND8 mice	↓	in the DG	Herring et al., 2009
APP/PS1 mice	↓	in the DG	Demars et al.2010
$APP_{Sw,Ind}$ mice	↑	in the DG	Jin et al. 2004a;
3xTg-AD mice (APP, PS1,tau)	↓	in the DG	Rodriguez et al. 2008
Tg2576	↓	in the DG	Haughey et al.2002
PDAPP mice	↓	in the DG	Donovan et al. 2006
in vivo and in vitro exposure to Aβ1-42	↑	in the SVZ	Sotthibundhu et al. 2009

Note: DG: Dentate gyrus; SVZ: Subventricular zone

The tissue samples used in the Jin et. al. study were from senile AD patients, which

points to increased AN in patients with sAD compared to normal group (Jin et al., 2004). However, the samples that showed no significant changes in AN were from presenile AD (fAD) cases (Boekhoorn et al., 2006). The controversy of AN changes in above mentioned studies may due to different types of disease analysed, fAD and sAD.

Using animal models for studying AN is an alternative. Most animal models used for studying AN are based on the amyloid cascade hypothesis by promoting Aβ plaques production in the brain. However, AN studies using transgenic mice which overexpress hAPP and/or PS1/2 could also not answer this question of a possible involvement of AN in the etiopathogenesis of AD (Marlatt and Lucassen, 2010, Mu and Gage, 2011). Just to give some examples: Some research groups revealed impaired AN, e.g. in the TCRND8 (TG) mice carrying the human APP 695 (hAPP) including the “Swedish” and “Indiana” mutations (hAPPSw,Ind mice) controlled by the Syrian hamster prion promoter (Herring et al., 2009), whereas other studies using APP23 mice with Swedish mutation (Ermini et al., 2008) as well as PDGF-APPSw,Ind mice, which also express the Swedish and Indiana APP mutations but under the control of a PDGF promotor (Jin, et al., 2004a) revealed increased AN in the hippocampus.

Many molecules involved in Aβ production and metabolism have been found to be linked to AN (Mu and Gage, 2011). Cleavage of APP by β- and γ-secretase as amyloidogenic pathway yields the APP intracellular domain (AICD) fragment in addition to Aβ, but the activity of α- and γ-secretase as a part of the non-amyloidogenic pathway generates the soluble fragment of APP (sAPPα) (Mu and Gage, 2011). Both, AICD and sAPPα, could influence AN. AICD expression decreases hippocampal progenitor cell proliferation and survival (Ghosal et al., 2010) and sAPPα protects neurons and AN (Han et al., 2005). Mutations of PS1 which is a component of γ-secretase decrease the production of new neurons (Wen et al., 2004). For Aβ itself, the role in regulating AN is complex. Low concentrations of

monomeric Aβ exerts a neurotrophic function on undifferentiated hippocampal neurons (Yankner et al., 1990) and stimulates primary neural progenitor cells` proliferation (Chen and Dong, 2009). A low micromolar concentration of oligomeric Aβ increased the proliferation of adult NSCs and high concentration was shown to be neurotoxic (Heo et al., 2007).

1.5 Hippocampus functional dissociation

The hippocampus, one of the neurogenic brain region in the adult brain, is a heterogeneous brain area, extends dorso-ventrally from the septal nuclei of the basal forebrain to the temporal lobe (Fig.7) (Tanti and Belzung, 2013). Along the septo-temporal (also called dorso-ventral, anterior-posterior or rostro-caudal) axis, the hippocampus is shown to have different functions (Tanti and Belzung, 2013, Strange et al., 2014). It's important to note that septal hippocampus in rodents represents the posterior hippocampus in humans and rodent temporal hippocampus represents the anterior hippocampus in humans (Fig.7) (Strange et al., 2014). The septal part of the hippocampus primarily plays a role in spatial learning based on the following evidences. Moser and colleagues found that septal hippocampal lesions consistently impaired spatial learning in a Morris water maze and the degree of impairment correlated with the lesion volume (Moser et al., 1993). In contrast, lesions of the temporal hippocampus spared both the rate and the precision of learning (Moser et al., 1993). The study of Vann and colleagues suggests that increased c-fos activation, an indirect marker of neuronal activity, was found in both septal and temporal hippocampus, but relative increase was significantly greater in the septal part after performing spatial tasks (Vann et al., 2000). The temporal hippocampus is more integrated into networks dealing with stress and emotion (Henke, 1990, Fanselow and Dong, 2010) and temporal hippocampal but not septal hippocampal lesions enhanced cold/restraint stress ulcers

(Henke, 1990). Temporal hippocampus seems to be preferentially involved in anxiety-like behavior studied in various tests like tone fear conditioning (Maren and Holt, 2004), social interaction test (McHugh et al., 2004), as well as contextual fear conditioning (Maren and Holt, 2004). Using brain tissue oxygen amperometry, McHugh et al. also confirm that septal hippocampus is associated with spatial processing and temporal hippocampus is associated with anxiety-related behavior (McHugh et al., 2011). These difference may due to the fact that septal hippocampus primarily receives afferents from the retrosplenial and from the anterior cingulate cortex (Cenquizca and Swanson, 2007), however, the temporal hippocampus receives inputs from areas linked with olfactory processing (Cenquizca and Swanson, 2007) and has reciprocal connections with the amygdala (Thierry et al., 2000).

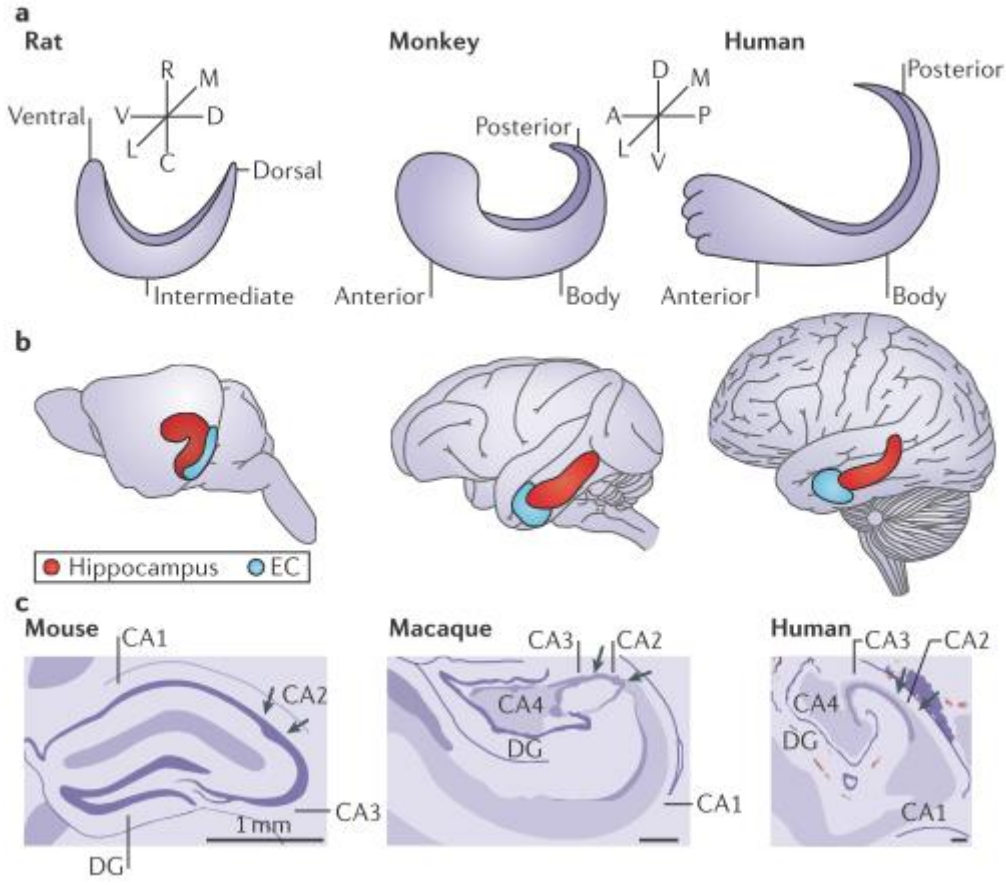


Fig.7. Schematic view of the orientation of the hippocampal axis in rats, macaque monkeys and humans (Strange et al., 2014)

The hippocampus is a heterogeneous brain area, extends dorso-ventrally from the septal nuclei of the basal forebrain to the temporal lobe. Septal hippocampus (also called dorsal hippocampus) in rodents represents the posterior hippocampus in humans and monkeys, and rodent temporal hippocampus (also called ventral hippocampus) represents the anterior hippocampus in humans and monkeys.

Interestingly, the characteristics of AN are also found to be different in the septal and temporal hippocampus (Piatti et al., 2011). The DG in septal hippocampus displays higher levels of basal network activity and faster rates of newborn neuron maturation than the temporal region (Piatti et al., 2011).

In recent years AD researchers started to analyze septal and temporal hippocampus separately (Fuster-Matanzo et al., 2011, Yiu et al., 2011). Fuster-Matanzo and coworkers demonstrated that increased neurodegeneration in consequence of GSK3ß overexpression in mice was detected exclusively in septal hippocampus (Fuster-Matanzo et al., 2011). In addition, locally and acutely increasing cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) function in the CA1 of septal hippocampus in TgCRND8 mice was sufficient to restore function in neuronal structure, network activity as well as learning and memory (Yiu et al., 2011).

1.6 Objective of the thesis

As STZ icv treated rats mirror an insulin resistant brain state and exhibit cognitive deficits resembling memory deficits in AD patients, they are proposed to be an animal model for sAD. Until now only very few studies have been reported on AN in STZ icv rat model and they analyzed AN exclusively in brains up to 3 weeks following STZ icv administration (Arabpoor et al., 2012, Qu et al., 2012). In this study, our goal was to examine short-term (1 month) and long-term (3 months) effects of STZ icv treatment on adult hippocampal neurogenesis. We performed a quantitative

immunohistochemistry study and double/triple immunofluorescence stainings using antibodies detecting different AN stages, e.g. proliferation of stem cells, differentiation and survival of adult-generated neurons. As it is suggested that the hippocampus is functionally different along its septo-temporal axis, we analyzed the septal and temporal part of the hippocampus separately.

In order to explore the mechanism of STZ effects on the proliferation of stem cells and the differentiation of newborn cells, I performed *in vitro* studies using cultured primary adult rat NSCs. The number and size of neurospheres in the presence of different concentrations of STZ as well as the migration of newborn differentiating cells was evaluated. Moreover, double immunofluorescence staining allowed us to detect whether STZ influences the differentiation fate of newborn cells. For the investigation of possible expression changes of molecules related to brain metabolism and/or especially to IR signaling pathways (such as GLUT1 and 3, IR, IGF-1) in consequence of STZ treatment quantitative real time polymerase chain reaction (qRT-PCR) for the detection of specific mRNAs was performed followed by immunofluorescence stainings for the detection of interesting proteins.

2. Material and Methods

2.1 Material

2.1.1 Brain tissue

Brain tissue of STZ icv treated rats – *in vivo* study. STZ icv treatment of rats for our *in vivo* study was performed by the research group of our collaboration partner Prof. Salkovic-Petrisic (Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia). Dissected and subsequently for 72 h in 4% PFA (dissolved in PBS, pH 7.5) fixed brains were then sent to the laboratory of the Department of Psychiatry, Psychosomatics and Psychotherapy of the University of Wuerzburg, Wuerzburg.

In brief, the STZ icv rat model was produced as described in the following: A total of 36 three- and five-month old male Wistar rats weighing 280–330 g were used for this study (Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia). Animals were kept in standardized cages (2-3 animals per cage) maintained under a 12/12h light/dark circle and provided with standard food pellets and water ad libitum. Experiments were carried out under the guidance of the Principles of Laboratory Animal care (NIH Publication No. 86-23, revised in 1985), conformed to Directive 2010/63/EU, and in line with the actual national law, the Animal Protection Act (NN135/06). Experiments were approved by the Ethical Committee of the University of Zagreb School of Medicine and the national regulatory body responsible for issuing ethical approval, Croatian Ministry of Agriculture (licence No. UP/I-322-01/11-01/100 to MSP for research approved by Croatian Ministry of Science, Education and Sport, project 108-1080003-0020 to MSP).

STZ icv injections were performed according to the procedure described first by Noble et al (Noble et al., 1967) and applied afterwards by Salkovic-Petrisic and

co-workers (Salkovic et al., 1995, Grunblatt et al., 2007, Salkovic-Petrisic et al., 2011, Salkovic-Petrisic et al., 2013b). Briefly, rats were given general anaesthesia [chloralhydrate, 300 mg/kg ip)], and STZ (1.5 mg/kg, dissolved in 0.05M citrate buffer pH 4.5) was injected into the left and right lateral ventricle (2 µl per ventricle) of the rat brain. STZ treatment was repeated on the 3rd day after the first injection. STZ was dissolved in 0.05 M citrate buffer, pH 4.5. Control animals received icv an equal volume of vehicle (citrate buffer). Additionally, a group of control animals was left intact without any icv treatment. Experimental rats were randomly divided into five experimental groups (see Fig. 8) with six to eight animals per group: Group 1 (control), intact rats without any icv treatment; Group 2 (vehicle 1 month), vehicle icv-treated rats at the age of 5 months; Group 3 (STZ 1 month), STZ icv-treated rats at the age of 5 months; Group 4 (vehicle 3 months), vehicle icv-treated rats at the age of 3 months; Group 5 (STZ 3 months), STZ icv-treated rats at the age of 3 months. Irrespective of treatment, all animals were sacrificed at the age of 6 months.

To label cells undergoing cell division, they used the exogenous marker 5-bromo-2`-deoxyuridine (BrdU), a thymidine analogue which incorporates into DNA during synthesis (Lehner et al., 2011). Each rat received ip injections of 50 mg/kg body weight BrdU (Roche Diagnostics, Mannheim, Germany; dissolved in PBS) and BrdU was applied once daily on 7 consecutive days. Rats were sacrificed 27 days after the first ip BrdU injection. The experimental timeline is provided in Fig.8.

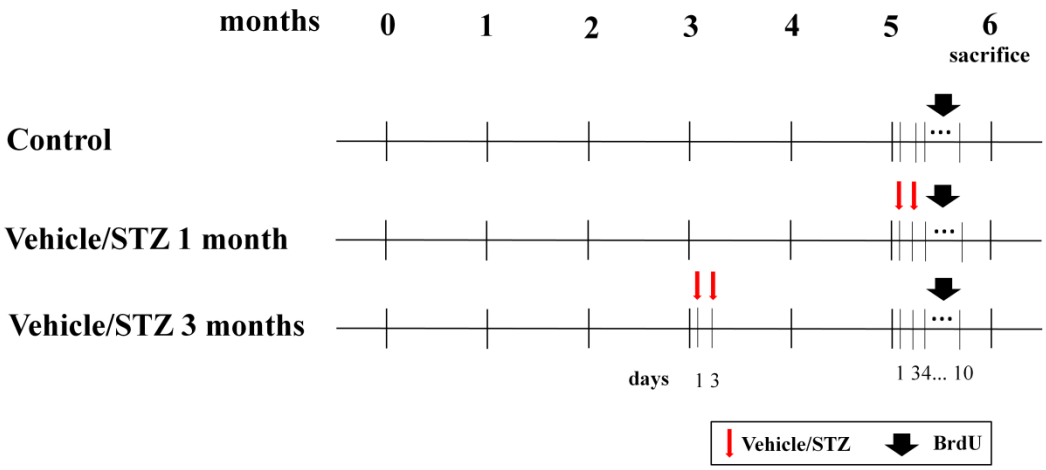


Fig.8. Experimental design

Rats were randomly divided into five groups (n=6-8 per group): two groups were STZ icv treated (STZ 1 month/STZ 3 months), two groups were vehicle icv treated (Vehicle 1 month/Vehicle 3 months) and one group was the absolute control group without any icv treatment. Vehicle/STZ 1 month: rats which received vehicle or STZ icv treatment at the age of 5 months. Vehicle/STZ 3 months: rats which received vehicle or STZ icv treatment at the age of 3 months. Rats were given general anesthesia (intraperitoneal (ip) injections of chloralhydrate 300 mg/kg body weight), and then STZ (1.5 mg/kg body weight) or vehicle (0.05 M citrate buffer, pH 4.5) was injected into the left and right lateral ventricle of the rat brain (indicated by a red arrow). STZ or vehicle treatment was repeated on the 3rd day after the first injection. Moreover, each rat received ip injections of 50 mg/kg body weight BrdU, which was applied once daily on seven consecutive days (indicated by an expanded black arrow). Rats were sacrificed 27 days after the first ip BrdU injection. Irrespective of treatment all animals were sacrificed with 6 months of age.

Brain tissue for the *in vitro* study. The 2 months old Wistar rats were bought from Charles River Laboratories (Sulzfeld, Germany). About 40 rats were used during our *in vitro* study. Animals were anesthetized by isoflurane and sacrificed by decapitation. Protocols fulfill the requirements of German law.

2.1.2 Antibodies

Antibodies	**Company**	**Dilution**
Goat anti-DCX	Santa Cruz	1:500
Goat anti-MCM2	Santa Cruz	1:1000
Goat anti-NeuroD	Santa Cruz	1:500
Mouse anti-APC	Calbiochem	1:200

Mouse anti-BrdU	Roche	1:400 (in vivo)/1:500 (in vitro)
Mouse anti-insulin receptor	Abcam	1:250
Mouse anti-Iba-1	Wako	1:1000
Mouse anti-Tuj-1	Abcam	1:250
Mouse anti-NeuN	Chemicon	1:250
Rat anti-BrdU	Serotec	1:300
Rabbit anti-GFAP	Dako	1:1000 (in vivo)/1:500 (*in vitro*)
Rabbit anti-Glucose transporter 3	Abcam	1:500
Rabbit anti-Nestin	Abcam	1:250
Alexa 350 donkey anti-rabbit antibody	Life technologies	1:500
Alexa 488 donkey anti-rat antibody	Life technologies	1:500
Alexa 555 donkey anti-mouse antibody	Life technologies	1:500
Biotinylated anti-goat IgGs antibody	Vector laboratories	1:1000
Biotinylated anti-mouse IgGs antibody	Vector laboratories	1:1000

2.1.3 Kits

Kits	**Source**	**Application**
3,3'-Diaminobenzidine (DAB) substrate kit	Roche	IHC
First strand cDNA synthesis kit With the following component: a: 5 × iScript reaction mix b: iScript reverse transcriptase c: Nuclease-free water	Bio-Rad	Real time PCR
NeuroCult™ NS-A Differentiation Kit (Rat) With the following	Stemcell	Cell culture

component: a: Neurocult NS-A differentiation supplement b: Neurocult NS-A basal medium		
NeuroCult™ NS-A Proliferation Kit (Rat) With the following component: a: Neurocult NS-A proliferation supplement b: Neurocult NS-A basal medium	Stemcell	Cell culture
Papain Dissociation System With the following component: a: Earle's Balanced Salt Solution (EBSS) b: Papain c: Deoxyribonuclease I (Dnase I) d: Ovomucoid protease inhibitor with bovine serum albumin	Worthington	Cell culture
Qiagen RNeasy Kit	Qiagen	Real time PCR
SYBR Select Master Mix for CFX	Thermo Fisher Scientific	Real time PCR
VECTASTAIN Elite ABC Kit	Vector laboratories	IHC

2.1.4 Primers

Primer	Sequence	Manufacturer	Source
GAPDH forward	**5'-tcaccaccatggagaaggc-3'**	**Metabion**	**(Bonefeld et al., 2008)**
GAPDH reverse	**5'- gctaagcagttggtggtgca-3'**	**Metabion**	**(Bonefeld et al., 2008)**

GLUT1 forward	**5'-agtatcgtggccatctttgg-3'**	**Metabion**	**(Weisova et al., 2009)**
GLUT1 reverse	**5'-cccacgatgaagtttgaggt-3'**	**Metabion**	**(Weisova et al., 2009)**
GLUT3 forward	**5'-cgcctgattattggcatctt-3'**	**Metabion**	**(Weisova et al., 2009)**
GLUT3 reverse	**5'-tccaaaccaaagacctgagc-3'**	**Metabion**	**(Weisova et al., 2009)**
IR forward	**5'-gctgtgtaaacttcagcttctgcc-3'**	**Metabion**	**(Nakazawa et al., 2005)**
IR reverse	**5'-aatgacgtattggtgacagcca-3'**	**Metabion**	**(Nakazawa et al., 2005)**
IGF1 forward	**5'-agggcgtagttgtagaagagtttcc-3'**	**Metabion**	**(Steen et al., 2005)**
IGF1 reverse	**5'-tacttgctgctgttccgagtgg-3'**	**Metabion**	**(Steen et al., 2005)**

2.1.5 Chemicals, reagents and solutions

Chemical/reagents	**Source**	**Application**
2x the saline-sodium citrate (SSC)	Sigma	IHC
4',6-diamidino-2-phenylindole (DAPI)	Molecular Probes	Marks nuclei
5-bromo-2`-deoxyuridine (BrdU)	Roche	Indicates DNA synthesis and therefore proliferative events
Basic fibroblast growth factor (FGF-b)	Peprotech	Cell culture
Bovine serum albumin (BSA)	Sigma	IHC/ICC
Dimethylformamide (DMF)	Molecular Probes	Cell culture

Dulbecco's Phosphate-Buffered Saline (D-PBS)	Gibco	Cell culture
Epidermal growth factor (EGF)	Peprotech	Cell culture
Ethylenglycol	Sigma	IHC
Fluoro mount	Dako	IHC/ICC: mounting medium
Formamid	Sigma	IHC
Glucose	Sigma	Cell culture
Glycerin	Applichem	IHC
H_3BO_3	Sigma	IHC/ICC
HCl	Applichem	IHC/ICC: adjust PH/break DNA double helix
Hydrogen peroxide	Sigma	IHC: blockade of endogenous peroxidase
KCl	Sigma	Buffer/solution preparation
KH_2PO_4	Sigma	Buffer/solution preparation
Laminin	Sigma	Coat coverslips
Isoflurane	Forene	For anesthesia
NaCl	Sigma	Buffer/solution preparation
NaH_2PO_4	Sigma	Buffer/solution preparation
Na_2HPO_4	Sigma	Buffer/solution preparation
NaOH	Applichem	Adjust PH
Neutral Protease (Dispase)	Worthington	Cell culture
Normal goat serum	Vector	IHC/ICC
Normal horse serum	Vector	IHC/ICC
Paraformaldehyde (PFA)	Sigma	Fixation of tissue/ cells
Penicillin/Streptomycin	Life technologies	Cell culture
Poly-L-Ornithine	Sigma	Coat coverslips
Tris	Sigma	IHC/ICC
Tris-EDTA (TE) buffer solution	Sigma	Real time PCR
Triton X-100	Sigma	IHC/ICC
Vitro Clud	R.Langenbrinck	IHC: mounting medium

2.1.6 Buffer and media

Buffer/medium	**Source**	**Application**
0.1 M borate buffer	Dissolve 3.09 g of boric acid (H_3BO_3) in 500 mL of water. The pH of the final solution will be 8.5	IHC
0.1 M phosphate buffer (PB)	Add 3.1 g of $NaH_2PO_4 \bullet H_2O$ and 10.9 g of Na_2HPO_4 (anhydrous) to distilled H_2O to make a volume of 1 L. The pH of the final solution will be 7.4. This buffer can be stored for up to 1 month at 4°C	IHC/ICC: Buffer/solution preparation
1x Phosphate buffered saline (PBS)	Dissolve the following in 800 ml distilled H_2O: 8 g of NaCl; 0.2 g of KCl; 1.44 g of Na_2HPO_4; 0.24 g of KH_2PO_4. Adjust pH to 7.4. Then adjust volume to 1L with distilled H_2O	IHC/ICC
1x Tris buffered saline (TBS)	0.15 M NaCl and 0.1 M Tris-HCl, pH 7.5	IHC/ICC
4% PFA	Pour 40 g PFA into a flask+500 ml of 1x PBS, and begin to stir. Place a thermometer into the solution to obtain continuous readings of temperature in the solution. Gradually start heating, but do not allow temperature to rise above 60°C. After solutions cleared up, add the remaining 500 ml 1x PBS PBS solution. Then filter the solution and adjust the pH of filtered solution to 7.5	Fixation of cultured cells and tissue
Blocking buffer I	5% normal goat (or horse) serum, 0.25% Triton X-100 and 2% BSA in TBS, pH 7.5	IHC/ICC
Blocking buffer II	2% normal goat (or horse) serum, 0.25% Triton X-100 and 2% BSA in TBS, pH 7.5	IHC/ICC
Sodium Citrate Buffer	Add 2.94 g Tri-sodium citrate to 1000 ml distilled H_2O to make a volume of 1 L. The pH of the final solution will be 8.5. This buffer can be stored for up to 3 month at 4°C	Antigen retrieval

Cryoprotectant solution	30 ml ethylenglycol and 25 ml glycerin, 45 ml 1x PBS	Long-term storage of brain sections at -20°C
DAPI stock Solution	10 mg DAPI, 2 ml DMF, mix to dissolve. Aliquot and store in -20°C	Immunofluorescence stainings
Differention cell culture medium	5 ml Neurocult NS-A differentiation supplement + 45 ml Neurocult NS-A basal medium + 500 µl penicillin/streptomycin	Cell culture
EGF stocking solution	50 µg EGF diluted in 5 ml PBS and 0.1% BSA and stored as aliquots at -20^{o}C	Cell culture
FGF stocking solution	50 µg FGF-b diluted in 5 ml PBS and 0.1% BSA and stored as aliquots at -20^{o}C	Cell culture
Inhibitor stock solution	32 ml EBSS reconstituted in a vial of ovomucoid protease inhibitor (10 mg) with bovine serum albumin (10 mg)	Cell culture
Inhibitor working solution	2.7 ml EBSS + 300 µl Inhibitor stock solution	Cell culture
Proliferation Cell culture medium	5 ml Neurocult NS-A proliferation supplement + 45 ml Neurocult NS-A basal medium +10 µl EGF stocking solution+5 µl FGF stocking solution +5 µl heparin +500 µl penicillin/streptomycin	Cell culture
PPD solution	Consisted of papain (2.5 U/ml) Neutral protease (1 U/ml Dispase), Dnase I (250 U/ml) in EBSS	Cell culture: Cell isolation

2.2 Methods used for the *in vivo* study

2.2.1 Immunohistochemistry as well as double- and triple-immunofluorescence stainings

Cutting of serial brain tissue sections. Fixed brains were transferred to 10 and 20% sucrose in PBS. Subsequently, brains were frozen in precooled isopentane and stored

at -80°C. Serial coronal sections of one hemisphere were cut at 50 µm on a freezing microtome (Microm, Germany) and placed in 24-well plates each well filled with 1x TBS. These free-floating sections were collected in a one-in-sixth series. For long-term storage, brain sections of one series were placed into cryoprotectant solution (30ml ethylenglycol and 25ml glycerin, 45 ml PBS) and could be stored at –20°C until further processing.

Single immunohistochemistry.

BrdU immunohistochemistry. In order to process brain slices for immunohistochemistry, brain sections were washed three times for 5 min with 1x TBS and subsequently incubated with 0.6% hydrogen peroxide in TBS for 30 min to inhibit endogenous peroxidases. After another washing step with 1x TBS, sections for quantitative detection of cells that incorporated BrdU were incubated in 50% formamid/2×SSC at 65°C for 2h. Then, sections were placed in 2 N HCl for 30 min at 65°C in order to break the hydrogen bonds and therefore denature the DNA double-helix. After rinsing the sections in 0.1 M boric acid (pH 8.5) for 10 min to neutralize the hydrochloric acid and washing them with 1x TBS, they were incubated in blocking solution I for 1.5 h at room temperature (RT). Thereafter, sections were incubated in blocking buffer I containing the monoclonal anti-BrdU antibody produced in mouse for 48 h at 4 °C. Afterwards, sections were washed again three times for 5 min in 1x TBS and incubated for 1.5 h in biotinylated anti-mouse IgGs made in goat diluted 1:600 in blocking solution II at RT for 2 h. Sections were then washed and processed with avidin-biotinylated horseradish peroxidase complex (ABC) in 1x TBS for 1 h at RT. After another washing step the binding sites of the antibodies were visualized using 3,3'-Diaminobenzidine (DAB Substrate Kit). The reaction was stopped by transferring the slices into 1x TBS. Subsequently, sections were mounted on slides, were left to dry at least overnight and were coverslipped with VitroClud.

