Title: Glucocorticoid induced leucine zipper in central nervous system health and disease.

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Abstract:

The central nervous system (CNS) is a large network of intercommunicating cells that function to maintain tissue health and homeostasis. Considerable evidence suggests that glucocorticoids exert both neuroprotective and neurodegenerative effects in the CNS. Glucocorticoids act by binding two related receptors in the cytoplasm, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). The glucocorticoid:receptor complex then mediate cellular responses by transactivating target genes or via protein:protein interactions. The paradoxical effects of glucocorticoids in mediating survival and death of neurons have been attributed to the concentration and the ratio of mineralocorticoid to glucocorticoid receptor activation. Glucocorticoid induced leucine zipper (GILZ) is a recently identified protein transcriptionally upregulated by glucocorticoids. It is constitutively expressed in many tissues including brain. Functionally while GILZ mimics many of the beneficial effects of glucocorticoids including the anti-inflammatory and anti-proliferative potential, it suppresses the adverse effects of glucocorticoids presumably by exerting differential effects on lineage development by pluripotent cells. As opposed to glucocorticoids, GILZ downregulates the adipogenic transcription factor peroxisome proliferator-activated receptor (PPAR)-γ and upregulates runt-related transcription factor 2 (RUNX2) widely implicated in osteogenesis and more recently in neurogenesis as well. Further, data on GILZ expression and effects following induced stress or spinal cord injury suggest potential roles in CNS diseases. Here we provide a short overview of GILZ expression in CNS health and discuss three potential rationales for its role in the pathogenesis of Alzheimer's disease, a common neurodegenerative pathology.

Introduction:

I. Glucocorticoids in the central nervous system (CNS):

Glucocorticoids (corticosterone in rodents and cortisol in humans) secreted in response to stress induced activation of the hypothalamo-pituitary-adrenal (HPA) axis exert a wide range of actions on most cell types including the cells of the central nervous system (CNS) [1, 2]. Upon binding the mineralocorticoid receptor (MR) or the glucocorticoid receptor (GR) in the cytoplasm, the glucocorticoid:receptor complex translocate to the nucleus and mediate cellular responses by genomic mechanisms via specific glucocorticoid response elements (GRE) in the DNA or by non-genomic protein:protein interactions. The receptors shuttle between the cytoplasm and the nucleus; their subcellular localization being determined by the equilibrium between nuclear import and export [3]. The negative feedback and circadian rhythmicity of circulating glucocorticoids are critical for maintaining the basal HPA axis activity and facilitate the termination of HPA activation [3, 4].

Glucocorticoids exhibit ten-fold higher affinity for MR than GR [5, 6]. This one ligand/ two receptor system works in balance, modulating a large spectrum of actions in the CNS. While MR is highly expressed in limbic areas such as the hippocampus and amygdala; GR is ubiquitously expressed throughout the brain [7, 8]. Sensitivity to glucocorticoids depends on the concentration and duration of exposure, bioavailability and the density of receptors. In physiological conditions and during circadian trough the high affinity MR is preferably occupied with less than 10% GR occupancy to help maintain low basal glucocorticoid levels [3, 9]. Several studies suggest that the MR

functionality is important for maintaining the basal HPA activity and preserving the normal metabolism of the neurons [10, 11]. Pathological effects of glucocorticoids are largely mediated by GR. While both receptors can modulate transcription of the same target genes, microarray studies suggest that approximately 20% of the expression profiles are uniquely modulated by GR [12]. This is largely attributed to the non-genomic functions, wherein nuclear GR tethers to and interferes with the functioning of other transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1) [8, 13].