MCM2, NeuroD and DCX immunohistochemistry. MCM2 indicated proliferating

cells, NeuroD is expressed in mitotic active cells of the neuronal lineage and DCX is primarily expressed in post mitotic immature neurons. The staining protocol resembles the BrdU immunohistochemistry protocol above with some changes as described in the following. After sections had been incubated in 0.6% H_2O_2 in 1x TBS for 30 min and washed with 1x TBS, an antigen retrieval was performed. For the detection of NeuroD and MCM2, sections were incubated with 10 mM sodium citrate buffer (pH 8.5) for 15 min at 90°C twice. For the detection of DCX, sections were incubated with 10 mM sodium citrate buffer (pH 8.5) for 35 min at 80°C. The primary antibodies used were polyclonal anti-MCM2 antibodies made in goat (1:1000), polyclonal anti-NeuroD antibodies produced in goat (1:600), and polyclonal anti-DCX antibodies made in goat (1:500). For all blocking solutions inclusive the antibody solution normal horse serum was used instead of normal goat serum. Subsequently, sections were incubated with biotinylated anti-goat IgGs made in horse (for NeuroD 1:500, MCM2 1:1000 and DCX 1:1000) (Vector Laboratories, Burlingame, CA, USA).

Double- and triple-immunofluorescence staining.

In order to study the final cell type, we performed a double and triple-immunoflurorescent labeling of BrdU with neuronal nucleus (NeuN) as a marker for mature neurons, with GFAP as a marker for astrocytes, with adenomatous polyposis coli (APC) as a marker for oligodendrocytes and with ionized calcium binding adaptor molecule 1 (Iba-1) as a marker for microglia. Brain sections were incubated in 50% formamid/2×SSC at 65°C for 2 h and then placed in a 2 N HCl solution for 30 min at 65°C. After rinsing of sections in 0.1 M boric acid (pH 8.5) for 10 min and washing with 1x TBS, sections were incubated then in blocking solution I for 60 min. Thereafter, sections were incubated in blocking buffer I containing a mixture of three different antibodies produced in different species (monoclonal rat anti-BrdU, monoclonal mouse anti-NeuN and polyclonal rabbit anti-GFAP) or mixture of two different antibodies produced in different species (monoclonal rat anti-BrdU and polyclonal rabbit anti-GFAP; monoclonal rat anti-BrdU and monoclonal mouse

anti-APC; monoclonal rat anti-BrdU and polyclonal rabbit anti-Iba-1) for 72h at 4°C. After removing the primary antibodies by washing the sections three times with 1x TBS, sections were incubated in blocking buffer II containing a mixture of three different secondary antibodies (Donkey anti-rat IgG conjugated with Alexa Fluor 488; Donkey anti-mouse IgG conjugated with Alexa Fluor 555 anti-mouse and Donkey anti-rabbit IgG conjugated with Alexa Fluor 350).

2.2.2 Quantification of immunolabeled cells

Cells immunolabelled for light microscopy by applying the ABC method and DAB as substrate of the peroxidase were counted in different layers of the DG [MCM2, NeuroD and DCX: in the subgranular zone (SGZ) and in the granule cell layer (GCL) (SGZ-GCL); BrdU: in the SGZ-GCL, molecular layer (ML), and in the hilus] using an unbiased stereology procedure (Stereo Investigator from MBF Bioscience). In more detail, immunoreactive (ir) cells were counted using the optical fractionator method (with a counting frame of 150 by 150 µm and a sampling grid of 150 by 150 µm). First section was randomly selected and every 6th succeeding section throughout the septal-temporal axis of the hippocampus was analyzed. To estimate the total number of new cells, the cell count for each hippocampus was multiplied by 6 since as only 1/6 of all sections were analyzed. We also analyzed the volume of hilus, SGZ-GCL and ML on the septal and temporal DG separately when counting the number of BrdU-positive cells with Stereo Investigator automatically.

Using *Stereo investigator* to perform the quantitative analysis allows an accurate, unbiased estimation of the number, length, area, and volume of cells in certain tissue (Keuker et al., 2001). We estimated cell number according to the following equation (West et al., 1991):

$$\mathrm{N} = \sum Q^{-} \times \frac{1}{ssf} \times \frac{1}{asf} \times \frac{1}{tsf}$$

In this equation, N is the estimate of total cell number; Q^- is the number of units counted; the section sampling fraction (ssf) corresponds to the ratio between the sections we analysed and the total number of sections needed to section the entire tissue; And the area sampling fraction (asf) represents the proportion of the sectional area which is investigated within the sampled sections; The thickness sampling fraction (tsf) is the ratio between the height of the disector and the section thickness. In our study, we chose the ssf according to the method mentioned in Keuker's paper (Keuker et al., 2001). In details, 15-20 sections at equally spaced intervals along the hippocampal formation should be selected and one hippocampus is on average of 115 sections, so every 6^{th} section was selected and about 20 sections for one study. Then the ssf was 1/6. The asf is the ratio of the counting frame area and the area between the optical disector positions in the x and y directions: asf=a(frame)/A(x,y step) (see Fig.9). In our case, a(frame) was equate to A(x,y step), so asf in our experiment is 1. The measured section thickness in our experiment was between 17 and 21 µm and we used an optical disector height of 15 µm. When we count the number of cells, we excluded the cell which touched the exclusion sides of the optical disector: the top, the front and the left side. In Fig. 9, no. 1 cell is not counted because it touches the left exclusion side. The cell of no. 2 is counted because it lies completely within the optical disector and no. 3 cell is counted because it is partly inside the optical dissector.

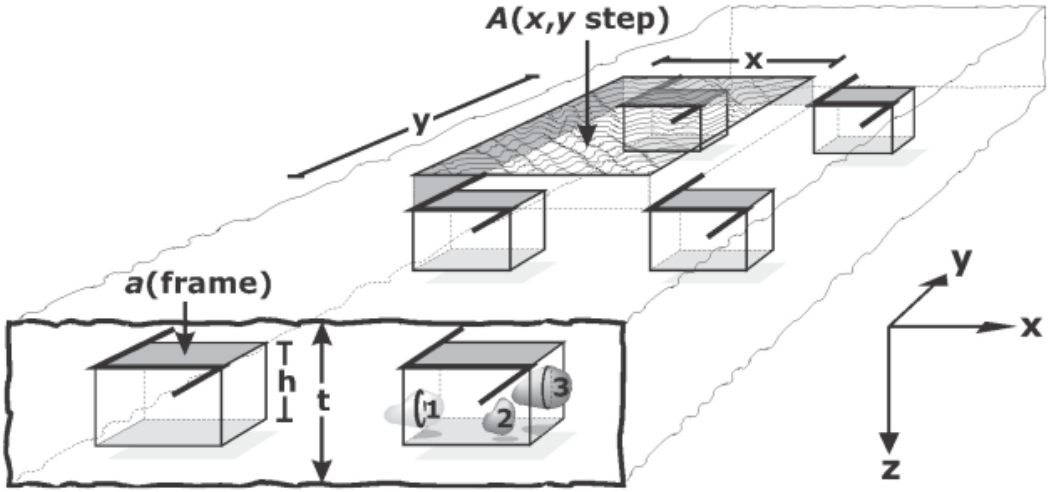


Fig.9. Schematic representation of a section with optical disector counting locations (Keuker et al., 2001)

For the quantification of BrdU/NeuN double stained cells, we counted BrdU-positive cells in the ML, SGZ, GCL and hilus which were co-labeled with NeuN or not. From each animal, 20 BrdU-positive cells within hilus, 20 BrdU-positive cells within SGZ, 10 BrdU-positive cells within GCL and 20 BrdU-positive cells within ML were randomly chosen and analyzed using a BX40 microscope from Olympus (Tokyo, Japan) equipped with a motorized stage controller and pictures were captured with the program “Cell” (Olympus). Then, the percentage of cells which were co-labeled with NeuN was calculated. All assessments were done by experimenters blind to the treatment group of each brain section.

2.2.3 Statistical Analysis

Data were expressed as mean ± standard error (SEM). As group size was limited to 6-8 rats and first analyses of results showed that obtained data were not normally distributed and variances of groups were not equal, we carried out statistical analyses using non-parametric tests (SPSS 16.0 software). Group-wise comparisons using the Mann-Whitney U-test were performed only

if a p-value of <0.1 in the Kruskal–Wallis test had indicated the presence of differences between the five experimental groups. P-values of <0.05 were considered to be statistically significant and 0.05<p<0.1 were defined as a trend.

2.3 Methods used for the *in vitro* study

2.3.1 Isolation and identification of the neural stem cells

Both hippocampi of Wistar Rat were dissected mechanically under Stereomicroscope (Olympus, Japan) and transferred to a 55 mm plate (CellStar, Carrollton, TX, USA) containing pre-cooled (using water ice) Dulbecco's Phosphate-Buffered Saline (D-PBS) plus 2% glucose. After dissections of all hippocampi had been completed, tissue was transferred in Papain-Protease-DNase (PPD) solution into a 15 ml conical tube and a 1 ml pipettor with sterile plastic tips was used to triturate tissue pieces approximately 50 times until a “milk”-like solution was formed. During titration, the pipettor was pressed against the bottom in order to generate resistance for a better breaking up of the tissue, and to avoid the production of bubbles. Then, we incubated the milk”-like solution in a 37°C water bath (the optimal working temperature of the PPD solution) for 30 min in order to break the tissue completely. Next, the tube was left for about 5 min undisturbed to allow the undispersed pieces of tissue to settle. Then, the supernatant was transfered to a new sterile 15 ml conical tube and one ml Inhibitor working solution was added to stop PPD digestion. Afterwards, we centrifuged this supernatant at 110 g (800 rpm using a centrifuge from Bio-Rad Laboratories, Hercules, CA, USA) for 7 min and discarded the supernatant. Pellets were then resuspended with 5 ml pre-warmed proliferation culture medium and cell suspension were let flew through a 40 µm cell strainer (without pressure) (BD Falcon, Durham, NC, USA). Next, additional 2 ml culture

medium were added and aspirated in total volume to a T25 flask (Corning, Tewksburg, MA, USA). Incubation was done in a 37°C incubator.

Cells were passaged every 7 days as described in the following:. We pipetted media into a 15 ml conical tube and washed back down the flask to harvest as many spheres as possible with 1 ml fresh pre-warmed proliferation culture medium. Solution was centrifuged at 110 g for 7 min and supernatant was discarded. Pellet was re-suspended with 1ml pre-warmed PPD solution and incubated for 10 min at 37°C Water Bath. After adding 1 ml inhibitor working solution, solution was centrifuged at 110 g for 7 min at RT and supernatant was discarded again. Then, pellet was re-suspended with 5 ml fresh pre-warmed proliferation culture medium to get a single cell suspension. We transferred all of the solution into a T25 flask (Corning, NY, USA), added additional 2 ml fresh culture medium and cells were incubated at 37°C and 5% CO_2.

To identify cultured cells, we stained neurospheres for nestin (a marker for neural stem cells) and studied whether cultured cells have pluripotent ability to differentiate into different types of cells (the method of differentiation see 2.3.5 Differentiation assay). After the neurospheres had been formed after approximately 2 days of incubation in proliferation medium, they were plated on poly-L-ornithine/laminin-coated coverslips and we let them grew in proliferation culture medium. After neurospheres had been attached on the coverslips (about 2h), they were fixed with 4% PFA and immunostained for nestin.

The method of coating coverslips is described as follows: we diluted poly-L-ornithine with D-PBS from the stock concentration (10 mg/ml) to yield 50 µg/ml working solution. 250 µl working solution was added per well in 24 well plate containing 12 mm round cover glass (Neuvitro, El Monte, CA, USA)

and incubated overnight. Then, coverslips were rinsed twice with D-PBS and incubated with laminnin (Sigma, St Louis, MO, USA) solution for at least 30 min. Laminin used for that had been pre-diluted to a final concentration of 5 µg/ml with D-PBS. After rinsing twice with D-PBS, we let them dry.

2.3.2 Treatment of NSCs with STZ

STZ was diluted in 0.1 M citrate buffer (pH 4.5) to a 0.5 M stocking solution. After cells had been passaged 2 times, they were incubated with STZ at concentrations of 0, 1, 2.5, 5 and 10 mM. With four days incubation in STZ containing medium the number of neurospheres was counted with the help of a BX40 microscope from Olympus (Tokyo, Japan). For further experiments, we selected a suitable concentration of STZ to study its effects on proliferation, differentiation as well as insulin/IGF signaling pathway changes.

2.3.3 Proliferation assay

In order to study the effect of STZ on the proliferation of NSCs, the number and size of neurospheres (size > 5 cells) and the absolute number of NSCs have been evaluated. After passaging the cells for 2 times, NSCs were seeded into Cellstart™ substrate-coated 96-well plates (Life Technologies, Gaithersburg, MD) with the density of 2,000 cells per well. For estimating the number of neurospheres per well, they were counted using the BX40 microscope at 10× magnification. Then, for evaluating the mean size of neurospheres, they were imaged (about 40 images per group) by the microscope equipped with a motorized stage controller and pictures were captured with the program “Cell” (Olympus) and then analyzed with Image-Pro-Plus 5.0 software (Media Cybernetics, Rockville, MD, USA).

Proliferation of the NSCs was also studied by using a BrdU incorporation assay. We dissected the neurospheres with PPD solution into single cell suspension and seeded these cells on poly-L-ornithine/laminin-coated coverslips at the density of 10,000 cells per well with or without 2.5 mM STZ. After two days incubation, 10 µM BrdU was applied to the cultured cells for 4 h to label proliferating cells. Before incubation was started, BrdU stocking solution (10 mM) had been diluted with D-PBS. Then, cells sticking on coverslips were fixed with 4% PFA and immunostained with mouse anti-BrdU antibody as decribed in the following: Coverslips were washed three times with TBS for 5 min and then were placed in 1 N HCl for 10 min at 37°C in order to break the hydrogen bonds and therefore denature the DNA double-helix. After rinsing the coverslips in 0.1 M boric acid (pH 8.5) for 10 min to neutralize the hydrochloric acid and washing the coverslips with 1x TBS for three times, they were incubated in blocking solution I for 1.5 h at RT. Thereafter, coverslips were incubated in blocking buffer I containing the monoclonal anti-BrdU antibody produced in mouse for 48 h at 4°C. Afterwards, coverslips were washed again three times for 5 min in 1x TBS and incubated for 1.5 h in Alexa 555 anti-mouse IgGs made in donkey diluted 1:600 in blocking solution II at RT for 2 h. After washing coverslips with 1x TBS three times, they were incubated with 300 nM DAPI for 5 min at RT, and then washed again. Finally, coverslips were mounted to slides with Fluoro mount.

2.3.4 Migration assay

We collected rat neurospheres and centrifuged at 110 g for 7 min. After removing all of the supernatant, the pellet was re-suspended with 5 ml differentiation culture medium. About 10-15 neurospheres/well were plated on poly-L-ornithine/laminin-coated coverslips in a 24-well plate (Sarstedt, Nümbrecht, Germany) and were incubated in differentiation culture medium. After neurospheres were attached on the coverslips (about 2 h), medium was

aspirated and changed to differentiation cell culture medium with or without 2.5 mM STZ. Two days later, pictures were taken using the BX40 microscope and then analyzed with Image-Pro-Plus 5.0 software.

In order to quantify the migration of newborn cells, we traced the outermost area, where to newborn cells had migrated to (starting from the neurosphere, which was located in the middle as shown in Fig.10A). Using the Image-Pro-Plus 5.0 software the average diameter (D1) of this area defined as S1 was estimated (Fig.10B). Then, we traced the area of the respective neurosphere (S2), the average diameter D2 could be calculated automatically by our program (Fig.10B). The average migration distance (R) for one neurosphere can be calculated according to following formula:

$$R = (D1 - D2)/2$$

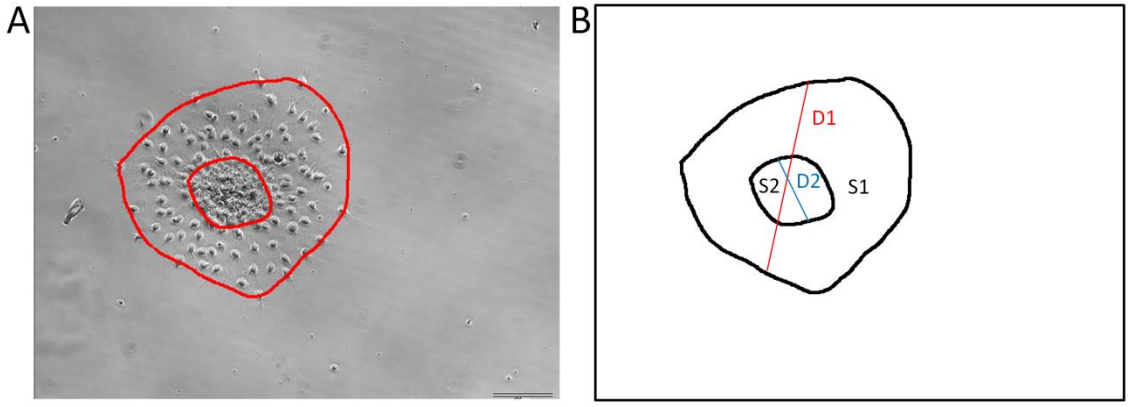


Fig.10. Method of estimation of newborn cell migration

The picture of neurosphere together with the cells migrated from the neurosphere was taken up by microscope (A). The area of neurosphere and migrating cells were traced by Image-Pro-Plus 5.0 software (A) and generated to trace image (B). The average diameter of neurosphere and migrating cells can be calculated automatically by program. The average migration distance (R) for one neurosphere was estimated according to following formula: $R = (D1 - D2)/2$. S1: the area of migrating cells. S2: the area of the neurosphere. D1: the average diameter of the area of migrating cells. D2: the average diameter of the area of the neurosphere.

2.3.5 Differentiation assay

To characterize the phenotype of differentiating NSCs and the effect of STZ on this differentiation process, NSCs were collected and then promoted to differentiate into different types of cells. In detail, after passaging for two times in proliferation culture medium, we collected rat neurospheres and centrifuged at 110 g for 7 min and discarded the supernatant. The pellet was re-suspended with 5 ml differentiation culture medium. Neurospheres were then transferred onto pre-coated cover glasses in a 24-well plate with the density of 10-15 neurospheres per well. After neurospheres were attached on the coverslips (about 2 h), the culture medium was exchanged to differentiation cell culture medium with or without 2.5 mM STZ. After cells were incubated in differentiation culture medium for 2 days, medium was removed from each well and .5 ml of the fixative 4% PFA was added. After an incubation for 30 min at RT in the fixative cells were immunostained using Tuj-1 and GFAP antibodies.

2.3.6 Detection of insulin receptor and glucose transporter 3 protein

We immunofluorescently stained the IR and GLUT3 proteins in both, NSCs and differentiated cells. For analyzing differentiated cells, the methods used are the same as in protocol above (2.3.5 Differentiation assay) and immunostained for IR as well as GLUT3. For the staining of NSCs, we generated a single cell suspension out of neurospheres with the help of PPD solution and seeded these cells on poly-L-ornithine/laminin-coated coverslips at the density of 10,000 cells per well with or without 2.5 mM STZ. Two days later, we fixed these cells with 4 % PFA and performed immunfluorscence stainings.

For immunofluorescence staining with antibodies against IR and GLUT3 proteins, protocols were similar to the BrdU staining protocol (see 2.3.3), but without 1 N HCl

for 10 min and boric acid incubation steps. For differentiation studies, coverslips were incubated in blocking buffer I containing the monoclonal anti-Tuj-1 antibody (produced in mouse) and polyclonal rabbit anti-GFAP for 48 h at 4°C after rinsing coverslips in 1x TBS three times for 5 min. Then coverslips were washed again three times for 5 min in 1x TBS and incubated for 1.5 h with Alexa 488 anti-mouse IgGs made in donkey and Alexa 555 anti-rabbit IgGs made in donkey diluted 1:500 in blocking solution II at RT for 2 h. For the detection of Nestin, IR and GLUT3 protein expression in NSCs and differentiating cells (Nestin only used for immunostaining of NSCs), coverslips were incubated in blocking buffer I containing the monoclonal anti-Nestin produced in mouse, anti-IR antibody produced in mouse or polyclonal anti-GLUT3 antibody produced in rabbit for 48 h at 4°C. Then coverslips were washed again three times for 5 min in 1x TBS and incubated for 1.5 h with Alexa 555 anti-mouse IgGs made in donkey or Alexa 555 anti-rabbit IgGs made in donkey diluted 1:500 in blocking solution II at RT for 2 h.

2.3.7 Quantitative real time PCR

Rat neurospheres were incubated in 6-well plates (Corning, New York, USA) with or without STZ for 2 days. After collecting the neurospheres, total RNA was extracted using RNeasy kit from Qiagen following manufacturer's instructions.

cDNA was synthesized with a first strand cDNA synthesis kit from Bio-Rad.

For each reaction:

5 × iScript reaction mix	4 µl
iScript reverse transcriptase	1 µl
Nuclease-free water	variable
RNA template (200 ng)	variable
Total volume	20 µl

cDNA synthesis was performed in a PCR machine (e.g. Biometra, Göttingen, Germany) using following program: 25 °C, 5 min; 42 °C, 30 min; 85°C, 5 min and then 4 °C until further processing. Finally, cDNA was diluted 1:5with TE buffer.

The PCR was performed in 384-well plates (life technologies, Gaithersburg, USA). For each reaction:

SYBR Select Master Mix for CFX (2 ×)	5 µl
Forward and Reverse Primers (5uM)	1 µl
cDNA	1 µl
RNA template (200 ng)	3 µl
Total volume	10 µl

The PCR reaction was carried out using a CFX384 Real-Time system from Bio-Rad using the following conditions: 95 °C, 2 min; 45 cycles of 95 °C, 5 sec; 60 °C, 30 sec; 95 °C, 10 min; lastly 65°C, 5 sec.

C_T values were calculated using the $2^{\Delta\Delta CT}$ method ($^{\Delta\Delta CT}$ = $^{\Delta CT}$sample – $^{\Delta CT}$control) for relative quantification. ΔC_T was calculated by normalized C_T values of samples minus C_T values of GAPDH (as a reference gene). C_T values were analyzed with the help of LinRegPCR software.

2.3.8 Statistical analysis

Data were expressed as mean ± standard deviation (SD). For STZ concentration determination and migration study, the data were analyzed one way analysis of variance (ANOVA) followed by Bonferroni post hoc test using SPSS 16.0. For the others, we performed a Student's t-test. P-values of <0.05 were considered to be statistically significant. Statistical significance is indicated as *, $p < 0.05$; **, $p < 0.01$.

3.Results

3.1 *In vivo* study results

3.1.1 The effect of STZ icv treatment on proliferating stem/progenitor cells in the SGZ

To determine the effect of STZ icv treatment on proliferating progenitor cells in the SGZ of the DG, hippocampal sections were stained with an antibody against MCM2, which is a G1-S phase-specific marker of cellular replication and can be used as an endogenous marker for proliferating cells (Kneissl et al., 2003). MCM2-immunosignals were detected in cell nuclei and often appeared in cluster (Fig.11A). MCM2-positive cells in the SGZ were stereologically quantified using the Stereo Investigator software (MBF Bioscience). Our results did not reveal any significant difference of the number of MCM2-positive cells between all treatment groups neither in septal, temporal nor in total hippocampus (Fig.11B-D). Even if significant group differences were missing, Fig. 11C depicts that the number of MCM2-positive cells seems to be increased in the temporal hippocampus of rats 1 month after STZ icv treatment compared to the vehicle 1 month group. Moreover, 3 months after STZ icv treatment the number of MCM2-ir cells was shown to be reduced by 37.1% in the septal (Fig.11B) and by 24.1% in the total hippocampus (Fig.11D).

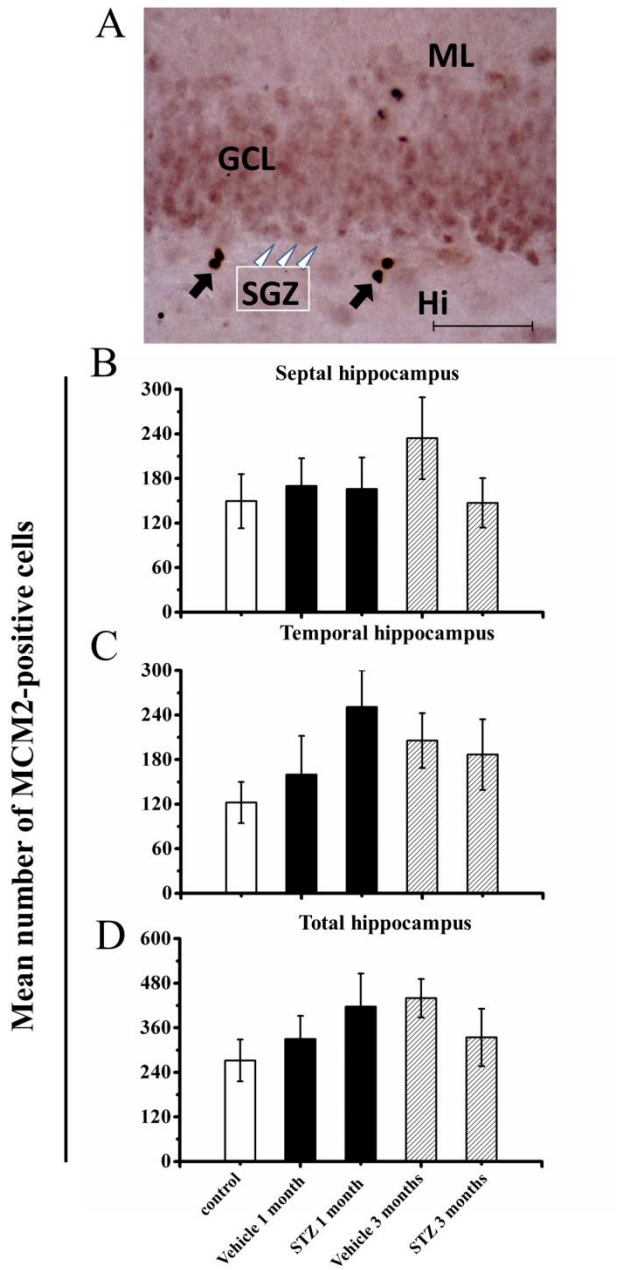


Fig.11. Effect of STZ icv treatment on the number of proliferating cells in the subgranular zone

A: Representative photomicrographs display MCM2-ir cells (indicated by arrows) in the dentate gyrus. Notice the subcellular distribution of the MCM2 in different cell nuclei. We quantified MCM2-positive cells which are localized in the subgranular zone (SGZ, indicated by arrowheads) of the septal (B), temporal (C) and total (D) hippocampus. Statistical analysis did not reveal any significant difference between groups (n=6-8 rats per group). Data are presented as the arithmetic mean of MCM2-positive cells per hemisphere ± standard error of mean (SEM). Hilus, Hi; Molecular layer, ML; granule cell layer, GCL. Scale bar in A represents 50 µm.

3.1.2 The number of immature neurons is decreased in 3 months, but not 1 month after STZ icv treatment

To determine possible effects of STZ icv treatment on the differentiation of newborn cells to neurons, we analyzed intermediate stages of AN using two different marker for immature neurons, NeuroD and DCX. As shown in Fig.12A and 13A, NeuroD as well as DCX-positive cells were primarily located in the SGZ of the DG. The NeuroD-protein was exclusively detected in cell nuclei, whereas DCX-immunoreactivity was found in the cytoplasm of the cell body (without immunostaining of the nucleus) and the dendrites extending through the GCL into the ML. NeuroD is a marker for mitotic active cells of the neuronal lineage and DCX marks primarily post mitotic immature neurons. Quantitative immunohistochemistry using both AN markers resulted in significant group differences in the SGZ of septal (but not temporal) hippocampus with p= 0.04 for NeuroD (Fig.12B) and p=0.01 for DCX (Fig.13B). In the total hippocampus, statistical analysis of DCX data revealed a trend for group differences (p=0.062; Fig.13D). As shown in Fig.12B, Fig.13C,D *post hoc* groupwise comparisons revealed that in the SGZ of the septal hippocampus STZ icv 3 months rats have significantly less NeuroD-ir cells (a decrease of 66.7%; p=0.017; Fig.12B) and highly significant less DCX-positive cells (a decrease of 72.5%; p=0.002; Fig.13C) than in vehicle icv 3 months rats. Besides these significant differences, a trend towards lower numbers of immunopositive cells in the STZ icv 3 months group compared to the STZ icv 1 month group could be shown for NeuroD in the septal hippocampus (p=0.073; Fig.12B) and for DCX in total hippocampus (0.053; Fig.13D). Comparing the data from the STZ icv 1 month rats with their appropriate vehicle control group and all other experimental groups, no significant differences could be revealed, neither analyzing NeuroD nor DCX.