I.a: <u>Glucocorticoids in Alzheimer's disease</u>

Both lack and overexposure of glucocorticoids increases the risk for neurodegenerative diseases. Excessive glucocorticoid alters MR/GR ratio, disrupts nucleocytoplasmic shuttling of the receptors and dysregulates HPA activity [1, 4]. Clinical observations of elevated plasma cortisol and/or changes in circadian rhythm of glucocorticoid release in Alzheimer's disease (AD) and Parkinson's disease (PD) support a role for high glucocorticoids in neurodegenerative pathologies [14-16]. In AD patients, the higher plasma glucocorticoids correlated with decreased hippocampal volume, a region of the brain critical for learning and memory [15, 16]. Mechanistically, highly elevated glucocorticoids have been shown to accelerate the beta amyloid (Aβ) plaque formation and tau phosphorylation, two major pathological hallmarks of AD, and thereby enhance the vulnerability of neuronal cells to toxic stimuli [17]. In triple transgenic mouse model of AD which develops both Aβ and tau pathologies in a progressive and region-specific manner, dexamethasone treatment upregulated steady state levels of amyloid precursor

protein (APP), β-site APP cleaving enzyme (BACE-1) and enhanced Aβ generation [18, 19]. Blockade of glucocorticoid:GR but not glucocorticoid:MR interaction abrogated the dexamethasone mediated upregulation of Aβ secretion [20].

Both stress and Aβ aggregates precipitate oxidative stress, considered as one of the early events in AD pathology as evidenced by the increased presence of reactive oxygen species in vulnerable neurons [21, 22]. Chronic stress has been shown to increase lipid peroxidation in the hippocampus in normal and AD mice with three fold higher upregulation in the diseased animals [23]. It has been observed that the oxidative stress mediate negative regulation of GR and allow retention of the glucocorticoid:GR complex in the cytoplasm [23, 24]. This reduces the ability of glucocorticoids to repress transcription factors NF-κB and AP-1 leading to inflammatory distress. Increased oxidative stress further enhances Aβ accumulation initiating a vicious cycle [21]. In AD mice, the Aβ induced oxidative stress could be suppressed by pharmacological blockade of corticosterone release suggesting that the elevated glucocorticoids and Aβ act synergistically to enhance the vulnerability of neurons to apoptosis [20].

II. Glucocorticoid induced leucine zipper (GILZ)

GILZ is a recently identified protein that has been shown to mediate many of the cellular effects of glucocorticoids. It is a member of the TSC22D (transforming growth factor β1-stimulated clone 22 domain) family of proteins that potentially impact multiple biological processes [25, 26]. Four different isoforms have been identified arising as splice

variants from a single gene [27]. Structurally, the GILZ protein has an amino terminal leucine zipper motif for dimerizing and a carboxy terminal proline rich region for protein-protein interactions [25, 26]. Constitutive expression of GILZ has been reported in many cell types and multiple organs including skeletal muscle, lungs, intestine, kidney and brain [27]. With six GRE in its promoter region, GILZ is strongly induced by glucocorticoids [25]. Much like other glucocorticoid responsive genes such as the tyrosine aminotransferase, glutamine synthetase, cholesterol-7α-hydroxylase and the glucocorticoid receptor, GILZ exhibits circadian rhythm of expression in response to the rhythmic release of endogenous glucocorticoid [27].

II.a. GILZ in the CNS

Endogenous GILZ is widely expressed throughout the brain and spinal cord suggesting a physiological role in the CNS [27]. In mice exposed to water immersion restraint stress, activation of HPA activity and increased glucocorticoid secretion correlated with elevated GILZ expression in medial pre-frontal cortex and hippocampal neurons [28, 29]. The stress induced GILZ upregulation was abrogated in adrenalectomized mice attributing the response to the increase in glucocorticod:GR mediated transactivation [29]. Constitutive expression of GILZ has been observed in murine microglial cells. Further, similar to the observations in macrophages and lymphocytes, microglial GILZ expression correlated negatively with inflammatory cytokines and was downregulated following induced anxiety [28, 30].

III. Plausible roles of GILZ in AD:

Three distinct rationales are postulated for potential roles of GILZ in AD pathogenesis. 1) A role in Aβ regulation supported by the ubiquitous expression of GILZ in neurons and glia [27, 29]; 2) GILZ as an anti-inflammatory molecule that modulates the processes of neuroinflammation and neurodegeneration in AD [31, 32] and 3) a role in neurogenesis based on potential cross-talk between GILZ and other nuclear transcriptional hormone receptors such as peroxisome proliferator-activated receptor (PPAR)-γ and CCAAT/enhancer-binding protein (C/EBP)-δ [33].

Hypothesis 1: Constitutive GILZ regulates physiological Aβ in CNS health.

Normal brain contains both soluble and neuronal Aβ. Picomolar concentration of Aβ exhibit neurotrophic properties, promote neurogenesis and contribute to normal synaptic activity and memory [34]. Mechanistically physiological Aβ has been shown to mediate tyrosine phosphorylation and increase phosphatidylinositol-3-kinases (PI3K) activity in neuronal cells [22, 35, 36]. The PI3K pathway has been implicated in modulating autophagy, neuronal survival, neurite extension and synaptic plasticity in the brain. Blockade of this pathway has been shown to increase apoptosis in Aβ exposed neuronal cells [37, 38].

As stated above, considerable data suggest essential roles for MR, the high affinity receptor for glucocorticoids, in neuronal survival and neurogenesis [10]. In health under low GC conditions, ligand activated MR not only induces BACE-1 transcription and inflammatory cytokines that facilitate Aβ production, but also upregulates many neuroprotective molecules [4, 39]. In PC12 neuronal cells overexpression of MR has

been shown to enhance neuron specific genes such as microtubule associated protein-2 (MAP2) and β-tubulin III, increase the ratio of anti-apoptotic (Bcl2 and Bcl$_{xL}$) to pro-apoptotic (Bax and Bak) proteins and diminish caspase 3 cleavage [40, 41]. While exposure to Aβ reduced MR and increased nuclear GR in cultured rat hippocampal and cortical neurons with consequent increase in cell death, activation of MR reduced the vulnerability of the cultured neurons to apoptosis [41, 42]. Tauroursodeoxycholic acid (TUDCA), a cholesterol derived endogenous molecule, has been shown to exhibit neuroprotective effects by selectively binding MR and increasing its nuclear translocation with subsequent transactivation of Bcl-2 and other anti-apoptotic genes [43]. It has been suggested that an optimal balance between MR and GR activation with MR predominating in the hippocampus is necessary for best emotional and cognitive function [1, 10, 11].

GILZ is one of the transcripts upregulated by both MR and GR [31, 44]. Mechanistically much like glucocorticoids, GILZ has been shown to enhance expressions of anti-apoptotic molecules such as Bcl-2 and Bcl-$_{XL}$ in different cell types including cardiomyocytes, epithelial cells and many human cancer cells [31, 45-47]. GILZ has also been shown to modulate the PI3K pathway, suppress cytokines and inhibit inflammation in activated lymphocytes and epithelial cells [48, 49]. Taken together it is plausible that the constitutive GILZ induced in neuronal cells under low GC conditions may act in concert with picomolar Aβ in regulating autophagy and neuronal survival by increasing anti-apoptotic factors and suppressing excessive cytokines. Furthermore, since PI3K signaling has been shown to play critical roles in regulating survival of Aβ

exposed neurons, it is tempting to speculate that the regulation of PI3K signaling by constitutive GILZ in neurons may indirectly control the threshold of Aβ accumulation and CNS health.

Hypothesis 2: Exogenous GILZ is an attractive strategy to suppress neuroinflammation.

Neuroinflammation plays critical roles in the initiation and/or progression of most neurodegenerative diseases including AD [50-52]. Aging and chronic stress are significant risk factors. In addition, persistent stress mediated increase in glucocorticoids enhances the neuronal cell vulnerability to glutamate, the excitatory neurotransmitter and Aβ induced neurotoxicity [1, 19]. Aβ exposure activates the p65:p50 NF-κB dimers in glia and post-mitotic neurons and enhance transactivation of inflammatory and apoptotic genes [53]. Elevated Bax (pro-apoptotic) to Bcl-2 (anti-apoptotic) ratio has been observed in Aβ stimulated neuronal cells [54-56]. Increased presence of IL-1β, IL-6, and TNF-α has been reported in the affected tissues, serum and cerebrospinal fluid of AD patients [57]. However, exogenous glucocorticoids, the prototype anti-inflammatory agents have been largely ineffective and even counter-productive in the treatment of AD. The low therapeutic efficacy of glucocorticoids is correlated with a disproportionate increase in the glucocorticoid:GR complex with enhanced susceptibility to apoptosis [58].