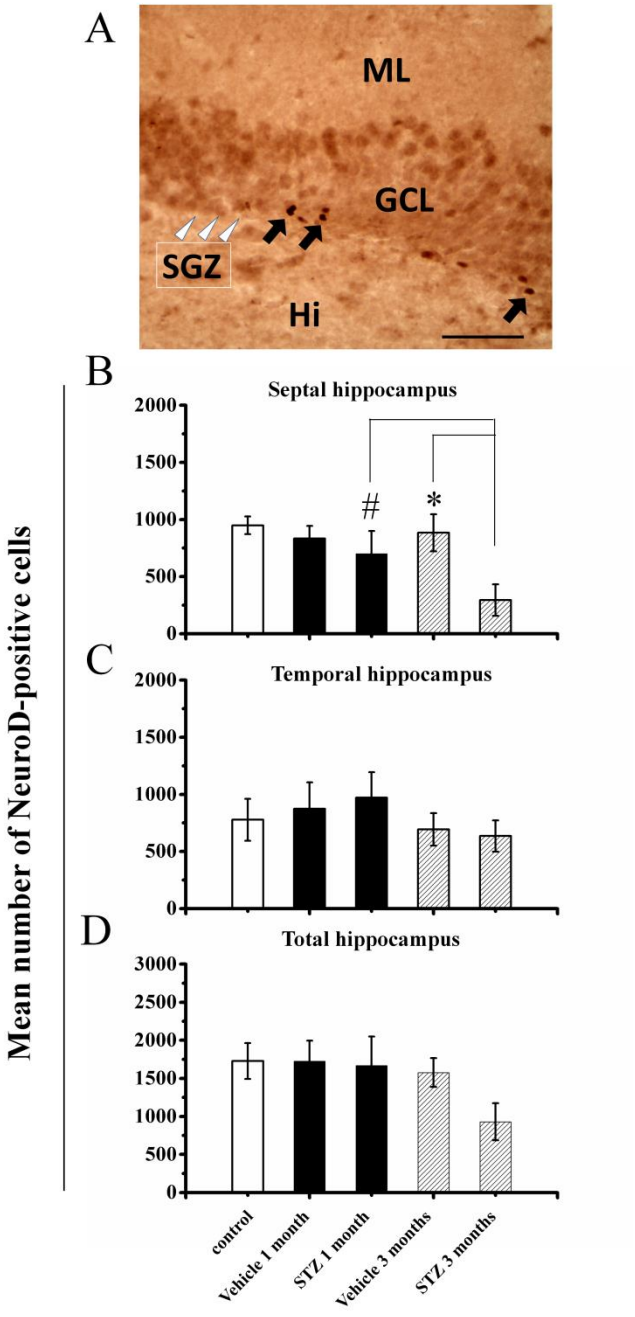


Fig.12. The number of NeuroD positive cells is reduced in the septal, but not in the temporal and total hippocampus, 3 months after STZ icv treatment

A. Representative microphotographs display NeuroD-ir cells (indicated by arrows) primarily located in the subgranular zone (SGZ, indicated by arrowheads) of the DG. Notice that the NeuroD-protein is exclusively detected in cell nuclei. We quantified NeuroD- (B,C,D) an the SGZ of the septal (B), temporal (C) and total (D) hippocampus using the StereoInvestigator software. Group differences were mainly revealed in the septal hippocampus for NeuroD (p=0.04). B: Groupwise comparisons showed that the number of NeuroD-ir cells in the septal hippocampus is significantly decreased by 66.7% (p=0.017) in rats sacrificed 3 months after STZ icv injections (STZ 3 months) compared to rats 3 months

after the vehicle icv treatment (Vehicle 3 months). A trend or tendency towards less NeuroD-ir cells in the STZ 3 months group compared to the STZ 1 month group could also be found. Data are presented as the arithmetic mean of NeuroD-positive cells per hemisphere ± standard error of mean (SEM). #: p-value of Mann-Whitney U- test <0.1 and >0.05; *: p-value <0.05; **: p-value <0.01. Hi, Hilus; Scale bar in A represents 200 µm.

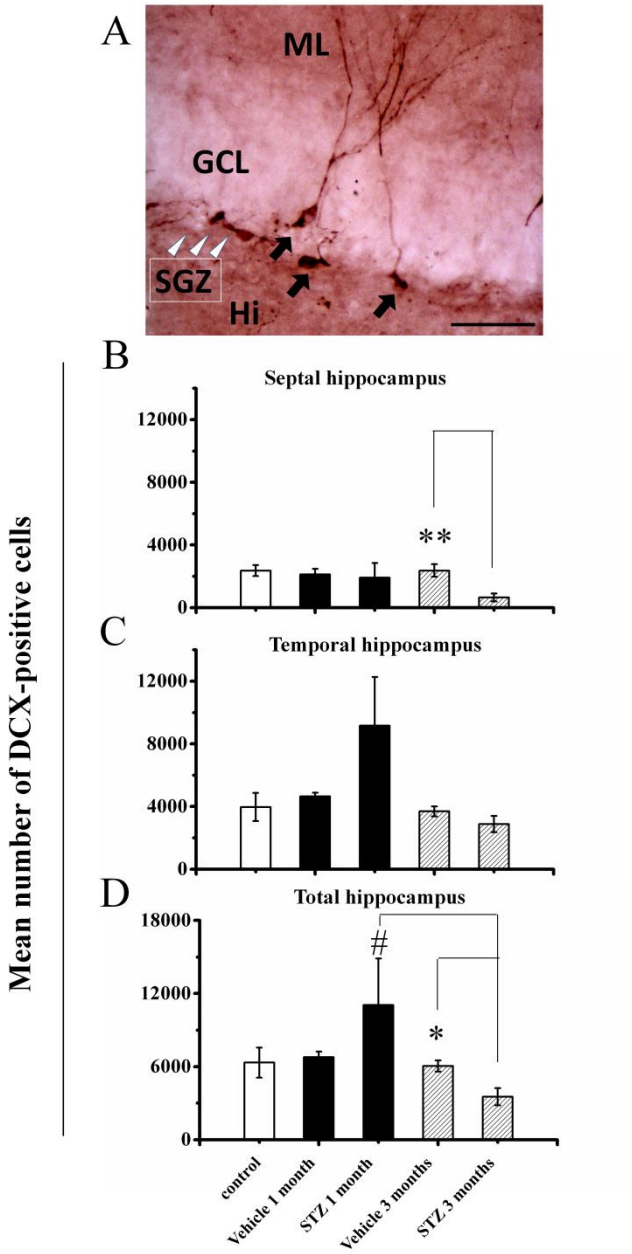


Fig.13. The number of DCX positive cells is reduced in the septal, but not in the temporal and total hippocampus, 3 months after STZ icv treatment

A: Representative microphotographs display DCX-ir cells (indicated by arrows) primarily located in the subgranular zone (SGZ, indicated by arrowheads) of the DG. Notice that the DCX-immunoreactivity is found in the cytoplasm of the cell body (without immunostaining of the nucleus) and the dendrites extending through the granule cell

layer (GCL) into the ML. We quantified DCX-positive cells in the SGZ of the septal (B), temporal (C) and total (D) hippocampus using the StereoInvestigator software. Group differences were mainly revealed in the septal hippocampus for DCX (p=0.01), and for DCX (p=0.062) also in the total hippocampus (n=6-8 rats per group). B: Groupwise comparisons revealed that highly significant less DCX-ir cells (up to 72.5% less; p=0.002) were detected in the STZ 3 months compared to the Vehicle 3 months group. C: Groupwise comparisons of the number of DCX-ir cells in the total hippocampus revealed significantly less DCX-ir-cells (p=0.021) in the STZ 3 month group in comparison to the Vehicle 3 months group. A trend or tendency towards less DCX-ir cells in the STZ 3 months group compared to the STZ 1 month group could also be detected (p=0.053). Even if significant group differences were missing, the number of DCX-positive cells seems to be increased in the hippocampus of rats of the STZ 1 month group compared to the Vehicle 1 month group (most pronounced in the temporal hippocampus). Data are presented as the arithmetic mean of DCX-positive cells per hemisphere ± standard error of mean (SEM). #: p-value of Mann-Whitney U- test <0.1 and >0.05; *: p-value <0.05; **: p-value <0.01. Hi, Hilus; Scale bar in A represents 200 µm.

3.1.3 STZ icv treatment differently impacts the survival of newborn cells in various dentate gyrus regions

Furthermore, we analyzed the effect of STZ icv treatment on the survival of newborn cells. For that, experimental rats received BrdU ip injections on 7 consecutive days (once daily) and were sacrificed 27 days after the first injection. As shown in Fig.14A and B immunohistochemistry using BrdU antibodies resulted in several immunopositive cell nuclei in the SGZ and GCL, the two main regions where AN happens, but also in the hilus and ML of the DG. As we detected many BrdU-ir cells in these latter regions, we quantified the number of BrdU-positive cells not only in the SGZ including the GCL (=SGZ/GCL), but also in the hilus and ML (Fig.14). Interestingly, we detected more BrdU-ir cells in the hilus and ML than in the SGZ/GCL irrespectively of the treatment group. In the SGZ/GCL (Fig.14 D,G,J) stereological quantification of BrdU-positive cell nuclei and applying the Kruskal-Wallis test resulted in significant group differences in the septal hippocampus with p=0.049 (Fig.14D), but not in temporal (Fig.14G) and total hippocampus (Fig.14J). Subsequent

post hoc groupwise comparisons revealed that in the septal hippocampus STZ 3 months rats have significantly less BrdU-immunoreactive (ir) cells (a decrease of 36.3%; p=0.048) than Vehicle 3 months rats (Fig.14D). Significant differences between the STZ 1 month rats and the Vehicle 1 month control group or the absolute control group (without any icv treatment) in the septal hippocampus were missing. In hilus (Fig.14C,F,I), a trend for a group difference applying the Kruskal-Wallis test was only found in the temporal hippocampus with p=0.051. As shown in Fig. 8F subsequent *post hoc*-analysis revealed a trend for more BrdU-positive cells in rats of the STZ 1 month icv group compared to the Vehicle 1 month icv group (p=0.052) and compared to the STZ 3 months icv group (p=0.073). In the ML (Fig.14E,H,K), significant group differences were found in the temporal (p=0.039; Fig.14H) and total hippocampus (p=0.049; Fig.14H). In the temporal hippocampus (Fig.14H), pairwise comparisons revealed a trend towards higher number of BrdU-positive cells in the STZ 1 month icv group compared to the Vehicle 1 month group (p=0.052) and compared to the STZ 3 months group (p=0.051). Moreover, a trend towards lower number of BrdU-positive cells in the Vehicle 1 month group compared to the absolute control group with p=0.093 was found (Fig.14H). In the total hippocampus (Fig.14K), pairwise comparisons revealed a trend towards higher number of BrdU-positive cells in the STZ 1 month group compared to the Vehicle 1 month group (p=0.082) and a trend towards lower number of BrdU-positive cells in the Vehicle 1 month group compared to the absolute control group with p=0.093. Notice, that in the hilus and ML much more BrdU-ir cells were detected than in the SGZ/GCL.

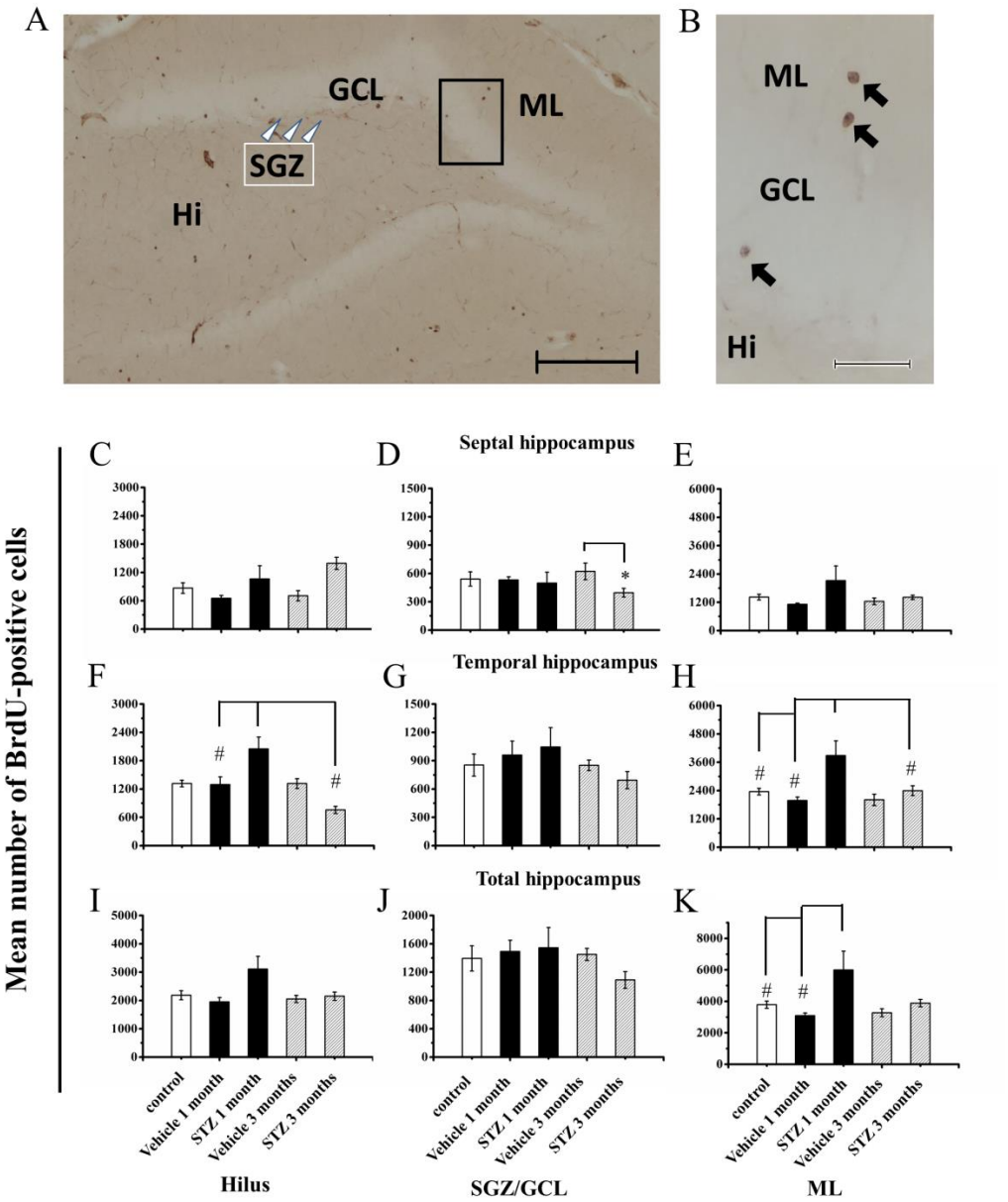


Fig.14. The effect of STZ icv treatment on the number of survived BrdU-positive cells in the dentate gyrus

A,B: Representative photomicrographs with different magnification of coronal sections through the DG immunostained with BrdU antibody. As experimental rats received daily BrdU ip injections on 7 consecutive days and were sacrificed 27 days after the first BrdU application, immunohistochemistry detection followed by quantification of BrdU-ir cells was performed to assess the effect of STZ icv treatment on surviving newborn cells. Notice that the BrdU is exclusively detected in cell nuclei (as indicated by arrows in B). BrdU-positive cells were found and quantified in the hilus (Hi; C,F,I), in the subgranular zone (SGZ, indicated by arrowheads) including the granule cell layer (SGZ/GCL; D,G,J) and in the molecular layer (ML; E,H,K) of the DG (n=6-8 rats per group). Moreover, we quantified BrdU-positive cells in the septal (C,D,E), the ventral (F,G,H), and the total hippocampus separately. Notice that we counted more BrdU-positive cells in the hilus and in the ML than in the SGZ/GCL layer, where stem cells are and neurogenesis primarily

takes place in the adult hippocampus. C,F,I: In Hilus, a trend towards more BrdU-positive cells in the STZ 1 month group compared to the Vehicle 1 month group as well as compared to the STZ 3 months group could be shown exclusively in the temporal hippocampus. D,G,J: Excusively in the SGZ/GCL of the septal hippocampus, the STZ 3 month group possessed significantly less BrdU-positive cells than the Vehicle 3 months group (p=0.048). E,H,K: In the ML, only small differences could be revealed. In the temporal hippocampus a trend towards more BrdU-ir cells was detected in the STZ 1 month group compared to the Vehicle 1 month and compared to the STZ 3 months group. Additionally, a trend towards lower BrdU numbers in the Vehicle 1 month group compared to the absolute control group was shown. Data are presented as the arithmetic mean of BrdU-positive cells per hemisphere ± standard error of mean (SEM). #: p-value of Mann-Whitney U- test <0.1 and >0.05; *: p-value <0.05. Scale bar in A represents 200 µm and in B represents 50 µm.

3.1.4 STZ icv treatment did not alter the rate of neuronal differentiation in the subgranular zone and granule cell layer

Triple stainings using antibodies detecting BrdU, NeuN (a marker for mature neurons), and GFAP (a marker for astrocytes) were performed to analyze the final phenotype of newborn BrdU-ir cells which survived up to 27 days. As shown in Fig.15A-C, single stained BrdU-positive cells (Fig.15A,C) but also double stained BrdU/NeuN-positive cells (Fig.15B) could be found. In a quantitative approach we focused on the neuronal phenotype and exclusively analyzed the percentage of BrdU-positive cells that expressed NeuN (Fig.15D-K). Statistical analyses applying the Kruskal-Wallis test did not reval any significant differences between experimental groups neither in Hilus, SGZ, GCL, and ML nor in septal or temporal hippocampus. Notice that BrdU-ir/NeuN-ir cells were almost exclusively found in the SGZ (Fig.15E,I) and GCL (Fig.15F,J), the neurogenic regions of the DG, and not in the hilus (Fig.15D,H) and ML (Fig.15G,K).

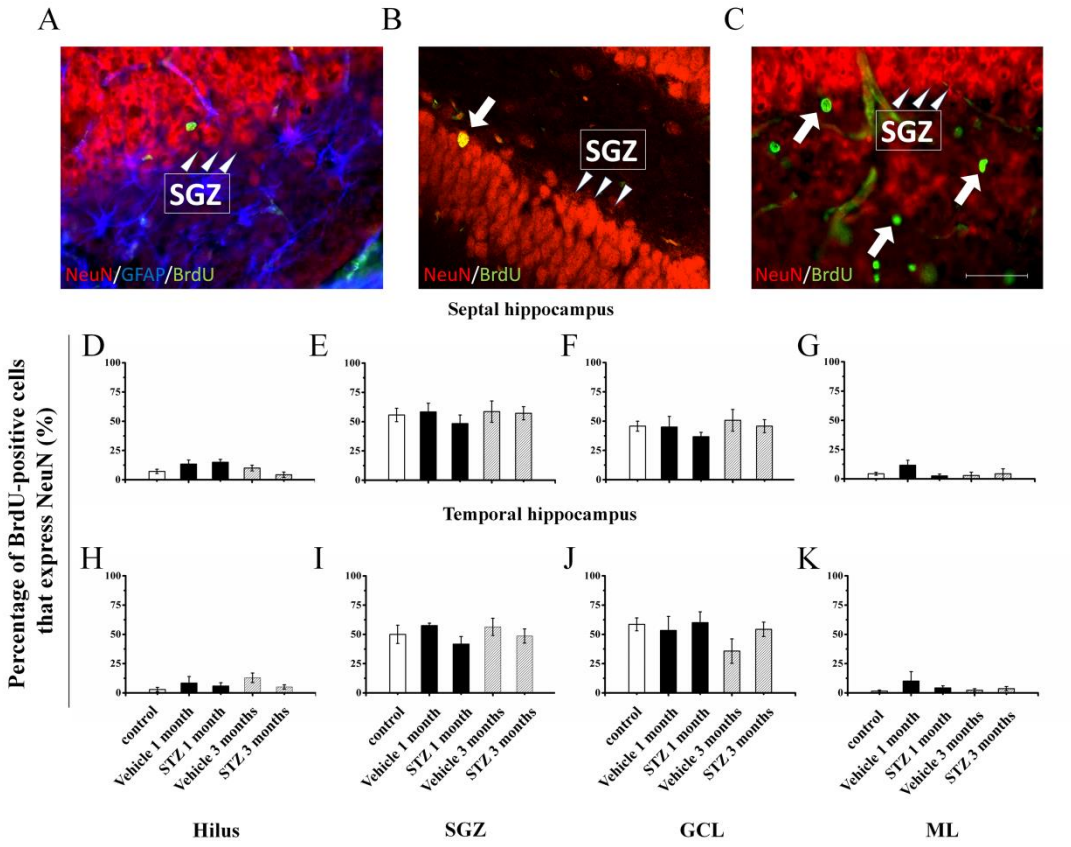


Fig.15. The percentage of newborn neurons in the dentate gyrus is not affected by STZ icv treatment

A-C: Phenotype analysis of BrdU-ir cell nuclei in the DG. Representative photomicrographs display BrdU/NeuN/GFAP triplestaining (A) and BrdU/NeuN double stainings (B, C). A: Merged image of BrdU (green), NeuN (red) and GFAP (blue) did not show any co-localization. B,C: Merged images display one cell in the subgranular zone (SGZ, indicated by arrowheads) double labeled for BrdU and NeuN (yellow cell nucleus in B, indicated by an arrow) and several BrdU-positive cells in the SGZ and the hilus without double labeling (green nuclei in C, indicated by arrows). D-K: Quantitative estimation of the percentage of BrdU-positive cells that express NeuN detected in hilus (D,H), SGZ (E,I), granule cell layer (GCL; F,J) and molecular layer (ML; G,K) didn`t resulted in any differences between experimental groups (n=6-8 rats per group), neither in the septal hippocampus (D-G) nor in the temporal hippocampus (H-K). Notice that the highest number of BrdU/NeuN double stained cells was detected in the SGZ and GCL, where adult neurogenesis happens. Data are presented as mean percentage of BrdU/NeuN double stained cells ± standard error of mean (SEM). Scale bar in C represents 50 µm for A-C.

3.1.5 Phenotype analysis of BrdU-positive cells in the hilus and molecular layer revealed newborn oligodendrocytes but not newborn microglia

As the quite numerous BrdU-positive cells localized in the hilus and ML, which had survived up to 27 days, were found to have no neuronal phenotype (see Fig.15D,H and G,K), double staining using antibodies detecting BrdU and APC (a marker for oligodendrocytes) and detecting BrdU and Iba-1 (a marker for microglia) were performed in addition to the already shown triple staining of BrdU/NeuN/GFAP (see Fig.15A) to further analyze their possible cellular phenotype. As shown in Fig.16, some BrdU-ir cells in ML displayed also APC immunoreactivity (Fig.16F), but we could not detect BrdU/GFAP-double stained cells (Fig.16C) and BrdU/Iba1 double stained cells (Fig.16I) in the ML. Thus, with exception of some newborn oligodendrocytes we could not identify the cell type of most of the BrdU-positive cells found in the ML.

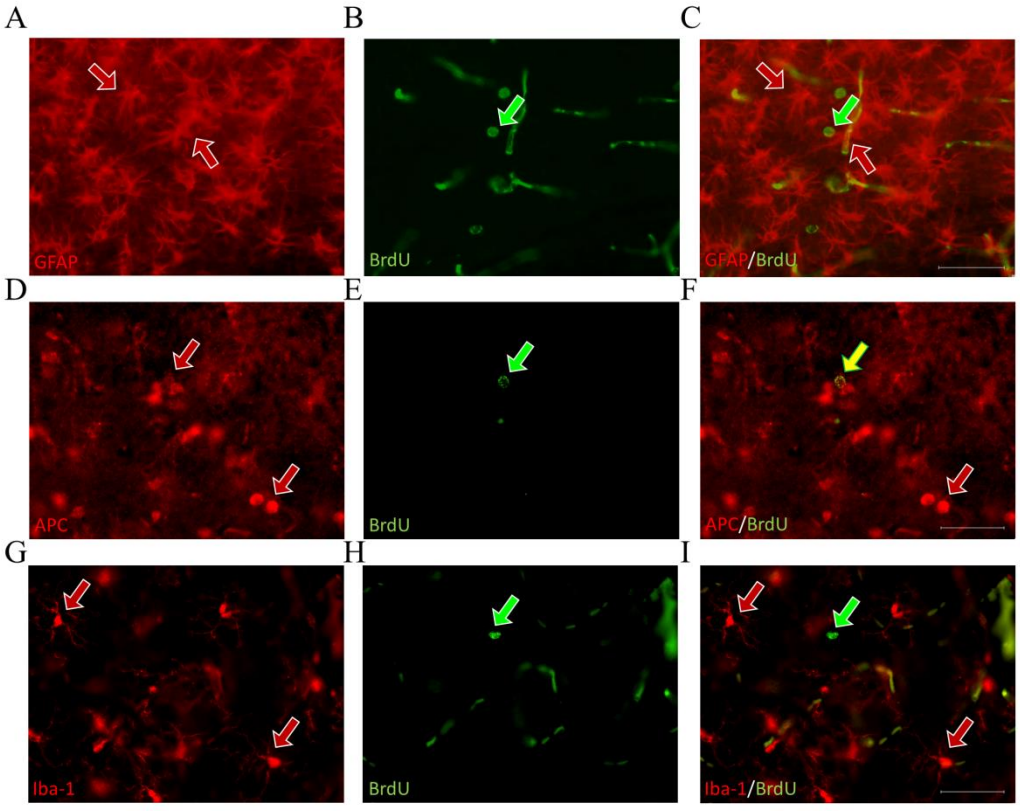


Fig.16. Some newborn cells in the molecular layer are oligodendrocytes

Phenotype analysis of BrdU-positive cells in the ML and in the hilus. Phenotype analysis

was performed for approximately 27 days survived cells using double labeling immunofluorescence staining. (A-C) Representative images display a section labeled for GFAP, a marker for astrocytes (A, red, indicated by red arrows) and BrdU (B, green, indicated by a green arrow). In B unspecific stained small vessels were visible. The merged image (C) shows that these newborn cells are located in close vicinity of both, GFAP-positive processes and small vessels (see unspecific labeling of small vessels in yellow), but BrdU+/GFAP+ cells were not observed. (D-F) Representative images show a section labeled for APC, a marker protein of oligodendrocytes (D, red, indicated by red arrows) and BrdU (E, green, indicated by a green arrow). The merged image (F) shows one cell double labeled for BrdU and APC (indicated by a yellow arrow) in addition to single stained APC-ir cells (indicated by red arrow). (G-I) Representative images show a section labeled for Iba-1, a microglia marker protein (G, red, indicated by red arrows) and BrdU (H, green, indicated by a green arrow). The merged image (F) does not show any double labeling (red arrows indicate single-stained Iba-1 positive microglial cells and the green arrow indicates the single-stained BrdU-ir cell nucleus).

3.1.6 The volume of different dentate gyrus subregions is not affected by the STZ icv treatment

In addition to the counting of BrdU-positive cells in all subregions of the DG (hilus, SGZ/GCL and ML), we estimated the volume of these subregions using stereological methods as outlined in Materials and Methods. Statistical analysis, however, did not reveal volume differences as depicted in Fig.17. Thus, STZ icv treatment did not produce overall changes in the volume of these different DG subregions, neither in the septal nor in the temporal hippocampus.

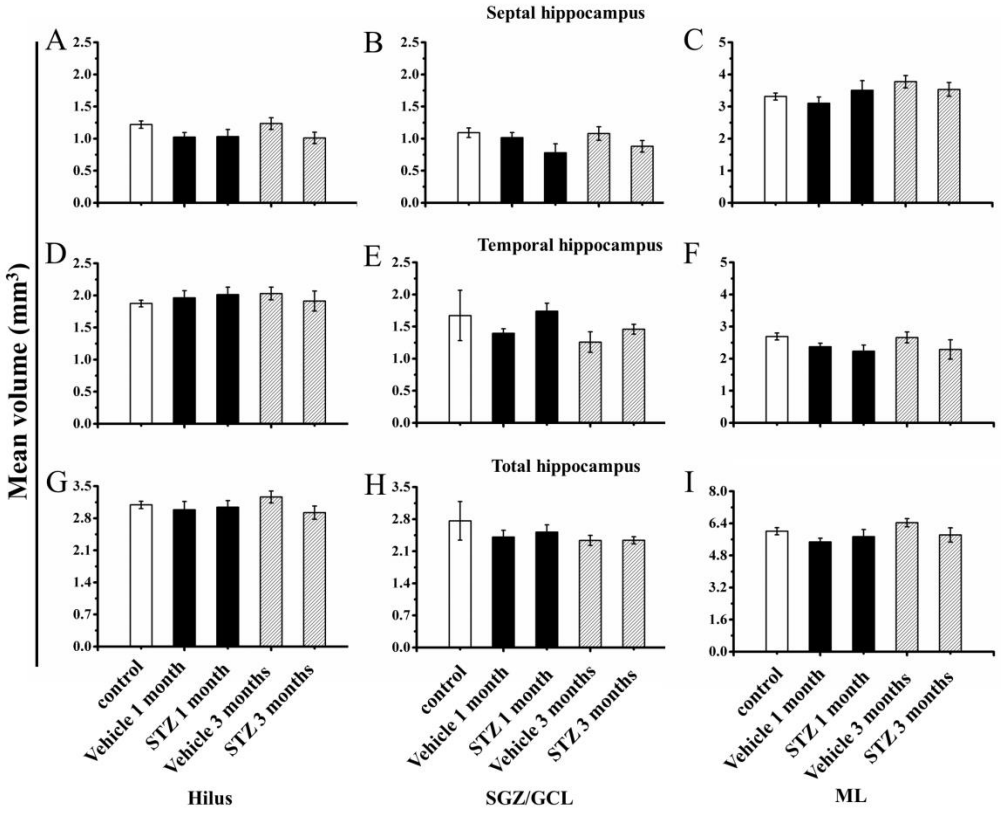


Fig.17. The volume of different dentate gyrus subregions is not affected by the STZ icv treatment

The volume of the hilus (A,D,G), subgranular zone including the granule cell layer (SGZ/GCL; B,E,H) and the molecular layer (ML; C,F,I) in the DG of each brain was assessed with the help of the StereoInvestigator software. Statistical evaluation of these measurements did not reveal any differences between experimental groups (n=6-8 rats per group), neither in the septal (A-C), the temporal (D-F), nor in the total hippocampus (G-I). Data are presented as the mean volume in mm^3 per hemisphere ± standard error of mean (SEM).

3.2 *In vitro* study results

3.2.1 Identification of neural stem cells and their progeny

In the presence of the "complete" proliferation cell culture medium, the hippocampus-derived NCSs proliferated quickly forming small free-floating clusters of NSCs first, and then forming larger so-called neurospheres after several days (Fig.18A). Neurosphere cells could be stained by an anti-nestin antibody (Fig.18B). Nestin is a marker for neural progenitor cells, which give rise to neurons as well as glial cells (Wiese et al., 2004). After replacing the proliferation cell culture medium to the differentiation cell culture medium, cultivation of neurospheres continued for 2 days. During this period of time differentiation and migration of these cells had been initiated and then verified via immunofluorescence staining using antibodies detecting for Tuj-1 (a marker for immature neurons) and GFAP (a marker for astrocytes) (Fig.18C).

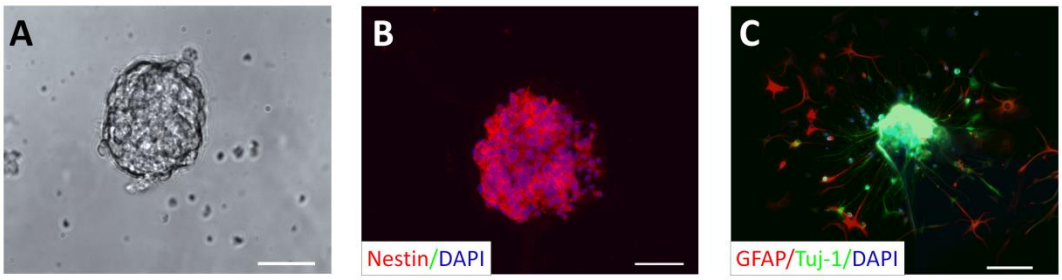


Fig.18. Identification of the cultured neural stem cells and their progeny
(A): Cells originating from adult rat hippocampus proliferated and formed free-floating neurospheres already one week after cultivating had started. (B): A representative neurosphere after the second passage shown to be immunoreactive for nestin, a marker for neural stem cells (red) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). (C): A neurosphere after the second passage followed by cultivating in differentiation culture medium for 2 additional days. Some cells originating from the neurosphere display immunoreactivity of Tuj-1, a marker for immature neurons (green) and most of them display immunoreactivity for GFAP, a marker for astrocytes (red). DAPI was used for counterstaining (blue) (C). Scale bar in A, B and C represents 50 µm.

3.2.2 STZ reduces the proliferation of neural stem cells

To determine the STZ concentration with optimal effectiveness on the proliferation of neural stem cells *in vitro*, separated neural stem cells (after the second passage) were seeded in a 96 well plate and treated with different concentrations of STZ (0, 1, 2.5, 5 and 10 mM). After four days, dose-dependent effects of STZ on the number of neurospheres were analyzed (Fig.19). Compared to the control group (0 mM STZ) treatment with different STZ concentrations decreased the number of neurospheres in a dose-dependent manner (1mM: 29.3%; 2.5 mM: 53.3%; 5 mM: 67.3% and 10 mM: 79.0%) (Fig.19). Considering middle-sized effects of STZ trying to avoid toxic effects of STZ, we chose a concentration of 2.5 mM STZ for all subsequent experiments.

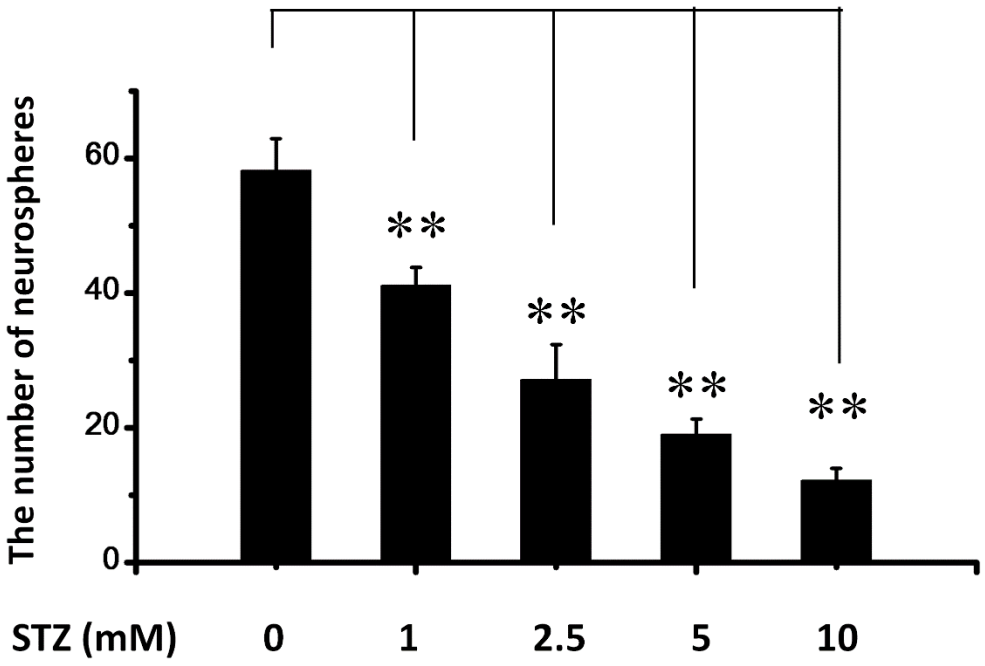


Fig.19. The number of neurospheres was decreased at various doses of STZ
Single cells separated from neurospheres after the second passage were incubated with various concentrations of STZ (0, 1, 2.5, 5 and 10 mM). After four days, STZ exhibits a dose-dependent effect on the form of neurospheres. Data are presented as the number of neurospheres per well ± standard deviation (SD). *: p-value <0.05; **: p-value <0.01.

One experiment dealt with the time-dependency of the effectiveness of STZ on the

proliferation of these neural stem cells (=on the generation of new neurospheres). As shown in Fig.20, from experimental day 2 on highly significant less neurospheres were counted in the STZ-containing culture medium compared to neurospheres in medium without STZ, whereupon the difference in number between these two treatment groups increased with the number of days of incubation (2 days by 25.1%; 4 days by 53.85%; 6 days by 59.8%; 8 days by 62.8%).

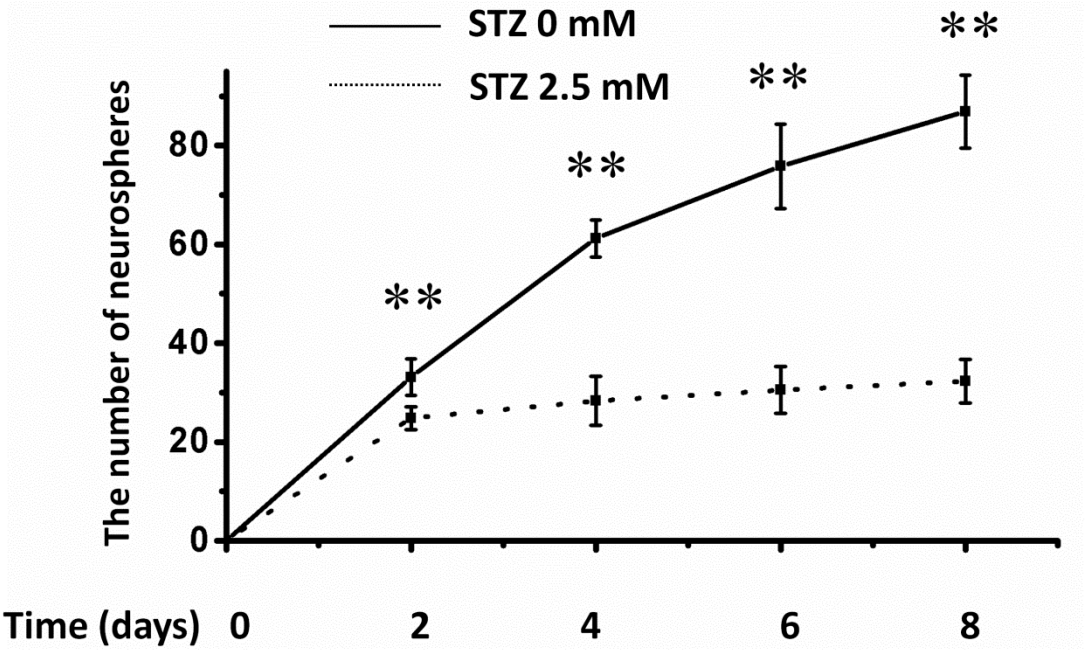


Fig.20. The number of neurospheres was decreased at various days of STZ
Single Cells separated from neurosphere after the second passage were incubated with 2.5 mM of STZ for different days. STZ exhibits a time-dependent effect on the form of neurospheres. Data are presented as the number of neurosphere per well ±standard deviation (SD). *: p-value <0.05; **: p-value <0.01.

As the size of each neurosphere reflects the proliferative capacity of neural stem cells each neurosphere is composed of, we measured the diameter of neurospheres after 2 days of incubation with or without STZ. A dramatically reduced mean size (by 32.5%) of neurospheres incubated in medium containing STZ compared to neurospheres incubated in medium without STZ was observed (Fig.21).

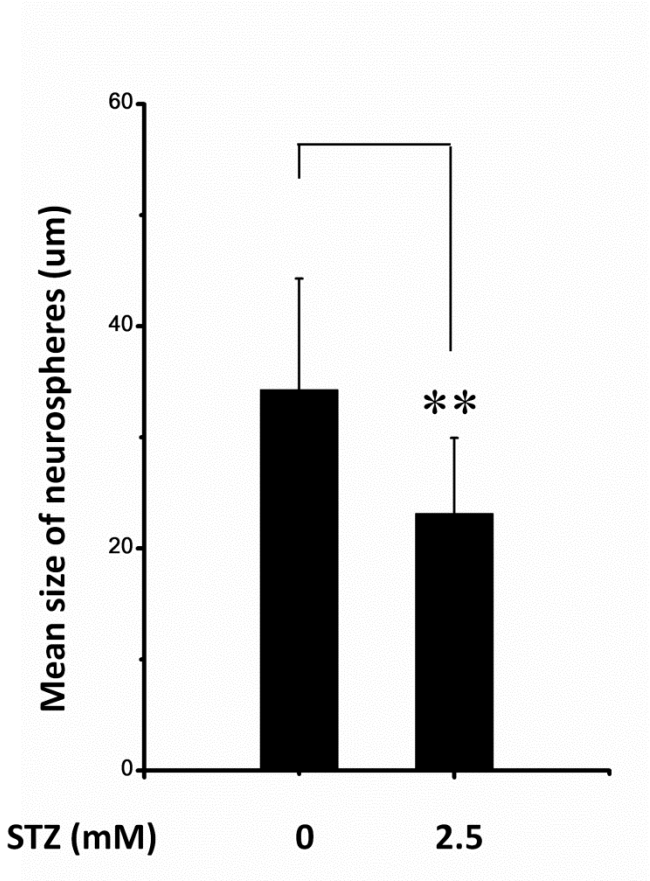


Fig.21. The diameter of neurospheres was decreased in consequence of STZ treatment
Single cells separated from neurospheres after their second passage were incubated in medium with 2.5 mM STZ for 2 days. Then, resulting neurospheres were pictured and the size of each neurosphere was analyzed with Image-Pro-Plus 5.0 software. Data are presented as the mean diameter of neurospheres ± standard deviation (SD). *: p-value <0.05; **: p-value <0.01.

BrdU is widely used to monitor cell proliferation as given in excess it incorporates into the DNA of dividing cells during their S-phase (Taupin, 2007). Under control conditions (without STZ treatment) about 20% of neural stem cells incorporated BrdU in 4 h as verified by immunofluorescence staining using an antibody detecting BrdU and DAPI counterstaining (Fig.22). When treated with STZ for 2 days, the fraction of BrdU-positive cells was decreased significantly (p=0.000661) and only about 12.9% of all cultured stem cells incorporated BrdU in 4 h (Fig.22C).

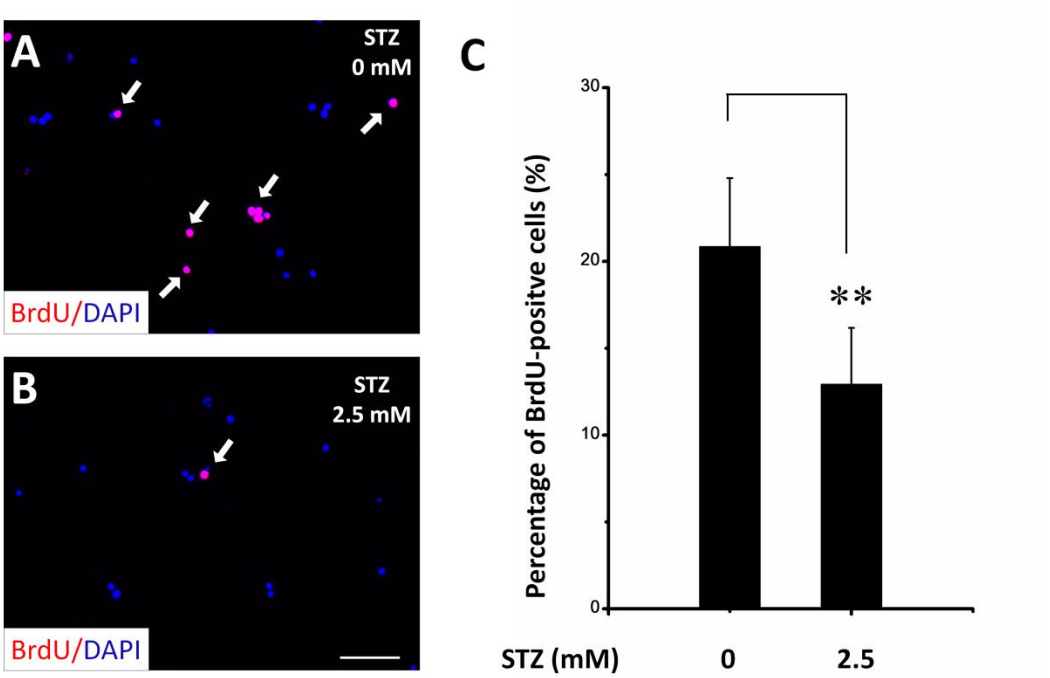


Fig.22. STZ treatment decreases the number of Bromodeoxyuridine (BrdU)-positive neural stem cells

Cells from neurospheres after their second passage were incubated in medium with (B) or without (A) 2.5 mM STZ for 2 days. These cells were then pulsed with BrdU (10 µM) for 4 h and fixedin 4% PFA for immunofluorescence stainings. Then, cells were pictured and analyzed with Image-Pro-Plus 5.0 software. Proliferation of neural stem cells was measured by BrdU-immunopositive nuclei, which were shown by red fluorescent immunostaining. The total cells were shown by staining with 4',6-diamidino-2-phenylindole (DAPI) (Blue). (C): The percentage of BrdU-positive cells from 10 replicate images was determined from different areas. Data are presented as the percentage of BrdU-positive cells of DAPI-positive cells ± standard deviation (SD). *: p-value <0.05; **: p-value <0.01. Scale=50 µm.

3.2.3 Effect of STZ on the migration of newborn differentiating cells

In SGZ, neural stem cells are shown to differentiate into immature neurons and then newly generated neurons migrate a short distance to the GCL to become granule neurons (Ming and Song, 2005). In order to study whether STZ treatment influences the migration capabilities of cells starting to differentiate, 10-15 neurospheres were plated onto poly-L-ornithine/laminin-coated coverslips in 24 well plates and were incubated in differentiation culture medium with or without

different concentrations of STZ for 2 days. As shown in Fig.23, STZ treatment for 2 days did not significantly affect the migration of newborn cells.

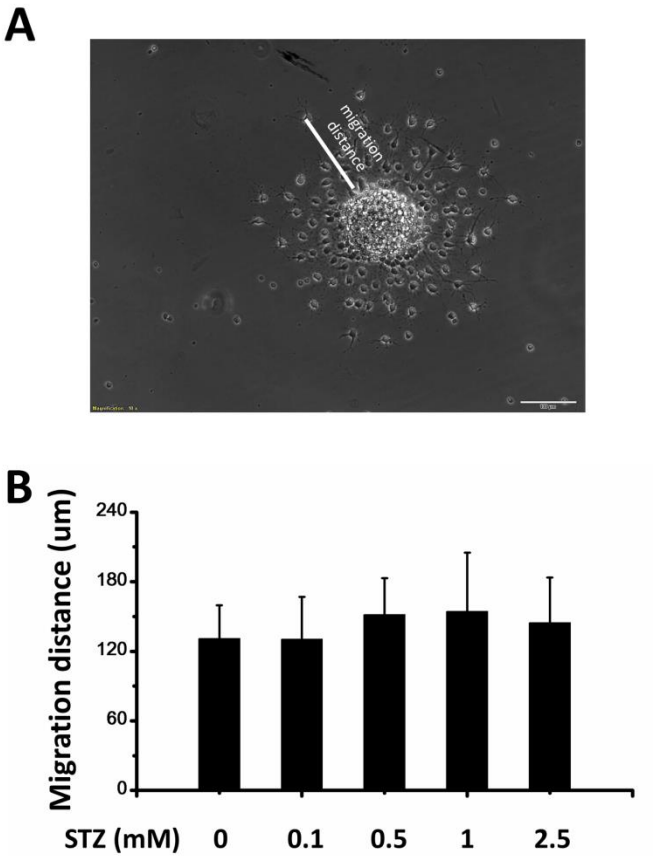


Fig.23. STZ does not influence the migration of differentiating cells
10-15 neuropheres were plated onto poly-L-ornithine/laminin-coated coverslips in 24 well plates and were incubated in differentiation culture medium with or without STZ for 2 days. Then microscope images were taken and analyzed with Image-Pro-Plus 5.0 software. (A): Representative image of migrating cells originating from a neurosphere. Scale bar represents 100 µm. (B): Average migration distances of neural stem cells with (0.1 mM, 0.5 mM, 1.0 mM and 2.5 mM) or without (0 mM) STZ treatment. Data are presented as the distance measured from the respective cell and the rim of the neurosphere ± standard deviation (SD). *: p-value <0.05; **: p-value <0.01.

3.2.4 STZ affects the neuronal fate of newborn cells

The neuronal phenotype of differentiating cells was determined by using Tuj-1 as a marker of immature neurons and GFAP as a marker for astrocytes. After 2 days of incubation in the differentiation medium, Tuj-1- and GFAP-positive cells were found

in immediate vicinity of the neurospheres (Fig.18C). Our results demonstrate that the percentage of cells immunoreactive for Tuj-1 were significantly lower in the STZ treatment group in comparison to those in the normal control group (a decrease of 45.5%; p=0.003) (Fig.24C). However, the percentages of cells immunopositive for GFAP were not significantly different between the STZ treatment and the control group (Fig.24D).

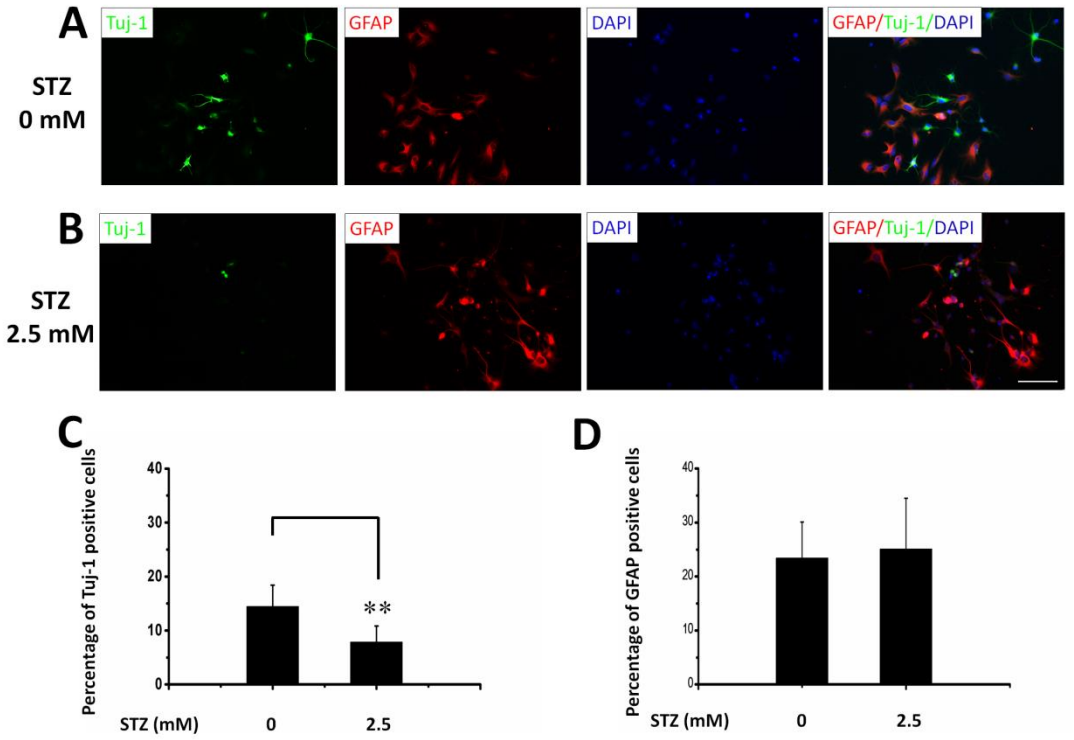


Fig.24. STZ decreases the number of newborn cells differentiating to neurons but has no effect on the differentiation to astrocytes

Cells originating from neurospheres (after the second passage) were incubated in differentiation culture medium with or without 2.5 mM STZ for 2 days, then stained with antibodies. Microscope images were taken and analyzed with Image-Pro-Plus 5.0 software. (A-B): Representative images of already differentiated cells as determined by Tuj-1 (green) and GFAP (red) immunocytochemistry after 2 days of incubation with (B) or without STZ (A). Nuclei of all cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). (C): The percentage of Tuj-1-positive cells using 10-12 replicate images was determined. (D): The percentage of GFAP-positive cells from 10-12 replicate images was determined from the periphery of the neurosphere colony area. In both cases, C and D, the analysed area comprises the periphery of the respective neurosphere. Data are presented as the percentage of Tuj-1 (C) or GFAP (D) positive cells of all DAPI positive cells ± standard deviation (SD). *: p-value <0.05; **: p-value <0.01. Scale bar represents 50 µm.

Furthermore, after two days of differentiation in the presence of STZ, the number and length of Tuj-1-positive cell processes seemed to be reduced compared to the number and length of cell processes of the control group (without STZ treatment) (Fig.25B).

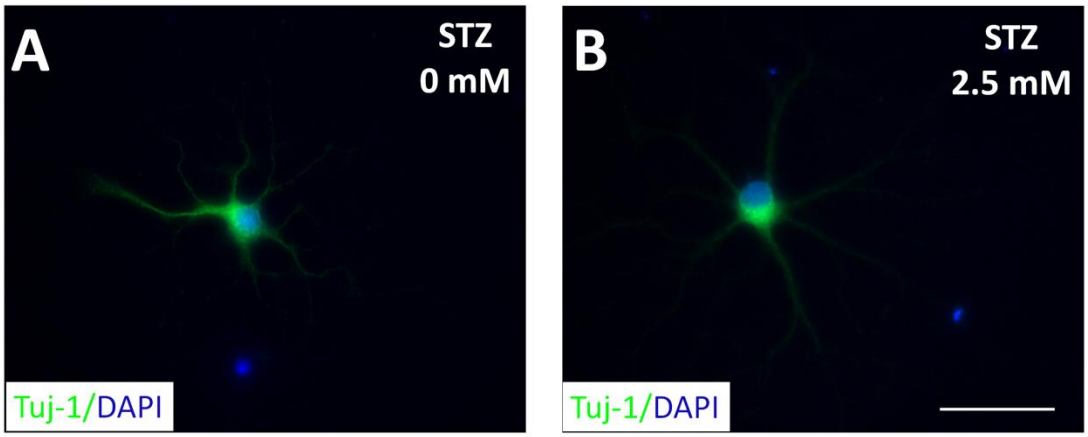


Fig.25. STZ impaired the growth of processes in Tuj-1 positive cells
Cells originating from neurospheres (after the second passage) were incubated in differentiation medium containing 0 or 2.5 mM STZ for 2 days, then stained with antibodies detecting Tuj-1. Representative image of processes in Tuj-1 positive cells (green) after 2 days of treatment with (B) or without STZ (A) counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Blue). Scale=20 µm.

3.2.5 Effect of STZ on insulin receptor, insulin-like growth factor 1 and glucose transporter 1 and 3 mRNA expression in neural stem cells

In the STZ icv treatment rat model (*in vivo*), brain glucose/energy metabolism abnormalities were found in all hippocampal subfields, such as decreased glucose utilization (Duelli et al., 1994). GLUT3 is most known for its specific expression in neurons and has originally been designated as the neuronal glucose transporter (Kayano et al., 1988). But, GLUT3 has been found to be expressed in adult NSCs, too (Maurer et al., 2006). Furthermore, in the STZ icv rat model, insulin system dysfunction was found and insulin receptor expression decreased significantly in hippocampus (Grunblatt et al., 2007). In order to study the effect of STZ on the expression of glucose metabolism related genes,

we collected neurospheres after incubation in proliferation medium for 2 days and then performed qRT-PCR for the detection of relative mRNA expression of IR, IGF1, GLUT1 as well as GLUT3. In Fig.26 the relative expression levels of GLUT3 mRNA is shown to be reduced by 46.4% (p=0.041) in consequence of STZ treatment. Besides this significant difference, a trend towards lower relative mRNA expression was foung for IGF1 in the STZ treatment group compared to the control group (p=0.057; Fig.26). Other than these, the expression of IR, IGF1 and GLUT1 in NSCs seems to be unaffected by STZ treatment (Fig.26).