GILZ represents a potentially efficacious alternative anti-inflammatory molecule to suppress neuroinflammation. Mechanistically the anti-inflammatory and anti-apoptotic effects of both glucocorticoids and GILZ are largely attributed to the suppression of NF-

κB mediated transactivation [59, 60]. While the glucocorticoid:GR complex tether to and interfere with NF-κB-DNA binding, GILZ binds to and sequesters the p65 subunit of NF-κB in the cytoplasm, thereby preventing nuclear translocation and transactivation of pathological mediators [7, 26, 61]. Overexpression of GILZ has been shown to suppress inflammatory cytokines and ameliorate pathology in models of colitis, multiple sclerosis and spinal cord injury [32, 62, 63]. We observed that selective blockade of activated p65 by as a synthetic peptide mimic of the p65 binding domain of GILZ suppressed Aβ mediated toxicity in neuroblastoma cells and in primary human neuronal cells [64, 65]. Taken together, it is hypothesized that by virtue of binding the transactivation domain of p65 exposed only in activated cells, exogenous GILZ represents an attractive strategy to suppress neuroinflammatory-neurodegenerative diseases including AD (Fig 2).

Hypothesis 3: GILZ plays a role in neurogenesis.

Neurogenesis involves proliferation, survival, migration and lineage differentiation of neural stem/progenitor cells. Continuous stimulation including cognitive functions elicits protective responses that avoid tissue damage, promote neurogenesis and maintain homeostasis in the CNS. This involves complex signaling pathways that are interlinked and tightly regulated [66]. Glucocorticoid and NF-κB signaling are two cross-talking pathways shown to play crucial roles in adult neurogenesis [67, 68]. In health, stimulation of NF-κB signaling by low concentrations of proinflammatory cytokines such as TNF-α or the excitatory neurotransmitter glutamate has been shown to increase proliferation and differentiation of neural stem cells and promote neuronal survival [67, 69]. It has been suggested that neuroprotection is largely attributed to the activation of

NF-κB C-rel dimers [70]. Similarly at physiological concentrations, glucocorticoids signaling via MR increased the proliferation and differentiation of human hippocampus-derived stem cells. However, excessive inflammation or high glucocorticoids largely inhibited neuronal stem cells [68, 71]. GILZ as an interface molecule that connects glucocorticoid and NF-κB pathways could potentially play a role in modulating the neural stem cell responses and hence neurogenesis [59].

In addition to NF-κB, the GILZ also interacts with other transcription factors implicated in adult neurogenesis, the PPAR-γ and C/EBP-δ [72, 73]. The basal expression of both C/EBPδ and PPAR-γ in the adult CNS is relatively low [74, 75]. While NF-κB activation and cytokines such as TNF-α enhance C/EBPδ expression, sustained elevation of inflammatory cytokines suppress its transcription [76]. Glucocorticoids enhance the transcription of both C/EBPδ and PPAR-γ [77, 78]. Further, C/EBPδ has also been shown to bind the C/EBP binding elements in PPAR-γ promoter and upregulate its transcription [75, 76]. Several reports substantiate a neurogenic role for PPAR-γ. Activation of PPAR-γ in neural stem cells has been shown to promote differentiation to mature neurons, astrocytes and oligodendrocytes [74]. Treatment with PPAR-γ agonists has been shown to induce neurite outgrowth and increased axonal length in hippocampal neurons [79].

GILZ has been shown to bind a tandem repeat of C/EBP binding sites and act as a sequence-specific trans-repressor of PPAR-γ in pluripotent mouse mesenchymal stem cells [33]. In human bone marrow derived stem cells dexamethasone induced GILZ has

been shown to suppress PPAR-γ2 and upregulate RUNX-2 transcription factor [73, 80]. Although a plethora of studies substantiate the critical involvement of RUNX2 in osteogenesis, recent reports suggest a potential role for the molecule in neuronal development [81]. Adult human hippocampus and cultured rat astrocytes have been shown to express RUNX2 transcript [82, 83]. In human astroglioma cells RUNX2 is significantly elevated and regulates the expression of galectin 3, a β galactoside-binding lectin that has been shown to promote neural cell adhesion and neurite growth [83].