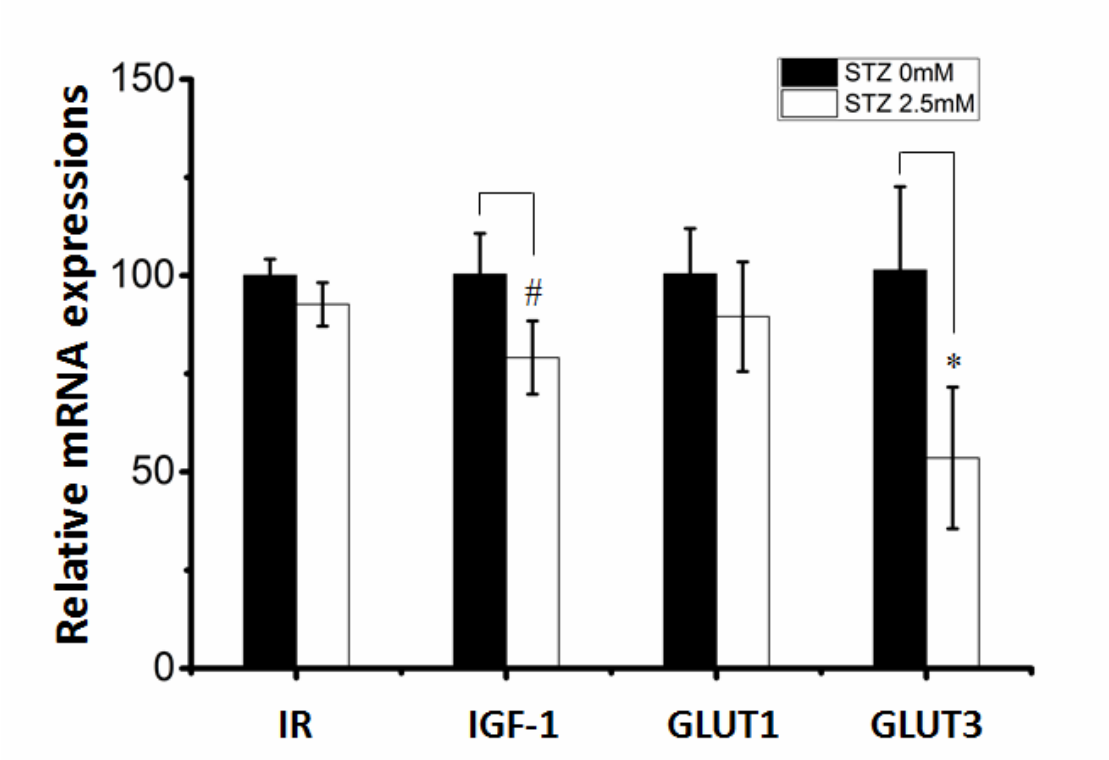


Fig.26. Effect of STZ on the gene expression of insulin receptor, insulin-like growth factor 1 and glucose transporter in neural stem cells

Cells originating from neurospheres (after the second passage) were incubated in proliferation culture medium in a 6-well plate with or without 2.5 mM STZ for 2 days, then cells were collected and analyzed via qRT-PCR. The relative mRNA expression of glucose transporter 3 (GLUT3) is reduced by 46.4% (p=0.041) in consequence of STZ treatment. No significant difference of the relative mRNA expression of insulin receptor (IR), insulin-like growth factor 1 (IGF1), glucose transporter 1 (GLUT1) in STZ treated cells compared to cells in the control medium without STZ was found. Data are presented as the relative mRNA expressions of IR, IGF1, GLUT1 or GLUT3 ± standard deviation (SD); *: p-value <0.05, #: 0.05<p-value <0.1.

3.2.6 Effect of STZ on the expression of insulin receptor protein in neural stem cells and differentiating cells

In order to study whether STZ could influence the expression of insulin receptor also *in vitro*, we stained NSCs (Fig.27) and NSCs after incubation in differentiation medium for 2 days (Fig.27) with antibodies detecting the insulin receptor and counterstained with DAPI. For the the investigation of NSCs, we separated neurospheres into single cells and seeded them in a 24-well plate with pre-coated coverslips at 20,000 cells/well. After 2 days of incubation in proliferation culture medium with or with 2.5 mM STZ, we stained them for IR. In Fig.27A it can be seen that most of the NSCs express IR, when STZ is absent in the culture medium. Thus, most NSCs express insulin receptor under normal conditions. However, incubation of NSCs culture medium with 2.5 mM STZ resulted in overall decreased number of NSCs and the percentage of NSCs with IR expression was decreased (to 42.2%; p=0.003) (Fig.27D, Fig.29).

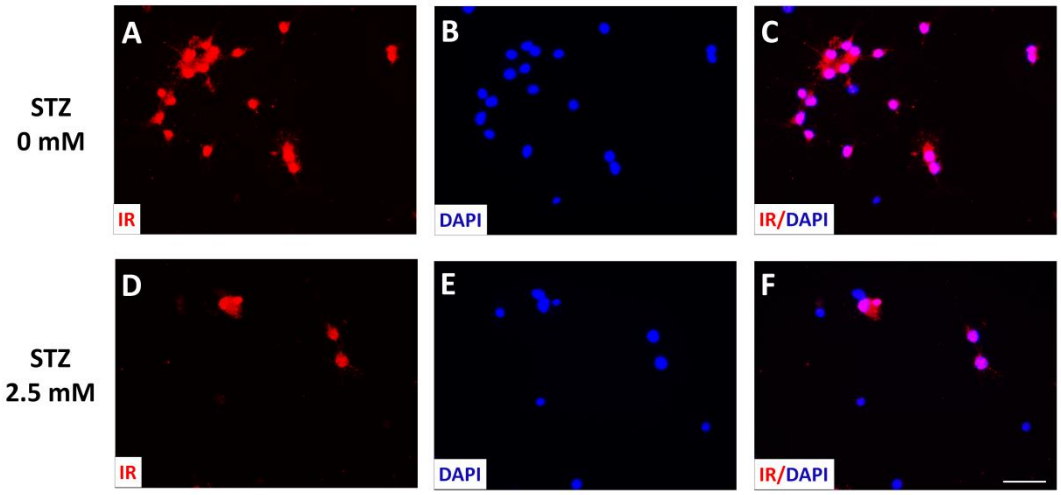


Fig.27. The effect of STZ on Insulin receptor expression in neural stem cells

We separated neurospheres into single cells and seeded them in a 24-well plate provided with pre-coated coverslips at 20,000 cells/well. After 2 days of incubation in proliferation culture medium without or with 2.5 mM STZ representative images (A-F) display that a high percentage of neural stem cells stained with 4',6-diamidino-2-phenylindole (DAPI) (B, Blue) were immunopositive for the insulin receptor (IR) (A, red; pink, C). After 2 days of incubation in medium containing 2.5 mM STZ not only the number DAPI-positive cells but also the number of double stained (IR and DAPI) cells is reduced. Scale bar in F=20 µm.

For the analysis of differentiating cells, 10-15 neuropheres were plated into poly-L-ornithine/laminin-coated coverslips in a 24-well plate and were grown/incubated in differentiation culture medium with or without 2.5 mM STZ for 2 days. Our results revealed that two days after differentiation had started, most cells still express IR (Fig.28A-C). Other than the effect of STZ on NSCs, the expression of IR in differentiating cells seems to be unaffected by STZ treatment (Fig.28A-F).

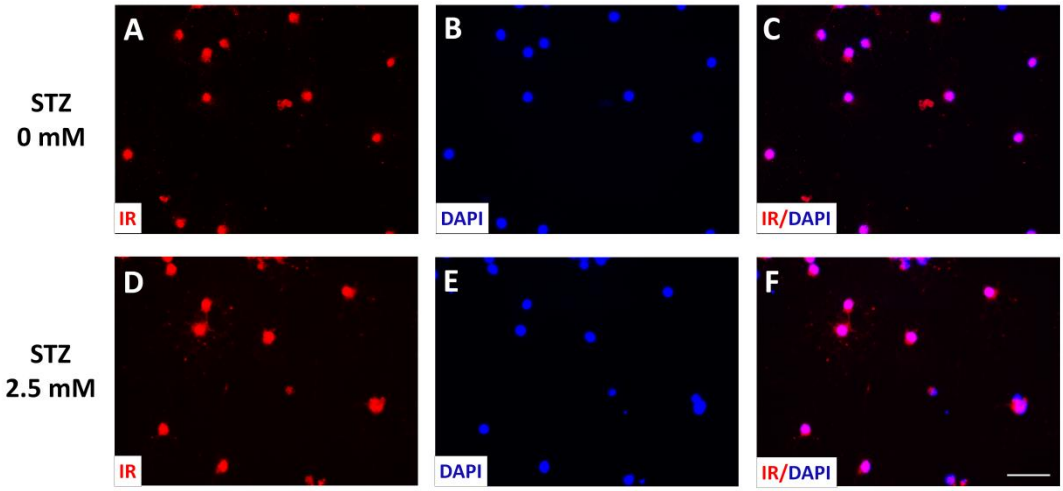


Fig.28. The effect of STZ on insulin receptor expression in differentiating cells
10-15 neuropheres were plated onto poly-L-ornithine/laminin-coated coverslips in a 24-well plate and were incubated in differentiation culture medium with or without 2.5 mM STZ for 2 days. We then immunostained them with insulin receptor antibody (IR, red) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). A-F: Representative images show that the number of differentiating cells and the expression of IR was not changed in STZ treated cells (D-F) compared to untreated cells (A-C). Scale bar in F=20 µm.

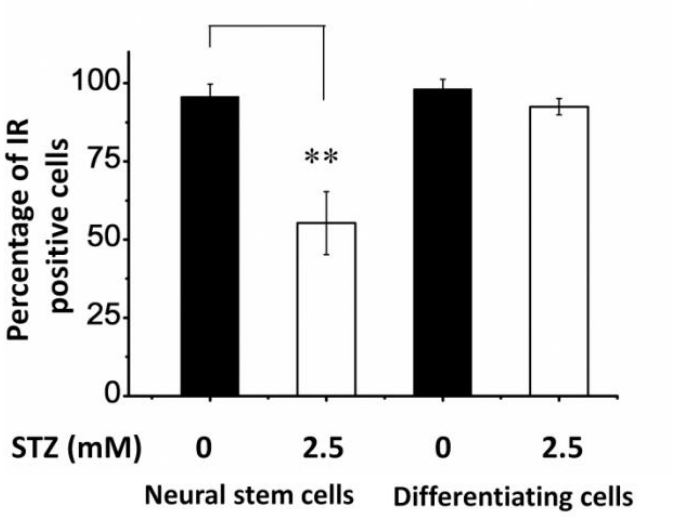


Fig.29. The impact of STZ on the percentage of Insulin receptor-positive neural stem cells and differentiating cells – a quantitative estimation

The percentage of insulin receptor (IR)-expressing neural stem cells is reduced by 42.2% (p=0.003) in consequence of STZ treatment. In differentiating cells, no significant difference of the percentage of IR expression in STZ treated cells compared to cells in the control medium without STZ was found. Data are presented as the percentage of IR-positive cells ± standard deviation (SD); **: p-value <0.01.

3.2.7 Effect of STZ on the expression of glucose transporter 3 protein in neural stem cells and differentiating cells

In our study, we stained GLUT3 using immunofluorescence staining in both, NSCs and differentiating cells, comparable to what we did in with antibodies detecting the insulin receptor above (see Fig.27 and 28). For NSCs, we separated neurospheres into single cells and seeded them in 24-well plates with pre-coated coverslips at 20,000 cells/well. After 2 days of incubation in proliferation culture medium with 0 mM or 2.5 mM STZ, we stained them for GLUT3 (Fig.30). As shown in Fig.30A, GLUT3 is expressed in nearly all NSCs. However, two days of incubation in cell culture medium containing 2.5 mM STZ resulted in dramatically decreased number of immunostained cells expressing GLUT3 (a decrease of 61.7%; p=0.000064) compared to the controls (Fig.30D, Fig.32).

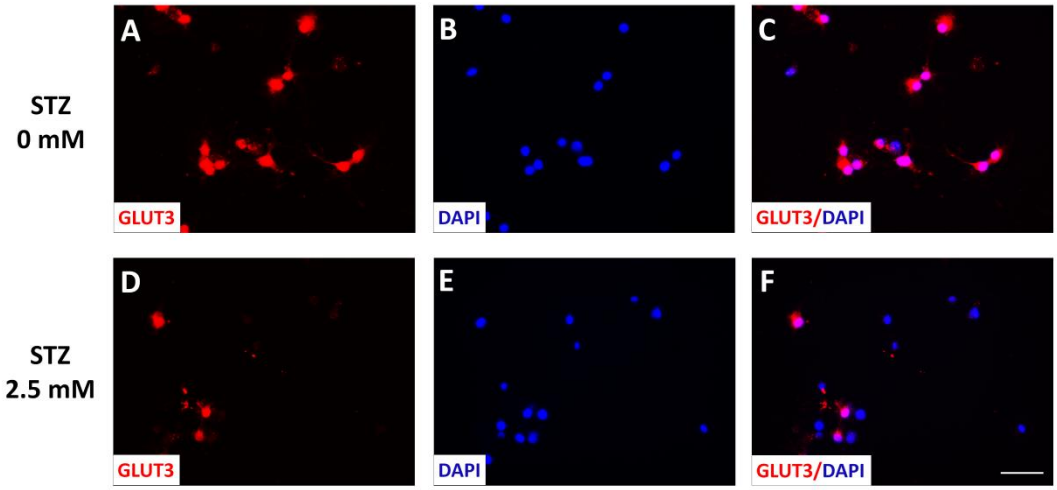


Fig.30. The effect of STZ on Glucose transporter 3 expression in neural stem cells

We separated neurospheres into single cells and seeded them in a 24-well plate provided with pre-coated coverslips at 20,000 cells/well. After 2 days of incubation in proliferation culture medium without (0 mM) or with 2.5 mM STZ we performed immunofluorescence staining using antibodies detecting the glucose transporter 3 (GLUT3; red) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Representative images of GLUT3-immunopositive NSCs display that under normal condition most neural stem cells express GLUT3 (A-C), and that STZ treatment did not only result in less GLUT3 expressing NSCs, the percentage of GLUT3 expressing NSCs cells is found to be reduced (D-F). Scale=20 µm.

For the analysis of GLUT3 expression in differentiating cells, we performed an experiment similar to our IR study in differentiating cells (see 3.26). 10-15 neuropheres were plated onto poly-L-ornithine/laminin-coated coverslips in 24 well plates and we let them grow in differentiation culture medium with or without 2.5 mM STZ for 2 days. Our results show that GLUT3 express in every differentiating cells (Fig.31A) and not only express in the cell body but also in the process of differentiating cells (Fig.31A). With 2.5 mM STZ treatment, some differentiating cells could not express GLUT3 (a decrease of 47.3%; p=0.015) (Fig.31D, Fig.32).

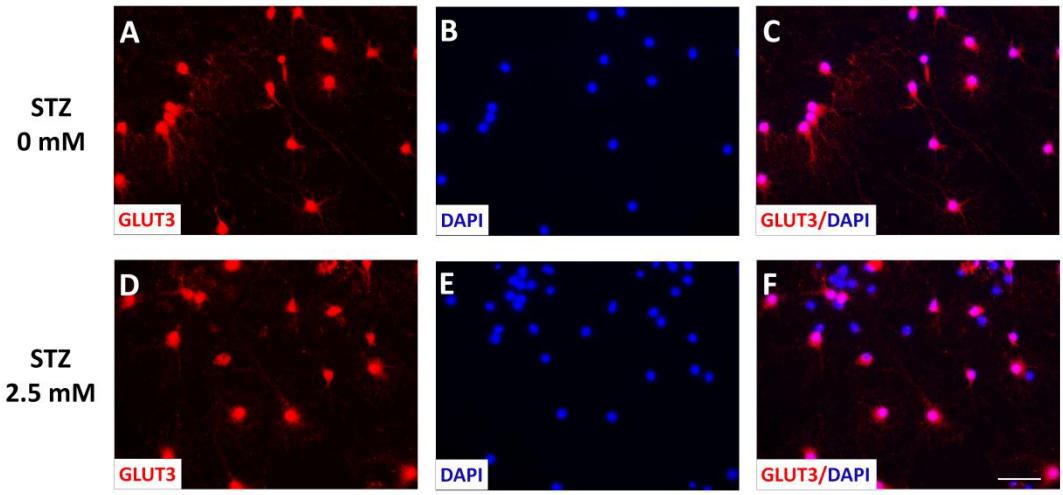


Fig.31. The effect of STZ on Glucose transporter 3 expression in differentiating cells

10-15 neuropheres were plated onto poly-L-ornithine/laminin-coated coverslips in a 24-well plate and were incubated in differentiation culture medium with or without 2.5 mM STZ for 2 days, so that the differentiation process started. These differentiating cells were then immunostained with glucose transporter 3 (GLUT3) antibodies (red) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Representative images of differentiating cells after GLUT3 immunofluorescence staining show that most of these cells express GLUT3 under normal conditions (A-C) and that STZ reduces the percentage of cells expressing GLUT3 (D-F). Scale bar =20 µm.

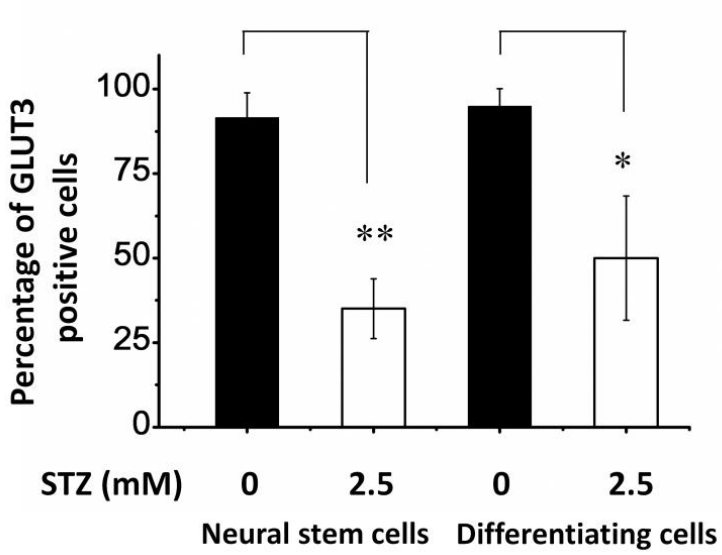


Fig.32. The impact of STZ on the percentage of Glucose transporter 3-positive neural stem cells and differentiating cells – a quantitative estimation

The percentage of Glucose transporter 3 (GLUT3)-expressing neural stem cells is reduced by 61.7% (p=0.000064) in consequence of STZ treatment. With regard to the percentage of differentiating cells a reduction of 47.3% (p=0.015) was found in STZ treated cells compared to cell in the control medium without STZ. Data are presented as the

percentage of GLUT3-positive cells± standard deviation (SD).*: p-value of t- test <0.05; **: p-value <0.05.

3.3 Summary

In summary, our *in vivo* study revealed, that even if STZ icv treatment does not seem to considerably influence stem cell proliferation in the hippocampus, neither over a short-term period (1 month after STZ icv treatment) nor in the long-term (3 months after STZ icv treatment), the generation of immature and adult neurons is diminished in the hippocampus of rats 3 months after STZ icv treatment. This reduction detected after 3 months was specific for the septal hippocampus, discussed to be important for spatial learning. Applying double immunofluorescence staining using antibodies detecting BrdU (applied appr. 27 days before sacrifice) and cell-type specific markers we found that STZ treatment does not affect the differentiation fate of newly generated cells. Phenotype analysis of BrdU-positive cells in the hilus and molecular layer revealed that some of the BrdU-positive cells are newborn oligodendrocytes but not newborn microglia.

However, results of our *in vitro* study showed that STZ in the cell culture medium affects AN at different stages, during the proliferation of NSCs and during differentiation of their progeny. In more detail, I could show that STZ inhibits the proliferation of NSCs in a dose-dependent and time-dependent manner. In addition to what we could show in the *in vivo* study, that STZ treatment affects the number of newly generated neurons, the *in vitro* approach showed that STZ treatment does not affect the generation of new astrocytes. Trying to reveal cellular mechanisms underlying the negative influence of STZ on hippocampal AN, we performed qRT-PCR and immunofluorescence staining and found that in NSCs GLUT3 expression was reduced at the mRNA as well as protein level, but that the IR was found to be diminished only at the protein level after STZ treatment. QRT-PCR using primer

pairs specific for the detection of IGF1 and GLUT1 didn`t show mRNA expression differences between differently treated NSCs. Interestingly, the effect of STZ on differentiating cells seems to be different compared to the effect of STZ on proliferating NSCs, as in differentiating cells IR protein levels were not significantly changed in consequence of STZ treatment but GLUT3 protein levels were shown to be decreased, too.

4. Discussion

4.1 *In vivo* study

In our *in vivo* study, we analyzed hippocampal AN in STZ icv treated rats, an animal model for sAD. We focused on different AN stages, such as cell (e.g. stem cell) proliferation, neuronal differentiation and cell survival to determine whether these AN phases are differentially affected by STZ icv treatment.

4.1.1 STZ treatment does not affect the cellular fate of newborn cells

We verified the birth of new neurons via double staining of BrdU-positive cells, which had survived up to 27 days, with NeuN, a marker for mature neurons, irrespective of the neuronal phenotype (e.g. excitatory glutamatergic neurons or inhibitory GABAergic neurons). As the percentage of newborn neurons (BrdU+/NeuN+) cells in relation to the number of BrdU+ cells was similar in all treatment groups, we could not reveal short-term or long-term effects of STZ icv treatment on the differentiation outcome. We disclosed that about 50% of all BrdU+ cells in the SGZ and GCL of the dentate gyrus also expressed NeuN, a percentage similar to the percentage reported by other groups (Aberg et al., 2000, Malberg et al., 2000, Schmitt et al., 2007, Winner et al., 2012). Interestingly, irrespectively of the treatment group we always detected more long-term survived BrdU-ir cells in the hilus and ML of the DG than in the GCL including the SGZ (=SGZ/GCL). This distribution pattern of long-term survived BrdU-positive cells in the rat DG (ML>hilus>SGZ/GCL) resembles the distribution pattern found by Mandyam and coworkers in mouse DG (ML>hilus≥oGCL) (Mandyam et al., 2007). In contrast, Donavan and coworkers detected most of the surviving BrdU-positive cells in the SGZ, many cells in the ML and fewest in the hilus and oGCL (SGZ≥ML>oGCL≥hilus) (Donovan et al., 2006). However, this almost exclusive distribution of BrdU+/NeuN+-cells in the

SGZ and GCL, and not in ML and hilus, reassured that the birth of neurons primarily happens in these neurogenic regions SGZ and GCL in rodents (Yagita et al., 2001, Donovan et al., 2006) as also found in humans (Eriksson et al., 1998) and macaque monkeys (Kornack and Rakic, 1999). This regional distribution of AN is further supported by the fact that marker for immature neurons such as NeuroD (mitotic) and DCX (postmitotic), we also used in this study, are exclusively expressed in the SGZ/GCL in rodents (Takasawa et al., 2002, Donovan et al., 2006), humans (Anacker et al., 2011) and monkeys (Ma et al., 2008). Although in our study the results of NeuroD and DCX stainings are similar, these two markers represent neuronal cells of different developmental stages.

In the ML and hilus, we could show that some of these newborn and 28 days survived cells are oligodendrocytes using double immunofluorescence staining with antibodies detecting BrdU and APC, a marker for oligodendrocytes. In accordance with these findings, Kornack and Rakic detected some BrdU-labeled cells which also express two different markers for oligodendrocytes, O4 and CNP, in the hilus and ML of adult macaque monkeys (Kornack and Rakic, 1999). In a mouse study, cells co-labeled for BrdU and the oligodendrocyte marker NG2, were almost exclusively found in the ML (Bribian et al., 2012). This suggests that the birth of new oligodendrocytes in the ML and hilus may be a common phenomenon not only in our rat model, but also in mice (Bribian et al., 2012) and monkey (Kornack and Rakic, 1999). Loss of oligodendrocytes in the hippocampus in monkeys in consequence of STZ icv treatment has been found by Lee et al., whereas they did not show that STZ really affects the generation of new oligodendrocytes (Lee et al., 2014). In general, new oligodendrocytes can be generated from oligodendrocyte progenitor cells and NSCs In the adult brain, and several factors have been found to be involved in their production, such as EGF (Gonzalez-Perez et al., 2009), sonic hedgehog (Lu et al., 2000), platelet-derived growth factor

(Woodruff et al., 2004), chordin (Jablonska et al., 2010) and sirtuin 1 (Rafalski et al., 2013).

4.1.2 STZ icv treatment does not affect cell proliferation, but affects differentiation and survival of newborn cells, a later stage of adult neurogenesis

In our quantitative immunohistochemistry study using antibodies detecting the proliferation marker MCM2 we could not reveal statistically significant differences between cell proliferations in the SGZ of STZ icv treated rats and the respective controls, neither in STZ 1 month groups nor in STZ 3 months groups. In accordance with our results Arabpoor and coworkers didn`t detect significant differences in the number of Ki67-positive cells, another frequently used marker for the detection of proliferating cells, 1 month after STZ icv treatment (Arabpoor et al., 2012). In contrast to our findings, Qu and coworkers showed that 20 days after STZ icv treatment the number of BrdU-positive cells was decreased (Qu et al., 2012). As they applied BrdU daily on 12 consecutive days before rats had been sacrificed their experimental design was not aimed solely at the analysis of stem cell proliferation, whereas their study results cannot be compared directly with our results.

In contrast, we detected significantly decreased number of newly generated mitotic neuroblasts and postmitotic immature neurons as detected by NeuroD and DCX immunohistochemistry, respectively, in the septal hippocampus of rats 3 months after STZ icv treatment. This phenomenon of less immature neurons probably represents the source for the reduced number of BrdU-ir cells (survived for approximately 27 days) we have detected in the same part of the hippocampus. Based on this we conclude that the STZ icv treatment primarily affects AN at the level of differentiation and survival of newborn

cells. However, one month survival after STZ icv injections did not affect the number of NeuroD and DCX positive cells. Therefore, cognitive deficits already shown in rats 2-4 weeks after STZ icv injections (see introduction) do not correlate with AN changes in the brain of rats 1 month after STZ icv treatment, and at this early stage must have other neurobiological correlates than the decreasing number of differentiating newborn cells in rats 3 months after STZ icv treatment. As the concentrations of glucose and ATP are shown to be decreased in the cerebral cortex from 3 weeks following STZ icv administration on (Lannert and Hoyer, 1998), brain insulin receptor signaling dysfunction (Lannert and Hoyer, 1998), and a progressive increase of oxidative stress is also shown very early after STZ icv treatment (Ishrat et al., 2006, Khan et al., 2012, Mansouri et al., 2013, Zhou et al., 2013) these biochemical changes could be responsible for the early cognitive decline. However, Rai et al. (2014) suggested that post-synaptic neurotoxicity is one key factor in STZ induced memory impairment. They showed spatial memory deficits in rats 14–16 days after STZ icv administration accompanied by reduced expression of post-synaptic marker CaMKIIα and PSD-95 in cortex and hippocampus, while pre-synaptic marker such as synaptophysin and SNAP-25 remained unaltered (Rai et al., 2014).

As AN is decreased in rats 3 months, and not 1 month after STZ icv treatment, these changes resemble the timeline of morphological changes as congophilic Aβ aggregates in meningeal capillaries and intracellular Aβ42 accumulation in cerebral cortex were found not earlier than three months after STZ icv administration (Salkovic-Petrisic et al., 2006, Salkovic-Petrisic et al., 2007, Salkovic-Petrisic et al., 2011). Even more, diffuse Aß42-positive extracellular plaque-like formations in neocortex and hippocampus could not be detected earlier than 6 months (Knezovic et al., 2015). Therefore, only subtle effects of STZ icv treatment on Aß and Tau pathology, but extensive biochemical and behavioral changes are detectable already one month after STZ icv treatment,

whereas structural changes including the appearance of Aß42 accumulation and altered AN are detectable not before 3 months after STZ icv treatment. Probably, an early stage of reversible cognitive impairment initially caused by synaptic dysfunction (Rai et al., 2014), energy deficits (Lannert and Hoyer, 1998) and increasing oxidative stress (Ishrat et al., 2006, Khan et al., 2012, Mansouri et al., 2013, Zhou et al., 2013) and paralleled by subtle effects of STZ icv treatment on Aß and Tau patholoy could become irreversible in association with decreased AN and more comprehensive Aß42 changes. Therefore, reduced AN may maintain the cognitive decline and may contribute to its general progressivity and irreversibility found in later stages of AD.