Collectively, the physical interactions of GILZ with multiple transcription factors greatly enhances the number of potentially regulated genes or post-translational modifications that could crucially affect molecular mechanisms involved in the dynamic process of adult neurogenesis. It is postulated that under low glucocorticoid and inflammatory conditions, GILZ could facilitate neuronal differentiation of stem cells and promote hippocampal neurogenesis by upregulating RUNX-2 transcription. This is supported by the observations of inverse relationship between NF-κB and RUNX-2 transcription factors and elevated RUNX-2 in adult hippocampus [83, 84].

IV. Conclusion:

The effects of endogenous and exogenous glucocorticoids in neurogenesis, neuroinflammation and neurodegeneration in the CNS have been extensively investigated with some mechanistic elucidations, some contradictory findings and some as yet unanswered questions. It is tempting to speculate that some of the contradictory or unknown glucocorticoids effects could be attributed to the GILZ expression. The

uneven distribution and upregulated expression following HPA activation suggests multiple roles for GILZ in the CNS. Much like its effects on osteogenic differentiation, GILZ could potentially play a role in neuronal differentiation and development. In microglial cells a tightly controlled and coordinated action of MR and GR has been shown to regulate NF-κB functions [8]. At low ligand concentration or at early time points following activation, the effects of MR mediated transactivation of neurotrophic factors and anti-inflammatory GILZ could potentially supersede that of BACE1 and pro-inflammatory cytokines. In conditions of chronic stress and/or Aβ toxicity inflammatory mediators suppress GILZ. In this context, exogenous GILZ could represent an attractive strategy to suppress neuroinflammation. In conclusion, elucidation of the physiological role of GILZ in the CNS, its induction by other factors including microbial agents or free radicals, role in BACE-1 activity and Aβ homeostasis will enhance our understanding of the glucocorticoid signaling in the CNS and help in the identification of alternative therapeutic strategies to target neuroinflammation and neurodegeneration.

Figure legend:

Fig 1: Schematic representation of potential role of GILZ in regulating physiological amyloid beta in CNS health. Low glucocorticoid (GC) and picomolar concentration of amyloid beta (Aβ) are features of healthy CNS. Signaling via phosphatidylinositol-3-kinases (PI3 K) has been shown to critical for neutrophic and anti-apoptotic effects of picomolar Aβ. Blockade of PI3 K has been shown to abrogate such effects. Under low GC conditions, MR is preferentially occupied and mediates GILZ expression. In lymphocytes and epithelial cells, GILZ overexpression has been shown to suppress PI3 kinase activity and inhibit inflammatory responses. Micromolar concentration of Aβ is associated with enhanced production of anti-apoptotic factors via increased PI3 kinase signaling. This figure speculates that constitutive GILZ expression plays a role in modulating PI3 K signaling thereby regulating indirectly the physiological Aβ.

Fig 2: Schematic representation of exogenous GILZ as a potential therapeutic agent for AD. Aging, chronic stress, amyloid b aggregates or other factors mediated oxidative stress activate NF-κB p65 which in turn induces activation of pro-inflammatory (cytokines, glutamate) and proapoptotic factors that ultimately lead to cell death. Exogenous GILZ will bind p65 and prevent activation of deleterious mediators.

Fig 3: Potential role of GILZ adult neurogenesis: Schematic representation of some of the factors involved in adult neurogenesis. Toll like receptor (TLR) stimulation and cytokines such as TNF-α activate NF-κB transcription factor in adult hippocampal neural stem cells. While NF-

κB C-rel containing dimers are predominantly neuroprotective, p65 containing dimers promote transactivation of inflammatory cytokines that upregulate C/EBP δ transcription factor which in turn induces PPAR-γ. However, inflammatory cytokines can also downregulate both C/EBP δ and PPAR-γ. Glucocorticoids induced GILZ expression in neural stem cells can modulate this process at two steps (inhibitory actions shown in red): 1) blockade of NF-κB p65 and 2) suppression of PPAR-γ and transactivation of RUNX-2.

References:

1.   Abraham IM, Meerlo P, Luiten PG: **Concentration dependent actions of glucocorticoids on neuronal viability and survival**. *Dose Response* 2006, **4**(1):38-54.

2.   Brunson KL, Chen Y, Avishai-Eliner S, Baram TZ: **Stress and the developing hippocampus: a double-edged sword?** *Molecular neurobiology* 2003, **27**(2):121-136.

3.   Madan AP, DeFranco DB: **Bidirectional transport of glucocorticoid receptors across the nuclear envelope**. *Proc Natl Acad Sci U S A* 1993, **90**(8):3588-3592.

4.   Brureau A, Zussy C, Delair B, Ogier C, Ixart G, Maurice T, Givalois L: **Deregulation of hypothalamic-pituitary-adrenal axis functions in an Alzheimer's disease rat model**. *Neurobiology of aging* 2013, **34**(5):1426-1439.

5.   Grossmann C, Scholz T, Rochel M, Bumke-Vogt C, Oelkers W, Pfeiffer AF, Diederich S, Bahr V: **Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties**. *Eur J Endocrinol* 2004, **151**(3):397-406.

6.   Rupprecht R, Reul JM, van Steensel B, Spengler D, Soder M, Berning B, Holsboer F, Damm K: **Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands**. *Eur J Pharmacol* 1993, **247**(2):145-154.

7.   Newton R: **Molecular mechanisms of glucocorticoid action: what is important?** *Thorax* 2000, **55**(7):603-613.

8.   Ogita K, Sugiyama C, Acosta GB, Kuramoto N, Shuto M, Yoneyama M, Nakamura Y, Shiba T, Yamaguchi T: **Opposing roles of glucocorticoid receptor and mineralocorticoid receptor in trimethyltin-induced cytotoxicity in the mouse hippocampus**. *Neurosci Lett* 2012, **511**(2):116-119.

9.   De Kloet ER, Reul JM: **Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems**. *Psychoneuroendocrinology* 1987, **12**(2):83-105.

10.   Le Menuet D, Lombes M: **The neuronal mineralocorticoid receptor: from cell survival to neurogenesis**. *Steroids* 2014, **91**:11-19.

11.   ter Heegde F, De Rijk RH, Vinkers CH: **The brain mineralocorticoid receptor and stress resilience**. *Psychoneuroendocrinology* 2015, **52**:92-110.

12.   Chen DW, Lynch JT, Demonacos C, Krstic-Demonacos M, Schwartz JM: **Quantitative analysis and modeling of glucocorticoid-controlled gene expression**. *Pharmacogenomics* 2010, **11**(11):1545-1560.

13.   Chantong B, Kratschmar DV, Nashev LG, Balazs Z, Odermatt A: **Mineralocorticoid and glucocorticoid receptors differentially regulate NF-kappaB activity and pro-inflammatory cytokine production in murine BV-2 microglial cells**. *Journal of neuroinflammation* 2012, **9**:260.

14.   Giubilei F, Patacchioli FR, Antonini G, Sepe Monti M, Tisei P, Bastianello S, Monnazzi P, Angelucci L: **Altered circadian cortisol secretion in Alzheimer's disease: clinical and neuroradiological aspects**. *J Neurosci Res* 2001, **66**(2):262-265.

15.   Huang CW, Lui CC, Chang WN, Lu CH, Wang YL, Chang CC: **Elevated basal cortisol level predicts lower hippocampal volume and cognitive decline in Alzheimer's disease**. *J Clin Neurosci* 2009, **16**(10):1283-1286.

16.   Weiner MF, Vobach S, Olsson K, Svetlik D, Risser RC: **Cortisol secretion and Alzheimer's disease progression**. *Biol Psychiatry* 1997, **42**(11):1030-1038.

17.   Abraham I, Harkany T, Horvath KM, Veenema AH, Penke B, Nyakas C, Luiten PG: **Chronic corticosterone administration dose-dependently modulates Abeta(1-42)- and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis**. *J Neuroendocrinol* 2000, **12**(6):486-494.

18.   Baglietto-Vargas D, Chen Y, Suh D, Ager RR, Rodriguez-Ortiz CJ, Medeiros R, Myczek K, Green KN, Baram TZ, LaFerla FM: **Short-term modern life-like stress exacerbates Abeta-pathology and synapse loss in 3xTg-AD mice**. *Journal of neurochemistry* 2015, **134**(5):915-926.