In mammals, stem cells in adult organs reside in specialized niches in close proximity to blood vessels - in so-called angiogenic niches (Palmer et al., 2000). Therefore, AN is controlled by factors derived from blood vessels and their microenvironment such as secreted neurotrohic and angiogenic factors (e.g. BDNF, VEGF, TGF-ß) (Goldberg and Hirschi, 2009, Goldman and Chen, 2011). As congophilic Aβ aggregation in the meningeal capillaries as well as diminished AN was detected in brains of rats 3 months after STZ administration (Salkovic-Petrisic et al., 2011), cerebral amyloid angiopathy could be discussed to comprehensively impact the phenomenon of AN in this animal model of sAD. A possibly altered angiogenic niche in AD was pointed to by a study of Boekhoorn and coworkers using human post mortem tissue of presenile AD patients as they detected increased Ki67 immunoreactivity in endothelial structures of AD patients compared to respective controls (Boekhoorn et al., 2006). As vascular structure appears impaired in AD (Wu et al., 2005) increased Ki67 immunoreactivity in vascular cells may reflect an aberrant response that could contribute to vascular dysfunction. Boekhoorn and coworkers proposed that “vascular-associated Ki67 expression in the presenile AD hippocampus is either non-functional or even dysfunctional as vascular function is compromised in AD” (Boekhoorn et al., 2006).

That energy metabolism, especially glucose metabolism and insulin receptor signaling, could have an influence on AN is substantiated by microarray studies performed by Geschwind et al. (Geschwind et al., 2001) and Ivanova and coworkers. They showed that proliferation of neural stem cells (NSCs) and the transition from a newborn undifferentiated cell to a differentiated (mature) neuron, astrocyte, or oligodendrocyte is accompanied by many changes of the expression of metabolism related genes such as Insulin-like growth factor binding protein 3, Cytochome c oxidase (Geschwind et al., 2001), Enolase 1, Acetyl-coenzyme A synthetase 1 and Pyruvate dehydrogenase E1 alpha subunit (Karsten et al., 2003). The Insulin/IGF-1 signaling pathway is central in coordinating energy taking and expenditure (Rafalski and Brunet, 2011). Although IGF-1 mRNA was detected in all regions of the neonatal brain, there is considerable regional variation in the level of expression with highest expression levels in regions where neurogenesis persists after birth such as the hippocampus and the subventricular zone (Bartlett et al., 1991). The association of AN and insulin signaling pathways is substantiated by the fact that adult NSCs express the IGF-1 receptor (Aberg et al., 2003) and that the insulin gene can be activated by the transcription factor NeuroD (also called Beta2) (Naya et al., 1995).

4.1.3 Hippocampal subregions were differently influenced by STZ icv treatment – which points to different connectivities and functions

We analyzed the septal and temporal part of the hippocampus separately, as it is suggested that the hippocampus is functionally different along its septo-temporal axis (Moser and Moser, 1998) and that septal regions are involved mainly in learning (especially in spatial learning) and memory processes (Moser et al., 1995). The most prominent cortical projections from the septal CA1 and the septal parts of the subicular complex are to the retrosplenial and anterior cingulated cortices in rats

(Cenquizca and Swanson, 2007) and monkeys (Kobayashi and Amaral, 2007). These two cortical regions have been found involved primarily in the cognitive processing of visuospatial information and memory processing as well as environmental exploration (Fanselow and Dong, 2010). The temporal parts of hippocampal-subiculum complex send its projections to olfactory bulb together with several other primary olfactory cortical areas, and share massive bidirectional connectivity with amygdalar nuclei (Cenquizca and Swanson, 2007). These regions regulate the impact of emotional experiences and to control general affective states (Fanselow and Dong, 2010). Strongest long-term effects of STZ icv treatment on hippocampal AN were found in the septal hippocampus and STZ icv treatment is shown to result in spatial learning deficits which resembles the spatial memory deficits in AD patients. Our results regarding the different involvement of the septal and temporal hippocampus in neuroplasticity phenomena are in line with findings obtained by other AD researchers. Fuster-Matanzo and coworkers showed increased neurodegeneration in consequence of GSK3ß overexpression in mice exclusively in septal hippocampus (Fuster-Matanzo et al., 2011). In addition, increased CREB function was revealed exclusively in the CA1 region of septal hippocampus in TgCRND8 mice which was sufficient to restore function in neuronal structure, network activity as well as learning and memory (Yiu et al., 2011).

The importance of AN for spatial learning is substantiated by the finding that the performance in a spatial learning test correlates with the number of NeuroD-positive cells, which are mitotic active neuroblasts, a pre-stage of the postmitotic immature neurons (Karabeg et al., 2013). Moreover, the study of human hippocampal neurogenesis by Coras and colleagues revealed that patients with low numbers of proliferating progenitor cells and newborn neurons showed more severe learning and memory impairments (Coras et al., 2010). As shown by the study of McClean and coworkers AN could not only play a role in the etiopathogenesis of AD, but also in the improvement of cognition (McClean et al., 2011). These authors were able to reveal improved learning and memory performances in TgAPP/PS1 mice after treatment

with liraglutide, a glucagon-like peptide-1 analog used for the treatment of diabetes, in parallel to an AN increase in the hippocampus. Similar effects were shown in TgAPP/PS1 transgenic mice after treatment with the neuroprotective Gingko biloba extract EGb 761 (Tchantchou et al., 2007).

In conclusion, as hippocampal AN is demonstrated to be diminished in STZ icv treated rats (Fig. 30), a non-transgenic animal model for sAD, this *in vivo* study provides further evidence that hippocampal AN could be involved in the etiopathogenesis of sAD.

4.2 *In vitro* study

In our *in vitro* study, we analyzed the effect of STZ on the hippocampus-derived NSCs. We focused on different AN stages, such as stem cell proliferation, cell migration and neural differentiation, whether these AN phases are differentially affected by STZ treatment. Besides, we analyzed mRNA as well as protein expression of the insulin receptor (IR) and glucose transporter 3 (GLUT3) in NSCs and differentiating cells.

4.2.1 STZ treatment significantly decreases stem cell proliferation

In our study, the number and size of neurospheres, the percentage of BrdU-labeled proliferating cells were shown to be dramatically decreased after STZ treatment. Although we could not exclude the possibility that the decreasing number and size of neurospheres are the results of cell death, the decreased number of BrdU marked cells indicate that at least the inhibition of proliferation is partly participating in the reducing of NSCs numbers. Our findings of a negative effect of STZ on proliferating cells is similar to the findings of Qu and colleagues, who detected significantly decreased number of

BrdU-positive cells in consequence of STZ treatment , but the concentration of STZ they used was 8 mM which is much higher than us (Qu et al., 2012).

The decreasing of NSCs following STZ treatment may be linked to increased oxidative stress. Qu and colleagues showed that STZ elicits a striking increase of cellular reactive oxygen species (ROS) in NSCs (Qu et al., 2012). Although proliferative NSCs maintain high endogenous ROS status and pharmacological or genetic manipulations that diminished cellular ROS levels interfered with normal NSCs function both in *in vitro* and *in vivo* studies (Le Belle et al., 2011), too high ROS loading could cause cell death and have anti-proliferative effects on NSCs (Limoli et al., 2006). The *in vitro* study of Prozorovski and coworkers showed that NSCs exposed to pro-oxidative buthionine sulfoximine, an inhibitor of glutathione synthetase, that cause intracellular accumulation of ROS, causes a reduction in the number of NSCs (Prozorovski et al., 2008).

4.2.2 STZ treatment does not affect the migration of differentiating cells

According to our results, STZ does not affect migration of newborn cells. NSCs migration is an essential process for the development of the central nervous system (CNS) as well as for AN in the CNS of mammals (Hatten, 1999). Little is understood about the mechanism of controlling the migration of NSCs or neural progenitor cells in the adult brain. Neural progenitors transplanted into the brain were shown to migrate toward either localized (like stroke) (Arvidsson et al., 2002) or diffuse (like glioblastoma) (Glass et al., 2005) areas of brain damage. These studies suggest that factors such as neuroinflammatory response like tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and monocyte chemoattractant protein-1 (MCP-1) (Belmadani et al., 2006), associated with damaged areas, can influence the migration of these differentiating NSCs. The level of TNF-α in hippocampus and cerebral cortex (Rai et al., 2014), and IFN-γ in peripheral blood lymphocytes (Pandey and Bani,

2010) were found to be increased in STZ icv treated rats. Whether these elevated TNF-α and IFN-γ levels affect the migration of NSCs is still uncertain. The results of our in vitro-investigations revealed that STZ in the culture medium did not affect the migration of new born cells. However, our assay measured migration of all of the new born and differentiating cells, irrespective of the final neural phenotype. However, we cannot exclude that STZ regulates migration of a particular cell type.

4.2.3 STZ treatment affects the cellular fate of newborn cells in vitro

We investigated whether STZ influences the differentiation process of *in vitro* cultivated NSCs to neurons and astrocytes via staining of Tuj-1 (a marker for immature neurons) and GFAP (a marker for astrocytes), respectively. In our quantitative study, STZ was found to dramatically reduce the number of Tuj-1-positive cells, but not the number of GFAP-positive cells. Therefore, in an *in vitro* approach STZ seems to affect the differentiation fate of newly generated cells. Similar to our results, Qu and colleagues found that STZ decreased the generation of new neurons via immunostaining with three neuronal markers, Tuj-1, microtubule-associated protein 2 (MAP 2) and neurofilament 150 (NF 150) (Qu et al., 2012). In our study we revealed that without any treatment about 15% of cells could be staining with Tuj-1 after two days of incubation in the differentiation medium, a percentage lower than the finding of Dong's (34%, differentiation for 3 weeks) (Dong et al., 2012) and Qu's (20%, , differentiation for 7 days) (Qu et al., 2012). However, besides different times for the differentiation process in these studies, NSCs used of both of these two research groups were obtained from much younger rats (Qu: newborn rats at postnatal day 0; Dong: E17 rats). That is why their study results cannot be compared directly with our results. As we had been interested in AN we had to use older (young adult) rats and not rats of an age

the other researchers used. Different from our results with Tuj-1 staining, our study revealed that STZ treatment has no significant influence on the percentage of GFAP-positive cells. About 20% GFAP positive cells are detected in both treatment groups, the STZ treatment group and the control group. Due to different ages of animals used, different percentages of GFAP-positive cells were detected. However, all known *in vitro* studies (including our *in vitro* study) reported about higher number of astrocytes compared to neurons (Tropepe et al., 1999, Horie et al., 2004, Schultz et al., 2011, Liu et al., 2014). As already mentioned above, STZ treatment increases ROS content in cultured NSCs (Qu et al., 2012). As increasing ROS content resulting from treatment with pro-oxidative bothionine sulfoximine or diethyldithiocarbamate was shown to decrease the proportion of neurons after 7 days of incubation of NSCs in differentiation medium (Prozorovski et al., 2008) ROS seem contribute to neural-fate decision.

4.2.4 Insulin receptor and glucose transporter 3 is affected by STZ treatment

Almost all NSCs incubated in culture medium without STZ were shown to express IR and GLUT3 proteins. However, NSCs incubated in culture medium containing 2.5 mM STZ resulted in a reduced number of cells exhibiting IR and GLUT3 protein expression. Interestingly，the gene expression of IR in NSCs was not significantly affected by STZ treatment. Thus, STZ may influence the process of IR protein translation, post-translational modification or distribution. It has been found that in the adult brain the insulin signal is needed to keep NSCs alive and keeping the IR expression in the brain prevents the die-off of NSCs in drosophila (Siegrist et al., 2010). Activation of the PI3K/Akt pathway downstream of the insulin/IGF receptors activation promotes the self-renewal of NSCs (Groszer et al., 2006). Thus, the inhibition of NSCs` proliferation after STZ treatment may be linked to the down regulation of IR as observed in our study. Adult NSCs express GLUT1 and

GLUT3 but not GLUT2 and GLUT4 (Maurer et al., 2006), so the “fuel” for NSCs depends on the transport of glucose via GLUT1 and GLUT3. Maurer and colleagues found that high glucose concentrations and hypoxia increase GLUT3 protein concentration but do not significantly change the expression of GLUT1 in NSCs (Maurer et al., 2006). Thus, GLUT3 may be more sensitive to environmental changes. There is only few literature available dealing with the topic of a relationship between NSCs` proliferation and GLUT3 expression. But, in non-small cell lung cancer, GLUT3 was found to be induced and to promote tumor cell proliferation (Masin et al., 2014). GLUT3 may also regulate NSCs proliferation through enhancement of the transport of glucose into the cells providing energy.

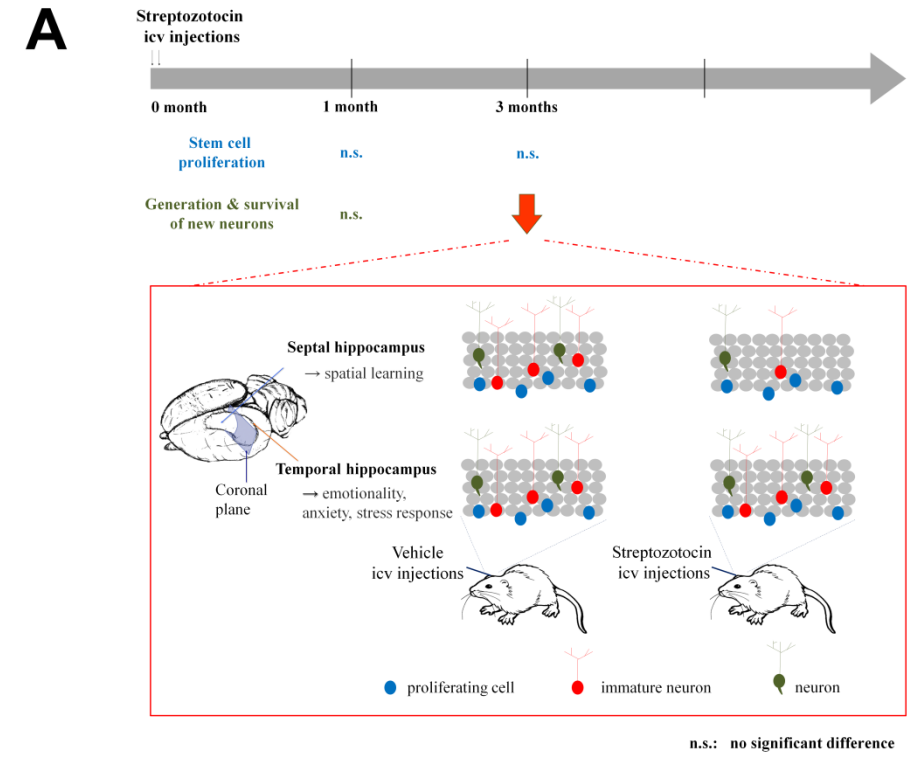
My immunostaining study with differentiating cells revealed that the expression of GLUT3 is decreased after STZ treatment, at least in some of the differentiating cells. This may be one out of several reasons why STZ affects the differentiation fate of NSCs. Both, GLUT1 and GLUT3, are expressed in NSCs (Maurer et al., 2006), however, GLUT1 is the main glucose transporter in the plasmamembrane of astrocytes, whereas GLUT3 is the main glucose transporter of neurons (McCall et al., 1996, Dienel, 2012). The decreasing of GLUT3 expression may reduce the potential of NSCs toward neuronal-oriented differentiation without affecting the differentiation to astrocytes.

4.3 The *in vivo* and *in vitro* study – similarities and differences of obtained results

Both, the *in vivo* as well as the *in vitro* study, point to the fact that STZ diminishes hippocampal AN. However, as displayed in Fig. 33 A, the *in vivo* study revealed that STZ icv treatment primarily affects the differentiation process and the survival rate of newborn cells, whereas the *in vitro* study, as displayed in Fig. 33B,

showed that STZ affects both, the proliferation of NSCs and the differentiation and survival of newborn cells.

Both approaches have their own advantages, e.g. in the *in vivo* study using hippocampal sections of the whole septal-temporal extension we were able to demonstrate that exclusively in the septal hippocampus (Fig. 33A) discussed to be important for spatial learning AN is hampered, and in the *in vitro* study cellular mechanisms underlying the observed impaired neurogenesis in the hippocampus of STZ-treated rats could be more easily studied. In this *in vitro* approach we were able to show that STZ inhibits the expression of IR in NSCs and GLUT3 in NSCs as well as in differentiation cells.



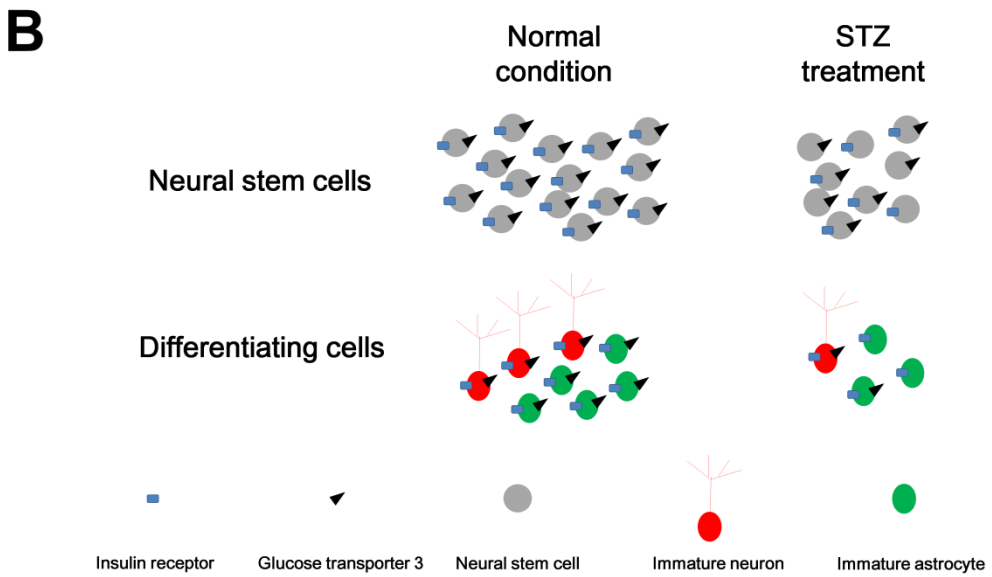


Fig.33 Schematic of *in vivo* (A) and *in vitro* (B) study results of streptozotocin treatment on adult neurogenesis in the rat hippocampus

A: The birth of new neurons in the adult hippocampus, is hampered in the hippocampus of rats 3 months, but not 1 month, after STZ icv treatment. Analyzing different AN-stages we revealed that STZ icv treatment primarily affects the differentiation process and the survival rate

of newborn cells. Both of long term and short term STZ icv treatment could not affect proliferating cells. We could also show that STZ exerts its biggest effects on the septal hippocampus, which is important for learning and memory processes and therefore is in line with spatial learning deficits obvious in AD patients.
B: In the *in vitro* approach STZ could inhibit AN in several different stages, including cell proliferation and differentiation. Moreover, we were able to show that STZ treatment seems to affect the differentiation fate of newly generated neurons but not of astrocytes. We could also show STZ inhibits the expression of IR in NSCs and GLUT3 in NSCs as well as in differentiation cells.

Different results from *in vivo* and *in vitro* studies are quite common in biological studies, especially in medical, pharmacological and toxicological research. Toxicological study revealed that non-genotoxic rat liver carcinogen methapyrilene alters the expression of the metabolizing genes sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 (SULT1A1) and 4-aminobutyrate aminotransferase (ABAT) as well as the growth arrest and DNA damage gene (GADD)34 in vitro, but not in vivo (Schug, et al., 2013). The observed *in vivo/in vitro* discrepancies always can be explained by different pharmacokinetics present *in vivo* and *in vitro* (Schug, et al., 2013). However, in other studies without involved in pharmacokinetics, discrepancy can also be found. For example, Desestret and coworkers showed that macrophages provided potent protection against neuron cell loss through a paracrine mechanism in the *in vitro* model of cerebral ischemia, however, in the *in vivo* model this cell-based treatment did not significantly improve any outcome measure compared with vehicle (Desestret, et al., 2013). The reason for the discrepancy in the studies like above may then be more complicated.

Discrepancy of our *in vivo* and *in vivo* results may originate in the different complexity of the NSCs proliferation and the growth and differentiation environment. In our rat model, STZ regulating AN may not target NSCs directly but also through effecting other cells, and influencing this micro-environment. However, for *in vitro* study, micro-environment for NSCs proliferation is almost missing and STZ targets NSCs and differentiating cells directly. In an *in vivo* condition, NSCs are surrounded by astrocytes, mature granule cells and resting

in close proximity to blood vessels and all these constitute a complex micro-environment for AN in the SGZ (Palmer et al., 2000). Micro-environment is important in the facilitation of the survival and self-renewing capacity of the stem cells (Kazanis et al., 2008) that depends on the change of vasculature (Palmer et al., 2000), growth and trophic factor (Anderson et al., 2002, Lee et al., 2002) and glial support cells (Morrens et al., 2012). In animal model studies, STZ does not only influence NSCs but also other type of cells like inducing neuronal apoptosis (Unsal et al., 2013), astrogliosis and microglial activation (Chen et al., 2013). Apoptotic neurons could secrete many factors [like high mobility group box 1 (HMGB1) and TNF-α (Kawabata et al., 2010)] and these factors are also shown to regulate AN. HMGB1 has been found to promote neurogenesis in later phases of intracerebral hemorrhage (Lei et al., 2015). Activated glial cells secrete cytokines like CD11b and TNF-α (Rai et al., 2014). Recently, It was reported that active microglia could enhance neurogenesis and pharmacological suppression of producing proinflammatory cytokines (i.e., IL-1β, IL-6, TNF-α, and IFN-γ) significantly inhibited neurogenesis in the SVZ (Shigemoto-Mogami et al., 2014). Furthermore, growth and trophic factors such as insulin/IGF (Grunblatt et al., 2007), NGF (Hellweg, 1994) as well as BDNF (Shonesy et al., 2012) are shown to be altered in the STZ icv rat model, which dramatically influence AN already described in our introduction part.

In summary, we speculate the discrepancy found in our *in vivo/in vitro* study may due to the complex micro-environment of AN niche in our *in vivo* study but missing in our *in vitro* study.

4.4 Outlook

As neuropathological changes already observed 3 months following STZ icv treatment are shown to become more and more apparent in brains of rats with increasing survival times, the analysis of hippocampal AN in rats 6 and 9 months after STZ icv treatment seems to be mandatory for a more comprehensive understanding of the involvement of AN in the etiopathogenesis of sAD. Regarding the *in vitro* study, there are still many unresolved issues needed to be further clarified. For instance, we only clarified that proliferation, differentiation as well as the expression of IR and GLUT3 protein were affected by the treatment with STZ. But, a causal relationship between them is not proven, yet. In future, we could continue to study these through gene knockout/overexpression of IR and GLUT3 and/or pharmacological inhibition of the IR [such as: insulin receptor-specific tyrosine kinase inhibitor, HNMPA-(AM)3].

In order to solve the discrepancies between the results of the *in vivo* study and *in vitro* study, we could use for future *in vitro* work the rat as old as *in vivo* study. We also could perform muti-cells co-culture systems, for instance, NSCs co-cultured with neurons, astrocyte or microglia. Then, the influence of micro-environment for NSCs could be studied in the treatment of STZ.

As factors and mechanisms underlying the formation and survival of new neurons in the adult brain certainly are of potential therapeutic importance especially for the treatment of sAD, in future, we could use our *in vivo* and *in vitro* system to help discovering new drugs. It would be worth testing drugs already shown to prevent cognitive deficits in this STZ icv model and cell model [such as treatment with galactose (Salkovic-Petrisic et al., 2014); curcumin (Isik et al., 2009); selenium (Ishrat et al., 2009); rhodiola crenulata extract (Qu et al., 2009); cyclooxygenase inhibitors (Dhull et al., 2012) on their effectiveness on AN.

References

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS (2000) Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:2896-2903.

Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS (2003) IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. Molecular and cellular neurosciences 24:23-40.

Aimone JB, Deng W, Gage FH (2010) Adult neurogenesis: integrating theories and separating functions. Trends in cognitive sciences 14:325-337.

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. The Journal of comparative neurology 124:319-335.

Alvarez-Buylla A, Lim DA (2004) For the long run: maintaining germinal niches in the adult brain. Neuron 41:683-686.

Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, Price J, Pariante CM (2011) Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Molecular psychiatry 16:738-750.

Anderson AJ, Su JH, Cotman CW (1996) DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:1710-1719.

Anderson MF, Aberg MA, Nilsson M, Eriksson PS (2002) Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. Brain research Developmental brain research 134:115-122.

Apelt J, Mehlhorn G, Schliebs R (1999) Insulin-sensitive GLUT4 glucose transporters are colocalized with GLUT3-expressing cells and demonstrate a chemically distinct neuron-specific localization in rat brain. Journal of neuroscience research 57:693-705.

Arabpoor Z, Hamidi G, Rashidi B, Shabrang M, Alaei H, Sharifi MR, Salami M, Dolatabadi HR, Reisi P (2012) Erythropoietin improves neuronal proliferation in dentate gyrus of hippocampal formation in an animal model of Alzheimer's disease. Advanced biomedical research 1:50.

Arsenijevic Y, Weiss S, Schneider B, Aebischer P (2001) Insulin-like growth factor-I is necessary for neural stem cell proliferation and demonstrates distinct actions of epidermal growth factor and fibroblast growth factor-2. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:7194-7202.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nature medicine 8:963-970.

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. Lancet 377:1019-1031.

Banks WA, Owen JB, Erickson MA (2012) Insulin in the brain: there and back again. Pharmacology & therapeutics 136:82-93.

Barilar JO, Knezovic A, Grunblatt E, Riederer P, Salkovic-Petrisic M (2015) Nine-month follow-up of the insulin receptor signalling cascade in the brain of streptozotocin rat

model of sporadic Alzheimer's disease. Journal of neural transmission 122:565-576.

Bartlett WP, Li XS, Williams M, Benkovic S (1991) Localization of insulin-like growth factor-1 mRNA in murine central nervous system during postnatal development. Developmental biology 147:239-250.

Bateman JM, McNeill H (2006) Insulin/IGF signalling in neurogenesis. Cellular and molecular life sciences : CMLS 63:1701-1705.

Belmadani A, Tran PB, Ren D, Miller RJ (2006) Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:3182-3191.

Bi X (2010) Alzheimer disease: update on basic mechanisms. The Journal of the American Osteopathic Association 110:S3-9.

Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH (1998) Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain research 800:125-135.

Biessels GJ, Kappelle LJ (2005) Increased risk of Alzheimer's disease in Type II diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? Biochemical Society transactions 33:1041-1044.

Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM (2005) Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45:675-688.

Binetti G, Magni E, Padovani A, Cappa SF, Bianchetti A, Trabucchi M (1996) Executive dysfunction in early Alzheimer's disease. Journal of neurology, neurosurgery, and psychiatry 60:91-93.

Blakesley VA, Scrimgeour A, Esposito D, Le Roith D (1996) Signaling via the insulin-like growth factor-I receptor: does it differ from insulin receptor signaling? Cytokine & growth factor reviews 7:153-159.

Blokland A, Jolles J (1993) Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin. Pharmacology, biochemistry, and behavior 44:491-494.

Blondel O, Portha B (1989) Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. Diabete & metabolisme 15:382-387.

Bloom GS (2014) Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA neurology 71:505-508.

Boekhoorn K, Joels M, Lucassen PJ (2006) Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiology of disease 24:1-14.

Bonefeld BE, Elfving B, Wegener G (2008) Reference genes for normalization: a study of rat brain tissue. Synapse 62:302-309.

Bonfanti L, Theodosis DT (1994) Expression of polysialylated neural cell adhesion molecule by proliferating cells in the subependymal layer of the adult rat, in its rostral extension and in the olfactory bulb. Neuroscience 62:291-305.

Borchelt DR, Ratovitski T, van Lare J, Lee MK, Gonzales V, Jenkins NA, Copeland NG, Price DL, Sisodia SS (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron

19:939-945.

Borta A, Hoglinger GU (2007) Dopamine and adult neurogenesis. Journal of neurochemistry 100:587-595.

Bozzola FG, Gorelick PB, Freels S (1992) Personality changes in Alzheimer's disease. Archives of neurology 49:297-300.

Braak H, Braak E (1985) On areas of transition between entorhinal allocortex and temporal isocortex in the human brain. Normal morphology and lamina-specific pathology in Alzheimer's disease. Acta neuropathologica 68:325-332.

Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta neuropathologica 82:239-259.

Braak H, Del Tredici K (2011) The pathological process underlying Alzheimer's disease in individuals under thirty. Acta neuropathologica 121:171-181.

Braak H, Del Tredici K (2012) Where, when, and in what form does sporadic Alzheimer's disease begin? Current opinion in neurology 25:708-714.

Brazel CY, Nunez JL, Yang Z, Levison SW (2005) Glutamate enhances survival and proliferation of neural progenitors derived from the subventricular zone. Neuroscience 131:55-65.

Breitner JC, Wyse BW, Anthony JC, Welsh-Bohmer KA, Steffens DC, Norton MC, Tschanz JT, Plassman BL, Meyer MR, Skoog I, Khachaturian A (1999) APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: the Cache County Study. Neurology 53:321-331.

Brentjens R, Saltz L (2001) Islet cell tumors of the pancreas: the medical oncologist's perspective. The Surgical clinics of North America 81:527-542.

Brezun JM, Daszuta A (2000) Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. The European journal of neuroscience 12:391-396.

Bribian A, Fontana X, Llorens F, Gavin R, Reina M, Garcia-Verdugo JM, Torres JM, de Castro F, del Rio JA (2012) Role of the cellular prion protein in oligodendrocyte precursor cell proliferation and differentiation in the developing and adult mouse CNS. PloS one 7:e33872.

Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. The Journal of comparative neurology 467:1-10.

Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, Yates J, Cotman C, Glabe C (1992) Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. The Journal of biological chemistry 267:546-554.

Cameron HA, Gould E (1994) Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 61:203-209.

Cameron HA, McEwen BS, Gould E (1995) Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:4687-4692.

Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, During MJ (2004) VEGF links hippocampal activity with neurogenesis, learning and memory. Nature genetics 36:827-835.

Carson MJ, Behringer RR, Brinster RL, McMorris FA (1993) Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. Neuron 10:729-740.

Cenquizca LA, Swanson LW (2007) Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain research reviews 56:1-26.

Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK (2000) Enhancement of hippocampal neurogenesis by lithium. Journal of neurochemistry 75:1729-1734.

Chen Y, Dong C (2009) Abeta40 promotes neuronal cell fate in neural progenitor cells. Cell death and differentiation 16:386-394.

Chen Y, Liang Z, Blanchard J, Dai CL, Sun S, Lee MH, Grundke-Iqbal I, Iqbal K, Liu F, Gong CX (2013) A non-transgenic mouse model (icv-STZ mouse) of Alzheimer's disease: similarities to and differences from the transgenic model (3xTg-AD mouse). Molecular neurobiology 47:711-725.

Chishti MA, Yang DS, Janus C, Phinney AL, Horne P, Pearson J, Strome R, Zuker N, Loukides J, French J, Turner S, Lozza G, Grilli M, Kunicki S, Morissette C, Paquette J, Gervais F, Bergeron C, Fraser PE, Carlson GA, George-Hyslop PS, Westaway D (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. The Journal of biological chemistry 276:21562-21570.

Clelland CD, Choi M, Romberg C, Clemenson GD, Jr., Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ (2009) A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325:210-213.

Coras R, Siebzehnrubl FA, Pauli E, Huttner HB, Njunting M, Kobow K, Villmann C, Hahnen E, Neuhuber W, Weigel D, Buchfelder M, Stefan H, Beck H, Steindler DA, Blumcke I (2010) Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans. Brain : a journal of neurology 133:3359-3372.

Correia SC, Santos RX, Perry G, Zhu X, Moreira PI, Smith MA (2011) Insulin-resistant brain state: the culprit in sporadic Alzheimer's disease? Ageing research reviews 10:264-273.

Cras P, Kawai M, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G (1991) Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proceedings of the National Academy of Sciences of the United States of America 88:7552-7556.

Cunnane S, Nugent S, Roy M, Courchesne-Loyer A, Croteau E, Tremblay S, Castellano A, Pifferi F, Bocti C, Paquet N, Begdouri H, Bentourkia M, Turcotte E, Allard M, Barberger-Gateau P, Fulop T, Rapoport SI (2011) Brain fuel metabolism, aging, and Alzheimer's disease. Nutrition 27:3-20.

Davies L, Wolska B, Hilbich C, Multhaup G, Martins R, Simms G, Beyreuther K, Masters CL (1988) A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. Neurology 38:1688-1693.

Davies P, Katzman R, Terry RD (1980) Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. Nature 288:279-280.

de la Monte SM (2009) Insulin resistance and Alzheimer's disease. BMB reports 42:475-481.

de la Monte SM, Wands JR (2008) Alzheimer's disease is type 3 diabetes-evidence reviewed. Journal of diabetes science and technology 2:1101-1113.

den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM (2003) Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. Diabetologia 46:1604-1610.

Deng W, Saxe MD, Gallina IS, Gage FH (2009) Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:13532-13542.

Desouza LA, Ladiwala U, Daniel SM, Agashe S, Vaidya RA, Vaidya VA (2005) Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. Molecular and cellular neurosciences 29:414-426.

Dhull DK, Jindal A, Dhull RK, Aggarwal S, Bhateja D, Padi SS (2012) Neuroprotective effect of cyclooxygenase inhibitors in ICV-STZ induced sporadic Alzheimer's disease in rats. Journal of molecular neuroscience : MN 46:223-235.

Dienel GA (2012) Fueling and imaging brain activation. ASN neuro 4.

Dodart JC, Meziane H, Mathis C, Bales KR, Paul SM, Ungerer A (1999) Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor protein. Behavioral neuroscience 113:982-990.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703-716.

Dong C, Rovnaghi CR, Anand KJ (2012) Ketamine alters the neurogenesis of rat cortical neural stem progenitor cells. Critical care medicine 40:2407-2416.

Donix M, Ercoli LM, Siddarth P, Brown JA, Martin-Harris L, Burggren AC, Miller KJ, Small GW, Bookheimer SY (2012) Influence of Alzheimer disease family history and genetic risk on cognitive performance in healthy middle-aged and older people. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry 20:565-573.

Donovan MH, Yazdani U, Norris RD, Games D, German DC, Eisch AJ (2006) Decreased adult hippocampal neurogenesis in the PDAPP mouse model of Alzheimer's disease. The Journal of comparative neurology 495:70-83.

Drucker DJ, Sherman SI, Gorelick FS, Bergenstal RM, Sherwin RS, Buse JB (2010) Incretin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and benefits. Diabetes care 33:428-433.

Duelli R, Kuschinsky W (2001) Brain glucose transporters: relationship to local energy demand. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society 16:71-76.

Duelli R, Schrock H, Kuschinsky W, Hoyer S (1994) Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 12:737-743.

Duman RS, Malberg J, Nakagawa S (2001) Regulation of adult neurogenesis by psychotropic drugs and stress. The Journal of pharmacology and experimental therapeutics

299:401-407.

Dupret D, Fabre A, Dobrossy MD, Panatier A, Rodriguez JJ, Lamarque S, Lemaire V, Oliet SH, Piazza PV, Abrous DN (2007) Spatial learning depends on both the addition and removal of new hippocampal neurons. PLoS Biol 5:e214.

Eckman CB, Mehta ND, Crook R, Perez-tur J, Prihar G, Pfeiffer E, Graff-Radford N, Hinder P, Yager D, Zenk B, Refolo LM, Prada CM, Younkin SG, Hutton M, Hardy J (1997) A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). Human molecular genetics 6:2087-2089.

Eileen Dolan M (1997) Inhibition of DNA repair as a means of increasing the antitumor activity of DNA reactive agents. Advanced drug delivery reviews 26:105-118.

Eisch AJ, Barrot M, Schad CA, Self DW, Nestler EJ (2000) Opiates inhibit neurogenesis in the adult rat hippocampus. Proceedings of the National Academy of Sciences of the United States of America 97:7579-7584.

El Messari S, Leloup C, Quignon M, Brisorgueil MJ, Penicaud L, Arluison M (1998) Immunocytochemical localization of the insulin-responsive glucose transporter 4 (Glut4) in the rat central nervous system. The Journal of comparative neurology 399:492-512.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nature medicine 4:1313-1317.

Ermini FV, Grathwohl S, Radde R, Yamaguchi M, Staufenbiel M, Palmer TD, Jucker M (2008) Neurogenesis and alterations of neural stem cells in mouse models of cerebral amyloidosis. The American journal of pathology 172:1520-1528.

Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7-19.

Fortini ME (2002) Gamma-secretase-mediated proteolysis in cell-surface-receptor signalling. Nature reviews Molecular cell biology 3:673-684.

Fukuyama H, Ogawa M, Yamauchi H, Yamaguchi S, Kimura J, Yonekura Y, Konishi J (1994) Altered cerebral energy metabolism in Alzheimer's disease: a PET study. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 35:1-6.

Fuster-Matanzo A, Llorens-Martin M, de Barreda EG, Avila J, Hernandez F (2011) Different susceptibility to neurodegeneration of dorsal and ventral hippocampal dentate gyrus: a study with transgenic mice overexpressing GSK3beta. PloS one 6:e27262.

Gage FH, Ray J, Fisher LJ (1995) Isolation, characterization, and use of stem cells from the CNS. Annual review of neuroscience 18:159-192.

Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. Nature 373:523-527.

Gerhart DZ, LeVasseur RJ, Broderius MA, Drewes LR (1989) Glucose transporter localization in brain using light and electron immunocytochemistry. Journal of neuroscience research 22:464-472.

Geschwind DH, Ou J, Easterday MC, Dougherty JD, Jackson RL, Chen Z, Antoine H, Terskikh A, Weissman IL, Nelson SF, Kornblum HI (2001) A genetic analysis of neural progenitor differentiation. Neuron 29:325-339.

Ghosal K, Stathopoulos A, Pimplikar SW (2010) APP intracellular domain impairs adult

neurogenesis in transgenic mice by inducing neuroinflammation. PloS one 5:e11866.

Gibson GE, Park LC, Zhang H, Sorbi S, Calingasan NY (1999) Oxidative stress and a key metabolic enzyme in Alzheimer brains, cultured cells, and an animal model of chronic oxidative deficits. Annals of the New York Academy of Sciences 893:79-94.

Glass R, Synowitz M, Kronenberg G, Walzlein JH, Markovic DS, Wang LP, Gast D, Kiwit J, Kempermann G, Kettenmann H (2005) Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:2637-2646.

Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron 3:519-526.

Goldberg JS, Hirschi KK (2009) Diverse roles of the vasculature within the neural stem cell niche. Regenerative medicine 4:879-897.

Goldman SA, Chen Z (2011) Perivascular instruction of cell genesis and fate in the adult brain. Nature neuroscience 14:1382-1389.

Goldstein BJ (2002) Insulin resistance as the core defect in type 2 diabetes mellitus. The American journal of cardiology 90:3G-10G.

Gonzalez-Perez O, Romero-Rodriguez R, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A (2009) Epidermal growth factor induces the progeny of subventricular zone type B cells to migrate and differentiate into oligodendrocytes. Stem Cells 27:2032-2043.

Gotz J, Probst A, Spillantini MG, Schafer T, Jakes R, Burki K, Goedert M (1995) Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. The EMBO journal 14:1304-1313.

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nature neuroscience 2:260-265.

Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:2492-2498.

Gould E, Tanapat P (1997) Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 80:427-436.

Gratzner HG (1982) Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. Science 218:474-475.

Gratzner HG, Leif RC, Ingram DJ, Castro A (1975) The use of antibody specific for bromodeoxyuridine for the immunofluorescent determination of DNA replication in single cells and chromosomes. Experimental cell research 95:88-94.

Graves AB, van Duijn CM, Chandra V, Fratiglioni L, Heyman A, Jorm AF, Kokmen E, Kondo K, Mortimer JA, Rocca WA, et al. (1991) Alcohol and tobacco consumption as risk factors for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. International journal of epidemiology 20 Suppl 2:S48-57.

Grillo CA, Piroli GG, Hendry RM, Reagan LP (2009) Insulin-stimulated translocation of

GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. Brain research 1296:35-45.

Gritti A, Frolichsthal-Schoeller P, Galli R, Parati EA, Cova L, Pagano SF, Bjornson CR, Vescovi AL (1999) Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:3287-3297.

Gritti A, Parati EA, Cova L, Frolichsthal P, Galli R, Wanke E, Faravelli L, Morassutti DJ, Roisen F, Nickel DD, Vescovi AL (1996) Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:1091-1100.

Groszer M, Erickson R, Scripture-Adams DD, Dougherty JD, Le Belle J, Zack JA, Geschwind DH, Liu X, Kornblum HI, Wu H (2006) PTEN negatively regulates neural stem cell self-renewal by modulating G0-G1 cell cycle entry. Proceedings of the National Academy of Sciences of the United States of America 103:111-116.

Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H (2001) Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 294:2186-2189.

Grunblatt E, Salkovic-Petrisic M, Osmanovic J, Riederer P, Hoyer S (2007) Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. Journal of neurochemistry 101:757-770.

Gylys KH, Fein JA, Yang F, Wiley DJ, Miller CA, Cole GM (2004) Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. The American journal of pathology 165:1809-1817.

Haber RS, Weinstein SP, O'Boyle E, Morgello S (1993) Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 132:2538-2543.

Han J, Wang B, Xiao Z, Gao Y, Zhao Y, Zhang J, Chen B, Wang X, Dai J (2008) Mammalian target of rapamycin (mTOR) is involved in the neuronal differentiation of neural progenitors induced by insulin. Molecular and cellular neurosciences 39:118-124.

Han P, Dou F, Li F, Zhang X, Zhang YW, Zheng H, Lipton SA, Xu H, Liao FF (2005) Suppression of cyclin-dependent kinase 5 activation by amyloid precursor protein: a novel excitoprotective mechanism involving modulation of tau phosphorylation. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:11542-11552.

Hartman RE, Izumi Y, Bales KR, Paul SM, Wozniak DF, Holtzman DM (2005) Treatment with an amyloid-beta antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:6213-6220.

Hatten ME (1999) Central nervous system neuronal migration. Annual review of neuroscience 22:511-539.

Hauser PS, Ryan RO (2013) Impact of apolipoprotein E on Alzheimer's disease. Current Alzheimer research 10:809-817.

Hellweg R (1994) Trophic factors during normal brain aging and after functional damage. Journal of neural transmission Supplementum 44:209-217.

Henke PG (1990) Hippocampal pathway to the amygdala and stress ulcer development. Brain research bulletin 25:691-695.

Heo C, Chang KA, Choi HS, Kim HS, Kim S, Liew H, Kim JA, Yu E, Ma J, Suh YH (2007) Effects of the monomeric, oligomeric, and fibrillar Abeta42 peptides on the proliferation and differentiation of adult neural stem cells from subventricular zone. Journal of neurochemistry 102:493-500.

Herring A, Ambree O, Tomm M, Habermann H, Sachser N, Paulus W, Keyvani K (2009) Environmental enrichment enhances cellular plasticity in transgenic mice with Alzheimer-like pathology. Experimental neurology 216:184-192.

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA (2001) Mitochondrial abnormalities in Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:3017-3023.

Hofmann C, Goldfine ID, Whittaker J (1989) The metabolic and mitogenic effects of both insulin and insulin-like growth factor are enhanced by transfection of insulin receptors into NIH3T3 fibroblasts. The Journal of biological chemistry 264:8606-8611.

Hoglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC (2004) Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nature neuroscience 7:726-735.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nature medicine 4:97-100.

Holtzman DM (2001) Role of apoe/Abeta interactions in the pathogenesis of Alzheimer's disease and cerebral amyloid angiopathy. Journal of molecular neuroscience : MN 17:147-155.

Horie N, Moriya T, Mitome M, Kitagawa N, Nagata I, Shinohara K (2004) Lowered glucose suppressed the proliferation and increased the differentiation of murine neural stem cells in vitro. FEBS letters 571:237-242.

Hoyer S (2004) Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. European journal of pharmacology 490:115-125.

Hoyer S, Nitsch R (1989) Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of Alzheimer type. Journal of neural transmission 75:227-232.

Hoyer S, Nitsch R, Oesterreich K (1991) Predominant abnormality in cerebral glucose utilization in late-onset dementia of the Alzheimer type: a cross-sectional comparison against advanced late-onset and incipient early-onset cases. Journal of neural transmission Parkinson's disease and dementia section 3:1-14.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice.

Science 274:99-102.

Hsieh J, Aimone JB, Kaspar BK, Kuwabara T, Nakashima K, Gage FH (2004) IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. The Journal of cell biology 164:111-122.

Ishrat T, Khan MB, Hoda MN, Yousuf S, Ahmad M, Ansari MA, Ahmad AS, Islam F (2006) Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. Behavioural brain research 171:9-16.

Ishrat T, Parveen K, Khan MM, Khuwaja G, Khan MB, Yousuf S, Ahmad A, Shrivastav P, Islam F (2009) Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain research 1281:117-127.

Isik AT, Celik T, Ulusoy G, Ongoru O, Elibol B, Doruk H, Bozoglu E, Kayir H, Mas MR, Akman S (2009) Curcumin ameliorates impaired insulin/IGF signalling and memory deficit in a streptozotocin-treated rat model. Age (Dordr) 31:39-49.

Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR (2002) A stem cell molecular signature. Science 298:601-604.

J SR-F, Sa-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, Moriguti JC, Roriz-Cruz M (2009) (Pre)diabetes, brain aging, and cognition. Biochimica et biophysica acta 1792:432-443.

Jablonska B, Aguirre A, Raymond M, Szabo G, Kitabatake Y, Sailor KA, Ming GL, Song H, Gallo V (2010) Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. Nature neuroscience 13:541-550.

Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA (2004) Increased hippocampal neurogenesis in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 101:343-347.

Johansson CB, Svensson M, Wallstedt L, Janson AM, Frisen J (1999) Neural stem cells in the adult human brain. Experimental cell research 253:733-736.

Kadowaki T, Kasuga M, Akanuma Y, Ezaki O, Takaku F (1984) Decreased autophosphorylation of the insulin receptor-kinase in streptozotocin-diabetic rats. The Journal of biological chemistry 259:14208-14216.

Kalluri HS, Vemuganti R, Dempsey RJ (2007) Mechanism of insulin-like growth factor I-mediated proliferation of adult neural progenitor cells: role of Akt. The European journal of neuroscience 25:1041-1048.

Kaplan MS, Hinds JW (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197:1092-1094.

Karabeg MM, Grauthoff S, Kollert SY, Weidner M, Heiming RS, Jansen F, Popp S, Kaiser S, Lesch KP, Sachser N, Schmitt AG, Lewejohann L (2013) 5-HTT deficiency affects neuroplasticity and increases stress sensitivity resulting in altered spatial learning performance in the Morris water maze but not in the Barnes maze. PloS one 8:e78238.

Karkkainen V, Pomeshchik Y, Savchenko E, Dhungana H, Kurronen A, Lehtonen S, Naumenko N, Tavi P, Levonen AL, Yamamoto M, Malm T, Magga J, Kanninen KM, Koistinaho J (2014) Nrf2 regulates neurogenesis and protects neural progenitor cells against Abeta toxicity. Stem Cells 32:1904-1916.

Karsten SL, Kudo LC, Jackson R, Sabatti C, Kornblum HI, Geschwind DH (2003) Global

analysis of gene expression in neural progenitors reveals specific cell-cycle, signaling, and metabolic networks. Developmental biology 261:165-182.

Kawabata H, Setoguchi T, Yone K, Souda M, Yoshida H, Kawahara K, Maruyama I, Komiya S (2010) High mobility group box 1 is upregulated after spinal cord injury and is associated with neuronal cell apoptosis. Spine 35:1109-1115.

Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI (1988) Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. The Journal of biological chemistry 263:15245-15248.

Kazanis I, Lathia J, Moss L, ffrench-Constant C (2008) The neural stem cell microenvironment. In: StemBook Cambridge (MA).

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-495.

Keuker JI, Vollmann-Honsdorf GK, Fuchs E (2001) How to use the optical fractionator: an example based on the estimation of neurons in the hippocampal CA1 and CA3 regions of tree shrews. Brain research Brain research protocols 7:211-221.

Khan MB, Khan MM, Khan A, Ahmed ME, Ishrat T, Tabassum R, Vaibhav K, Ahmad A, Islam F (2012) Naringenin ameliorates Alzheimer's disease (AD)-type neurodegeneration with cognitive impairment (AD-TNDCI) caused by the intracerebroventricular-streptozotocin in rat model. Neurochemistry international 61:1081-1093.

Kippin TE, Kapur S, van der Kooy D (2005) Dopamine specifically inhibits forebrain neural stem cell proliferation, suggesting a novel effect of antipsychotic drugs. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:5815-5823.

Kirby ED, Muroy SE, Sun WG, Covarrubias D, Leong MJ, Barchas LA, Kaufer D (2013) Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. eLife 2:e00362.

Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H (2002) Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Annals of internal medicine 137:149-155.

Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H, Nissinen A (2005) Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archives of neurology 62:1556-1560.

Kneissl M, Putter V, Szalay AA, Grummt F (2003) Interaction and assembly of murine pre-replicative complex proteins in yeast and mouse cells. Journal of molecular biology 327:111-128.

Knezovic A, Osmanovic-Barilar J, Curlin M, Hof PR, Simic G, Riederer P, Salkovic-Petrisic M (2015) Staging of cognitive deficits and neuropathological and ultrastructural changes in streptozotocin-induced rat model of Alzheimer's disease. Journal of neural transmission 122:577-592.

Kobayashi Y, Amaral DG (2007) Macaque monkey retrosplenial cortex: III. Cortical efferents.

The Journal of comparative neurology 502:810-833.

Kornack DR, Rakic P (1999) Continuation of neurogenesis in the hippocampus of the adult macaque monkey. Proceedings of the National Academy of Sciences of the United States of America 96:5768-5773.

Kuhn HG, Dickinson-Anson H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:2027-2033.

Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH (1997) Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:5820-5829.

Lannert H, Hoyer S (1998) Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behavioral neuroscience 112:1199-1208.

Le Belle JE, Orozco NM, Paucar AA, Saxe JP, Mottahedeh J, Pyle AD, Wu H, Kornblum HI (2011) Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. Cell stem cell 8:59-71.

Lee J, Duan W, Mattson MP (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. Journal of neurochemistry 82:1367-1375.

Lee Y, Kim YH, Park SJ, Huh JW, Kim SH, Kim SU, Kim JS, Jeong KJ, Lee KM, Hong Y, Lee SR, Chang KT (2014) Insulin/IGF signaling-related gene expression in the brain of a sporadic Alzheimer's disease monkey model induced by intracerebroventricular injection of streptozotocin. Journal of Alzheimer's disease : JAD 38:251-267.

Lehner B, Sandner B, Marschallinger J, Lehner C, Furtner T, Couillard-Despres S, Rivera FJ, Brockhoff G, Bauer HC, Weidner N, Aigner L (2011) The dark side of BrdU in neural stem cell biology: detrimental effects on cell cycle, differentiation and survival. Cell and tissue research 345:313-328.

Lei C, Wu B, Cao T, Zhang S, Liu M (2015) Activation of the high-mobility group box 1 protein-receptor for advanced glycation end-products signaling pathway in rats during neurogenesis after intracerebral hemorrhage. Stroke; a journal of cerebral circulation 46:500-506.

Lemere CA, Lopera F, Kosik KS, Lendon CL, Ossa J, Saido TC, Yamaguchi H, Ruiz A, Martinez A, Madrigal L, Hincapie L, Arango JC, Anthony DC, Koo EH, Goate AM, Selkoe DJ (1996) The E280A presenilin 1 Alzheimer mutation produces increased A beta 42 deposition and severe cerebellar pathology. Nature medicine 2:1146-1150.

Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Paul Murphy M, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nature genetics 25:402-405.

Leybaert L, De Bock M, Van Moorhem M, Decrock E, De Vuyst E (2007) Neurobarrier

coupling in the brain: adjusting glucose entry with demand. Journal of neuroscience research 85:3213-3220.

Li ZG, Zhang W, Grunberger G, Sima AA (2002) Hippocampal neuronal apoptosis in type 1 diabetes. Brain research 946:221-231.

Li ZG, Zhang W, Sima AA (2007) Alzheimer-like changes in rat models of spontaneous diabetes. Diabetes 56:1817-1824.

Like AA, Rossini AA (1976) Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science 193:415-417.

Limoli CL, Giedzinski E, Baure J, Doctrow SR, Rola R, Fike JR (2006) Using superoxide dismutase/catalase mimetics to manipulate the redox environment of neural precursor cells. Radiation protection dosimetry 122:228-236.

Liu Q, Wu D, Ni N, Ren H, Luo C, He C, Kang JX, Wan JB, Su H (2014) Omega-3 polyunsaturated fatty acids protect neural progenitor cells against oxidative injury. Marine drugs 12:2341-2356.

Lois C, Garcia-Verdugo JM, Alvarez-Buylla A (1996) Chain migration of neuronal precursors. Science 271:978-981.

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH (2000) Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron 25:317-329.

Ma D, Lu L, Boneva NB, Warashina S, Kaplamadzhiev DB, Mori Y, Nakaya MA, Kikuchi M, Tonchev AB, Okano H, Yamashima T (2008) Expression of free fatty acid receptor GPR40 in the neurogenic niche of adult monkey hippocampus. Hippocampus 18:326-333.

Magavi SS, Macklis JD (2008) Identification of newborn cells by BrdU labeling and immunocytochemistry in vivo. Methods Mol Biol 438:335-343.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:9104-9110.

Mandyam CD, Harburg GC, Eisch AJ (2007) Determination of key aspects of precursor cell proliferation, cell cycle length and kinetics in the adult mouse subgranular zone. Neuroscience 146:108-122.

Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Farbood Y, Sarkaki A, Bavarsad K (2013) Gallic acid prevents memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats. Pharmacology, biochemistry, and behavior 111:90-96.

Maren S, Holt WG (2004) Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. Behavioral neuroscience 118:97-110.

Marlatt MW, Lucassen PJ (2010) Neurogenesis and Alzheimer's disease: Biology and pathophysiology in mice and men. Current Alzheimer research 7:113-125.

Marson DC, Earnst KS, Jamil F, Bartolucci A, Harrell LE (2000) Consistency of physicians' legal standard and personal judgments of competency in patients with Alzheimer's disease. Journal of the American Geriatrics Society 48:911-918.

Masin M, Vazquez J, Rossi S, Groeneveld S, Samson N, Schwalie PC, Deplancke B, Frawley LE, Gouttenoire J, Moradpour D, Oliver TG, Meylan E (2014) GLUT3 is induced during epithelial-mesenchymal transition and promotes tumor cell proliferation in non-small cell lung cancer. Cancer & metabolism 2:11.

Maurer MH, Geomor HK, Burgers HF, Schelshorn DW, Kuschinsky W (2006) Adult neural stem cells express glucose transporters GLUT1 and GLUT3 and regulate GLUT3 expression. FEBS letters 580:4430-4434.

Mayer G, Nitsch R, Hoyer S (1990) Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. Brain research 532:95-100.

McCall AL, Van Bueren AM, Nipper V, Moholt-Siebert M, Downes H, Lessov N (1996) Forebrain ischemia increases GLUT1 protein in brain microvessels and parenchyma. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 16:69-76.

McClean PL, Holscher C (2014) Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. Neuropharmacology 76 Pt A:57-67.

McClean PL, Parthsarathy V, Faivre E, Holscher C (2011) The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:6587-6594.

McGovern SF, Hunter K, Holscher C (2012) Effects of the glucagon-like polypeptide-1 analogue (Val8)GLP-1 on learning, progenitor cell proliferation and neurogenesis in the C57B/16 mouse brain. Brain research 1473:204-213.

McHugh SB, Deacon RM, Rawlins JN, Bannerman DM (2004) Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. Behavioral neuroscience 118:63-78.

McHugh SB, Fillenz M, Lowry JP, Rawlins JN, Bannerman DM (2011) Brain tissue oxygen amperometry in behaving rats demonstrates functional dissociation of dorsal and ventral hippocampus during spatial processing and anxiety. The European journal of neuroscience 33:322-337.

Merchant C, Tang MX, Albert S, Manly J, Stern Y, Mayeux R (1999) The influence of smoking on the risk of Alzheimer's disease. Neurology 52:1408-1412.

Miller MW, Nowakowski RS (1988) Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. Brain research 457:44-52.

Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annual review of neuroscience 28:223-250.

Miyata T, Maeda T, Lee JE (1999) NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus. Genes & development 13:1647-1652.

Moroz N, Tong M, Longato L, Xu H, de la Monte SM (2008) Limited Alzheimer-type neurodegeneration in experimental obesity and type 2 diabetes mellitus. Journal of Alzheimer's disease : JAD 15:29-44.

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

Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:3916-3925.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8:608-619.

Moser MB, Moser EI, Forrest E, Andersen P, Morris RG (1995) Spatial learning with a minislab in the dorsal hippocampus. Proceedings of the National Academy of Sciences of the United States of America 92:9697-9701.

Mu Y, Gage FH (2011) Adult hippocampal neurogenesis and its role in Alzheimer's disease. Molecular neurodegeneration 6:85.

Mullen RJ, Buck CR, Smith AM (1992) NeuN, a neuronal specific nuclear protein in vertebrates. Development 116:201-211.

Murdoch BE, Chenery HJ, Wilks V, Boyle RS (1987) Language disorders in dementia of the Alzheimer type. Brain and language 31:122-137.

Muresan Z, Muresan V (2008) Seeding neuritic plaques from the distance: a possible role for brainstem neurons in the development of Alzheimer's disease pathology. Neuro-degenerative diseases 5:250-253.

Murray-Lyon IM, Eddleston AL, Williams R, Brown M, Hogbin BM, Bennett A, Edwards JC, Taylor KW (1968) Treatment of multiple-hormone-producing malignant islet-cell tumour with streptozotocin. Lancet 2:895-898.

Nakazawa T, Chiba T, Kaneko E, Yui K, Yoshida M, Shimokado K (2005) Insulin signaling in arteries prevents smooth muscle apoptosis. Arteriosclerosis, thrombosis, and vascular biology 25:760-765.

Naya FJ, Stellrecht CM, Tsai MJ (1995) Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes & development 9:1009-1019.

Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kovari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, Beach TG (2012) Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. Journal of neuropathology and experimental neurology 71:362-381.

Newman AB, Fitzpatrick AL, Lopez O, Jackson S, Lyketsos C, Jagust W, Ives D, Dekosky ST, Kuller LH (2005) Dementia and Alzheimer's disease incidence in relationship to cardiovascular disease in the Cardiovascular Health Study cohort. Journal of the American Geriatrics Society 53:1101-1107.

Nicolls MR (2004) The clinical and biological relationship between Type II diabetes mellitus and Alzheimer's disease. Current Alzheimer research 1:47-54.

Noble EP, Wurtman RJ, Axelrod J (1967) A simple and rapid method for injecting H3-norepinephrine into the lateral ventricle of the rat brain. Life sciences 6:281-291.

Nowakowski RS, Lewin SB, Miller MW (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an

anatomically defined population. Journal of neurocytology 18:311-318.

Nunan J, Small DH (2000) Regulation of APP cleavage by alpha-, beta- and gamma-secretases. FEBS letters 483:6-10.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409-421.

Otaegi G, Yusta-Boyo MJ, Vergano-Vera E, Mendez-Gomez HR, Carrera AC, Abad JL, Gonzalez M, de la Rosa EJ, Vicario-Abejon C, de Pablo F (2006) Modulation of the PI 3-kinase-Akt signalling pathway by IGF-I and PTEN regulates the differentiation of neural stem/precursor cells. Journal of cell science 119:2739-2748.

Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM (1999) Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology 53:1937-1942.

Packer MA, Stasiv Y, Benraiss A, Chmielnicki E, Grinberg A, Westphal H, Goldman SA, Enikolopov G (2003) Nitric oxide negatively regulates mammalian adult neurogenesis. Proceedings of the National Academy of Sciences of the United States of America 100:9566-9571.

Paez-Gonzalez P, Asrican B, Rodriguez E, Kuo CT (2014) Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. Nature neuroscience 17:934-942.

Paik JH, Ding Z, Narurkar R, Ramkissoon S, Muller F, Kamoun WS, Chae SS, Zheng H, Ying H, Mahoney J, Hiller D, Jiang S, Protopopov A, Wong WH, Chin L, Ligon KL, DePinho RA (2009) FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. Cell stem cell 5:540-553.

Palmer TD, Willhoite AR, Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. The Journal of comparative neurology 425:479-494.

Pandey A, Bani S (2010) Hydroxychavicol inhibits immune responses to mitigate cognitive dysfunction in rats. Journal of neuroimmunology 226:48-58.

Pardridge WM (1983) Brain metabolism: a perspective from the blood-brain barrier. Physiological reviews 63:1481-1535.

Parent JM, Lowenstein DH (1997) Mossy fiber reorganization in the epileptic hippocampus. Current opinion in neurology 10:103-109.

Payne J, Maher F, Simpson I, Mattice L, Davies P (1997) Glucose transporter Glut 5 expression in microglial cells. Glia 21:327-331.

Perry EK, Johnson M, Ekonomou A, Perry RH, Ballard C, Attems J (2012) Neurogenic abnormalities in Alzheimer's disease differ between stages of neurogenesis and are partly related to cholinergic pathology. Neurobiology of disease 47:155-162.

Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, Perry RH (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. British medical journal 2:1457-1459.

Piatti VC, Davies-Sala MG, Esposito MS, Mongiat LA, Trinchero MF, Schinder AF (2011) The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:7715-7728.

Plaschke K, Hoyer S (1993) Action of the diabetogenic drug streptozotocin on glycolytic and

glycogenolytic metabolism in adult rat brain cortex and hippocampus. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 11:477-483.

Pozniak CD, Pleasure SJ (2006) Genetic control of hippocampal neurogenesis. Genome biology 7:207.

Prozorovski T, Schulze-Topphoff U, Glumm R, Baumgart J, Schroter F, Ninnemann O, Siegert E, Bendix I, Brustle O, Nitsch R, Zipp F, Aktas O (2008) Sirt1 contributes critically to the redox-dependent fate of neural progenitors. Nature cell biology 10:385-394.

Qiu C, Kivipelto M, von Strauss E (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues in clinical neuroscience 11:111-128.

Qu ZQ, Zhou Y, Zeng YS, Li Y, Chung P (2009) Pretreatment with Rhodiola rosea extract reduces cognitive impairment induced by intracerebroventricular streptozotocin in rats: implication of anti-oxidative and neuroprotective effects. Biomedical and environmental sciences : BES 22:318-326.

Qu ZQ, Zhou Y, Zeng YS, Lin YK, Li Y, Zhong ZQ, Chan WY (2012) Protective effects of a Rhodiola crenulata extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. PloS one 7:e29641.

Raber J, Rola R, LeFevour A, Morhardt D, Curley J, Mizumatsu S, VandenBerg SR, Fike JR (2004) Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. Radiation research 162:39-47.

Rafalski VA, Brunet A (2011) Energy metabolism in adult neural stem cell fate. Progress in neurobiology 93:182-203.

Rafalski VA, Ho PP, Brett JO, Ucar D, Dugas JC, Pollina EA, Chow LM, Ibrahim A, Baker SJ, Barres BA, Steinman L, Brunet A (2013) Expansion of oligodendrocyte progenitor cells following SIRT1 inactivation in the adult brain. Nature cell biology 15:614-624.

Rai S, Kamat PK, Nath C, Shukla R (2014) Glial activation and post-synaptic neurotoxicity: the key events in Streptozotocin (ICV) induced memory impairment in rats. Pharmacology, biochemistry, and behavior 117:104-117.

Ray J, Gage FH (2006) Differential properties of adult rat and mouse brain-derived neural stem/progenitor cells. Molecular and cellular neurosciences 31:560-573.

Reaven GM, Ho H (1991) Low-dose streptozotocin-induced diabetes in the spontaneously hypertensive rat. Metabolism: clinical and experimental 40:335-337.

Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM (2000) A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism: clinical and experimental 49:1390-1394.

Reisberg B, Ferris SH, de Leon MJ, Crook T (1982) The Global Deterioration Scale for assessment of primary degenerative dementia. The American journal of psychiatry 139:1136-1139.

Reitz C, Brayne C, Mayeux R (2011) Epidemiology of Alzheimer disease. Nature reviews Neurology 7:137-152.

Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR, Fike JR (2004) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. Experimental neurology 188:316-330.

Ronnemaa E, Zethelius B, Sundelof J, Sundstrom J, Degerman-Gunnarsson M, Berne C, Lannfelt L, Kilander L (2008) Impaired insulin secretion increases the risk of Alzheimer disease. Neurology 71:1065-1071.

Sajan FD, Martiniuk F, Marcus DL, Frey WH, 2nd, Hite R, Bordayo EZ, Freedman ML (2007) Apoptotic gene expression in Alzheimer's disease hippocampal tissue. American journal of Alzheimer's disease and other dementias 22:319-328.

Salkovic-Petrisic M, Knezovic A, Hoyer S, Riederer P (2013a) What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. Journal of neural transmission 120:233-252.

Salkovic-Petrisic M, Osmanovic-Barilar J, Bruckner MK, Hoyer S, Arendt T, Riederer P (2011) Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: a long-term follow up study. Journal of neural transmission 118:765-772.

Salkovic-Petrisic M, Osmanovic-Barilar J, Knezovic A, Hoyer S, Mosetter K, Reutter W (2013b) Long-term oral galactose treatment prevents cognitive deficits in male Wistar rats treated intracerebroventricularly with streptozotocin. Neuropharmacology 77C:68-80.

Salkovic-Petrisic M, Osmanovic-Barilar J, Knezovic A, Hoyer S, Mosetter K, Reutter W (2014) Long-term oral galactose treatment prevents cognitive deficits in male Wistar rats treated intracerebroventricularly with streptozotocin. Neuropharmacology 77:68-80.

Salkovic-Petrisic M, Osmanovic J, Grunblatt E, Riederer P, Hoyer S (2009) Modeling sporadic Alzheimer's disease: the insulin resistant brain state generates multiple long-term morphobiological abnormalities including hyperphosphorylated tau protein and amyloid-beta. Journal of Alzheimer's disease : JAD 18:729-750.

Salkovic-Petrisic M, Osmanovic J, Siegfrid H, Riederer PF (2007) Time course of amyloid beta pathology in streptozotocin-rat model of sporadic Alzheimer's Disease. Parkinsonism Relat D 13:S129-S129.

Salkovic-Petrisic M, Tribl F, Schmidt M, Hoyer S, Riederer P (2006) Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway. Journal of neurochemistry 96:1005-1015.

Salkovic M, Sabolic I, Lackovic Z (1995) Striatal dopaminergic D1 and D2 receptors after intracerebroventricular application of alloxan and streptozocin in rat. Journal of neural transmission General section 100:137-145.

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, et al. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43:1467-1472.

Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD, Sofroniew MV, Kandel ER, Santarelli L, Hen R, Drew MR (2006) Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proceedings of the National Academy of Sciences of the United States of America 103:17501-17506.

Saxena G, Patro IK, Nath C (2011) ICV STZ induced impairment in memory and neuronal

mitochondrial function: A protective role of nicotinic receptor. Behavioural brain research 224:50-57.

Schmitt A, Benninghoff J, Moessner R, Rizzi M, Paizanis E, Doenitz C, Gross S, Hermann M, Gritti A, Lanfumey L, Fritzen S, Reif A, Hamon M, Murphy DL, Vescovi A, Lesch KP (2007) Adult neurogenesis in serotonin transporter deficient mice. Journal of neural transmission 114:1107-1119.

Schrijvers EM, Witteman JC, Sijbrands EJ, Hofman A, Koudstaal PJ, Breteler MM (2010) Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. Neurology 75:1982-1987.

Schultz KM, Banisadr G, Lastra RO, McGuire T, Kessler JA, Miller RJ, McGarry TJ (2011) Geminin-deficient neural stem cells exhibit normal cell division and normal neurogenesis. PloS one 6:e17736.

Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:7153-7160.

Sharma M, Gupta YK (2001) Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life sciences 68:1021-1029.

Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K (2014) Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:2231-2243.

Shimazu K, Zhao M, Sakata K, Akbarian S, Bates B, Jaenisch R, Lu B (2006) NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis. Learn Mem 13:307-315.

Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, Cross JC, Weiss S (2003) Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 299:117-120.

Shonesy BC, Thiruchelvam K, Parameshwaran K, Rahman EA, Karuppagounder SS, Huggins KW, Pinkert CA, Amin R, Dhanasekaran M, Suppiramaniam V (2012) Central insulin resistance and synaptic dysfunction in intracerebroventricular-streptozotocin injected rodents. Neurobiology of aging 33:430 e435-418.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. Nature 410:372-376.

Sidman RL, Miale IL, Feder N (1959) Cell proliferation and migration in the primitive ependymal zone: an autoradiographic study of histogenesis in the nervous system. Experimental neurology 1:322-333.

Siegrist SE, Haque NS, Chen CH, Hay BA, Hariharan IK (2010) Inactivation of both Foxo and reaper promotes long-term adult neurogenesis in Drosophila. Current biology : CB 20:643-648.

Simpson IA, Carruthers A, Vannucci SJ (2007) Supply and demand in cerebral energy metabolism: the role of nutrient transporters. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 27:1766-1791.

Snyder JS, Cameron HA (2012) Could adult hippocampal neurogenesis be relevant for human behavior? Behavioural brain research 227:384-390.

Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476:458-461.

Song J, Zhong C, Bonaguidi MA, Sun GJ, Hsu D, Gu Y, Meletis K, Huang ZJ, Ge S, Enikolopov G, Deisseroth K, Luscher B, Christian KM, Ming GL, Song H (2012) Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature 489:150-154.

Stafford MR, Bartlett PF, Adams DJ (2007) Purinergic receptor activation inhibits mitogen-stimulated proliferation in primary neurospheres from the adult mouse subventricular zone. Molecular and cellular neurosciences 35:535-548.

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? Journal of Alzheimer's disease : JAD 7:63-80.

Strange BA, Witter MP, Lein ES, Moser EI (2014) Functional organization of the hippocampal longitudinal axis. Nature reviews Neuroscience 15:655-669.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America 90:1977-1981.

Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, Ledermann B, Burki K, Frey P, Paganetti PA, Waridel C, Calhoun ME, Jucker M, Probst A, Staufenbiel M, Sommer B (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proceedings of the National Academy of Sciences of the United States of America 94:13287-13292.

Svendsen CN, Skepper J, Rosser AE, ter Borg MG, Tyres P, Ryken T (1997) Restricted growth potential of rat neural precursors as compared to mouse. Brain research Developmental brain research 99:253-258.

Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research / Academia Scientiarum Bohemoslovaca 50:537-546.

Takamura N, Nakagawa S, Masuda T, Boku S, Kato A, Song N, An Y, Kitaichi Y, Inoue T, Koyama T, Kusumi I (2014) The effect of dopamine on adult hippocampal neurogenesis. Progress in neuro-psychopharmacology & biological psychiatry 50:116-124.

Takasawa K, Kitagawa K, Yagita Y, Sasaki T, Tanaka S, Matsushita K, Ohstuki T, Miyata T, Okano H, Hori M, Matsumoto M (2002) Increased proliferation of neural progenitor cells but reduced survival of newborn cells in the contralateral hippocampus after focal cerebral ischemia in rats. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 22:299-307.

Takeda K, Araki W, Tabira T (2004) Enhanced generation of intracellular Abeta42 amyloid peptide by mutation of presenilins PS1 and PS2. The European journal of neuroscience 19:258-264.

Takeuchi H, Iba M, Inoue H, Higuchi M, Takao K, Tsukita K, Karatsu Y, Iwamoto Y, Miyakawa T, Suhara T, Trojanowski JQ, Lee VM, Takahashi R (2011) P301S mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating. PloS one 6:e21050.

Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. The Journal of clinical investigation 122:1316-1338.

Tanapat P, Hastings NB, Reeves AJ, Gould E (1999) Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:5792-5801.

Tandon A, Fraser P (2002) The presenilins. Genome biology 3:reviews3014.

Tanti A, Belzung C (2013) Neurogenesis along the septo-temporal axis of the hippocampus: are depression and the action of antidepressants region-specific? Neuroscience 252:234-252.

Taupin P (2007) BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain research reviews 53:198-214.

Tchantchou F, Xu Y, Wu Y, Christen Y, Luo Y (2007) EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 21:2400-2408.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Annals of neurology 30:572-580.

Teuchert-Noodt G, Dawirs RR, Hildebrandt K (2000) Adult treatment with methamphetamine transiently decreases dentate granule cell proliferation in the gerbil hippocampus. Journal of neural transmission 107:133-143.

Thierry AM, Gioanni Y, Degenetais E, Glowinski J (2000) Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. Hippocampus 10:411-419.

Tropepe V, Sibilia M, Ciruna BG, Rossant J, Wagner EF, van der Kooy D (1999) Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. Developmental biology 208:166-188.

Unsal C, Oran M, Albayrak Y, Aktas C, Erboga M, Topcu B, Uygur R, Tulubas F, Yanartas O, Ates O, Ozen OA (2013) Neuroprotective effect of ebselen against intracerebroventricular streptozotocin-induced neuronal apoptosis and oxidative stress in rats. Toxicology and industrial health.

Valente T, Gella A, Fernandez-Busquets X, Unzeta M, Durany N (2010) Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus. Neurobiology of disease 37:67-76.

van den Elzen P, Garg S, Leon L, Brigl M, Leadbetter EA, Gumperz JE, Dascher CC, Cheng TY, Sacks FM, Illarionov PA, Besra GS, Kent SC, Moody DB, Brenner MB (2005) Apolipoprotein-mediated pathways of lipid antigen presentation. Nature 437:906-910.

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature neuroscience 2:266-270.

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030-1034.

Vann SD, Brown MW, Erichsen JT, Aggleton JP (2000) Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:2711-2718.

von Bohlen und Halbach O (2011) Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. Cell and tissue research 345:1-19.

Wang Z, Gleichmann H (1998) GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes 47:50-56.

Waterhouse EG, An JJ, Orefice LL, Baydyuk M, Liao GY, Zheng K, Lu B, Xu B (2012) BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:14318-14330.

Weisova P, Concannon CG, Devocelle M, Prehn JH, Ward MW (2009) Regulation of glucose transporter 3 surface expression by the AMP-activated protein kinase mediates tolerance to glutamate excitation in neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:2997-3008.

Wen PH, Hof PR, Chen X, Gluck K, Austin G, Younkin SG, Younkin LH, DeGasperi R, Gama Sosa MA, Robakis NK, Haroutunian V, Elder GA (2004) The presenilin-1 familial Alzheimer disease mutant P117L impairs neurogenesis in the hippocampus of adult mice. Experimental neurology 188:224-237.

West MJ, Slomianka L, Gundersen HJ (1991) Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. The Anatomical record 231:482-497.

Whitmer RA (2007) Type 2 diabetes and risk of cognitive impairment and dementia. Current neurology and neuroscience reports 7:373-380.

Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM (2004) Nestin expression--a property of multi-lineage progenitor cells? Cellular and molecular life sciences : CMLS 61:2510-2522.

Willis SL, Allen-Burge R, Dolan MM, Bertrand RM, Yesavage J, Taylor JL (1998) Everyday problem solving among individuals with Alzheimer's disease. The Gerontologist 38:569-577.

Winner B, Regensburger M, Schreglmann S, Boyer L, Prots I, Rockenstein E, Mante M, Zhao C, Winkler J, Masliah E, Gage FH (2012) Role of alpha-synuclein in adult neurogenesis and neuronal maturation in the dentate gyrus. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:16906-16916.

Wiskott L, Rasch MJ, Kempermann G (2006) A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus. Hippocampus 16:329-343.

Woodruff RH, Fruttiger M, Richardson WD, Franklin RJ (2004) Platelet-derived growth factor

regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination. Molecular and cellular neurosciences 25:252-262.

Wu Z, Guo H, Chow N, Sallstrom J, Bell RD, Deane R, Brooks AI, Kanagala S, Rubio A, Sagare A, Liu D, Li F, Armstrong D, Gasiewicz T, Zidovetzki R, Song X, Hofman F, Zlokovic BV (2005) Role of the MEOX2 homeobox gene in neurovascular dysfunction in Alzheimer disease. Nature medicine 11:959-965.

Yagita Y, Kitagawa K, Ohtsuki T, Takasawa K, Miyata T, Okano H, Hori M, Matsumoto M (2001) Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. Stroke; a journal of cerebral circulation 32:1890-1896.

Yankner BA, Duffy LK, Kirschner DA (1990) Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. Science 250:279-282.

Ye P, Carson J, D'Ercole AJ (1995) In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:7344-7356.

Yiu AP, Rashid AJ, Josselyn SA (2011) Increasing CREB function in the CA1 region of dorsal hippocampus rescues the spatial memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:2169-2186.

Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ, Lee VM (2007) Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron 53:337-351.

Yu S, Ding WG (1998) The 45 kDa form of glucose transporter 1 (GLUT1) is localized in oligodendrocyte and astrocyte but not in microglia in the rat brain. Brain research 797:65-72.

Zhao M, Li D, Shimazu K, Zhou YX, Lu B, Deng CX (2007) Fibroblast growth factor receptor-1 is required for long-term potentiation, memory consolidation, and neurogenesis. Biological psychiatry 62:381-390.

Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K, Lein ES, Eadie BD, Willhoite AR, Muotri AR, Summers RG, Chun J, Lee KF, Gage FH (2003) Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. Proceedings of the National Academy of Sciences of the United States of America 100:6777-6782.

Zhou S, Yu G, Chi L, Zhu J, Zhang W, Zhang Y, Zhang L (2013) Neuroprotective effects of edaravone on cognitive deficit, oxidative stress and tau hyperphosphorylation induced by intracerebroventricular streptozotocin in rats. Neurotoxicology 38:136-145.

Zhu DY, Liu SH, Sun HS, Lu YM (2003) Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates neurogenesis in the adult rodent dentate gyrus. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:223-229.

Curriculum Vitae

Profile

Name: Ping Sun
DOB: December 4,1985
Gender: male
Address: Department of Psychiatry, Psychosomatics and Psychotherapy, Füchsleinstr. 15, 97080 Würzburg, Germany
E-mail: josp124@gmail.com / ping.sun@stud-mail.uni-wuerzburg.de

Education Resume

1. 2011.10 - present Ph.D., Neuroscience
   Department of Psychiatry, Psychosomatics and Psychotherapy
   University of Würzburg, Gemany
   Advisor: PD Dr. Angelika Schmitt

2. 2008.9 - 2011.7 Master, The basis of Integrative Medicine
   Beijing University of Chinese Medicine, China
   Joint training in Institute of Biophysics, Chinese Academy of Science
   Adivisor: Prof. Qian Hua

3. 2004.9 - 2007.7 Bachelor, Bioengineering
   Sichuan University, China
   Adivisor: Prof. Yongkui Zhang

Conferences and Schools

1. 2014, GK-Emotions Spring School, GK-Emotions summer School; 2013, GK-Emotions Spring School, GK-Emotions summer School
2. 2014, the 9th FENS Forum of Neuroscience (Milan, Italy)
3. 2013, the Congress of the German Neuroscience Society (Göttingen, Germany)
4. 2012, Route 28 summits in neurobiology in Frauenchiemsee, Germany
5. 2009, The 8th Biennial Conference of the Chinese Society for Neuroscience (CSfN) (Guangzhou,China)
6. 2008, The Third ISN Special Neurochemistry Conference (Beijing,China)

Place, Date Signature

Publications

Original contributions in international peer-reviewed journals

1. **Sun P**, Knezovic A, Parlak M, Cuber J, Karabeg MM, Deckert J, Riederer P, Hua Q, Salkovic-Petrisic M, Schmitt AG. Long-term effects of intracerebroventricular streptozotocin treatment on adult neurogenesis in the rat hippocampus. Curr Alzheimer Res. 2015 Jul 10.

3. **Sun P,** Ding H, Liang M, Li X, Mo W, Wang X, Liu Y, He R, Hua Q. Neuroprotective Effects of Geniposide in SH-SY5Y Cells and Primary Hippocampal Neurons Exposed to Aβ42. Biomed Res Int. 2014;2014:284314.

4. Yang K, Tan Y, Wang F, Zhang Q, **Sun P,** Zhang Y, Yao N, Zhao Y, Wang X, Fan A, Hua Q. The improvement of spatial memory deficits in APP/V717I transgenic mice by chronic anti-stroke herb treatment. Exp Biol Med (Maywood). 2014 May 28.

5. Li J, Wang F, Ding H, Jin C, Chen J, Zhao Y, Li X, Chen W, **Sun P,** Tan Y, Zhang Q, Wang X, Fan A, Hua Q. Geniposide, the component of the Chinese herbal formula Tongluojiunao, protects amyloid-β peptide (1-42-mediated death of hippocampal neurons via the non-classical estrogen signaling pathway. Neural Regen Res. 2014 Mar 1; 9(5):474-80.

6. **Sun P,** Chen JY, Li J, Sun MR, Mo WC, Liu KL, Meng YY, Liu Y, Wang F, He RQ, Hua Q. The protective effect of geniposide on human neuroblastoma cells in the presence of formaldehyde. BMC Complement Altern Med. 2013 Jul 1;13:152.

7. Li XJ, Hou JC, **Sun P,** Li PT, He RQ, Liu Y, Zhao LY, Hua Q. Neuroprotective effects of tongluojiunao in neurons exposed to oxygen and glucose deprivation. J Ethnopharmacol. 2012 Jun 14; 141(3):927-33.

8. Hua Q, Qing X, Li P, Li W, Hou J, Hu J, Hong Q, **Sun P,** Zhu X. Brain microvascular endothelial cells mediate neuroprotective effects on ischemia/reperfusion neurons. J Ethnopharmacol. 2010 Jun 16; 129(3):306-13.

Review article

Sun P, Hua Q, Schmitt AG. Energy metabolism, adult neurogenesis and their possible roles in Alzheimer's disease: A brief overview. Current Topics in Medicinal Chemistry.

In preparation

Sun P, Deckert J, Riederer P, Hua Q, Schmitt AG. Streptozotocin impairs proliferation and differentiation of adult hippocampal neural stem cells going along with insulin receptor and glucose transporter 3 expression changes - an in vitro study

Acknowledgements

First and foremost, I am very grateful to my supervisor PD Dr. Angelika Schmitt, who not only provide valuable suggestions groomed me into a researcher with good scientific, but also provided all kindness of help for my life in Germany. Thank PD Dr. Angelika Schmitt for keeping faith and trust in me. Her unconditional and unlimited support helped me in finishing my project successfully.

Secondly, I also want to thank Prof. Dr. Jürgen Deckert and Prof. Dr. Dr. h.c. Peter Riederer, whose diverse and intense knowledge helped me to lay the foundation of my study. And also, their inputs helped me to shape my project from elementary stage till last stage of my thesis submission. Furthermore, I would like to thank Prof. Melita Salkovic-Petrisic from Croatia and Prof. Qian Hua from China for their helpful discussion in our publication. I also want to thank other supervisors in my supervisory committee, Prof. Dr. Wolfgang Rössler and Dr. Camelia Monoranu for kind support and valuable suggestions.

Then, I want to thank all staffs of the Department of Psychiatry, Psychosomatics and Psychotherapy. They help me a lot in my PhD period, especially Gabi Ortega in real time PCR study, Milena Parlak and Jacqueline Cuber in section staining, Margherita M. Karabeg in data analysis.

Finally, I would like to give my sincere thanks to my parent and my family, who always supported me in last 4 years.

Affidavit

I hereby confirm that my thesis entitled " Alzheimer`s disease and brain insulin resistance: The diabetes inducing drug streptozotocin diminishes adult neurogenesis in the rat hippocampus – an *in vivo* and *in vitro* study " is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Place, Date Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation " Alzheimer-Krankheit und Insulinresistenz im Gehirn: Streptozotocin, das Änderungen im Insulinstoffwechsel hervorruft, reduziert die Neubildung von Neuronen im Hippocampus von adulten Ratten - *In vivo*- und *In vitro*-Untersuchungen" eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Ort, Datum Unterschrift