19.   Li WZ, Li WP, Yao YY, Zhang W, Yin YY, Wu GC, Gong HL: **Glucocorticoids increase impairments in learning and memory due to elevated amyloid precursor protein expression and neuronal apoptosis in 12-month old mice**. *Eur J Pharmacol* 2010, **628**(1-3):108-115.

20.   Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM: **Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease**. *J Neurosci* 2006, **26**(35):9047-9056.

21.   Sayre LM, Perry G, Smith MA: **Oxidative stress and neurotoxicity**. *Chem Res Toxicol* 2008, **21**(1):172-188.

22.   Swomley AM, Forster S, Keeney JT, Triplett J, Zhang Z, Sultana R, Butterfield DA: **Abeta, oxidative stress in Alzheimer disease: evidence based on proteomics studies**. *Biochimica et biophysica acta* 2014, **1842**(8):1248-1257.

23.   Rothman SM, Mattson MP: **Adverse stress, hippocampal networks, and Alzheimer's disease**. *Neuromolecular Med* 2010, **12**(1):56-70.

24.   Sotiropoulos I, Cerqueira JJ, Catania C, Takashima A, Sousa N, Almeida OF: **Stress and glucocorticoid footprints in the brain-the path from depression to Alzheimer's disease**. *Neurosci Biobehav Rev* 2008, **32**(6):1161-1173.

25.   Cannarile L, Zollo O, D'Adamio F, Ayroldi E, Marchetti C, Tabilio A, Bruscoli S, Riccardi C: **Cloning, chromosomal assignment and tissue distribution of human GILZ, a glucocorticoid hormone-induced gene**. *Cell death and differentiation* 2001, **8**(2):201-203.

26. Di Marco B, Massetti M, Bruscoli S, Macchiarulo A, Di Virgilio R, Velardi E, Donato V, Migliorati G, Riccardi C: **Glucocorticoid-induced leucine zipper (GILZ)/NF-kappaB interaction: role of GILZ homo-dimerization and C-terminal domain**. *Nucleic Acids Res* 2007, **35**(2):517-528.

27. Ayyar VS, Almon RR, Jusko WJ, DuBois DC: **Quantitative tissue-specific dynamics of in vivo GILZ mRNA expression and regulation by endogenous and exogenous glucocorticoids**. *Physiol Rep* 2015, **3**(6).

28. Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF: **beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat**. *J Neurosci* 2011, **31**(17):6277-6288.

29. Yachi K, Inoue K, Tanaka H, Yoshikawa H, Tohyama M: **Localization of glucocorticoid-induced leucine zipper (GILZ) expressing neurons in the central nervous system and its relationship to the stress response**. *Brain Res* 2007, **1159**:141-147.

30. Berrebi D, Bruscoli S, Cohen N, Foussat A, Migliorati G, Bouchet-Delbos L, Maillot MC, Portier A, Couderc J, Galanaud P *et al*: **Synthesis of glucocorticoid-induced leucine zipper (GILZ) by macrophages: an anti-inflammatory and immunosuppressive mechanism shared by glucocorticoids and IL-10**. *Blood* 2003, **101**(2):729-738.

31. Ayroldi E, Riccardi C: **Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action**. *FASEB J* 2009, **23**(11):3649-3658.

32. Fan H, Morand EF: **Targeting the side effects of steroid therapy in autoimmune diseases: the role of GILZ**. *Discov Med* 2012, **13**(69):123-133.

33. Shi XM, Blair HC, Yang X, McDonald JM, Cao X: **Tandem repeat of C/EBP binding sites mediates PPARgamma2 gene transcription in glucocorticoid-induced adipocyte differentiation**. *J Cell Biochem* 2000, **76**(3):518-527.

34. Atwood CS, Obrenovich ME, Liu T, Chan H, Perry G, Smith MA, Martins RN: **Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta**. *Brain Res Brain Res Rev* 2003, **43**(1):1-16.

35. Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ: **Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release**. *Brain research* 1998, **785**(2):195-206.

36. Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, Carmona-Cuenca I, Caballero C, Ruano D, Gutierrez A *et al*: **Age-dependent accumulation of soluble amyloid beta (Abeta) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP(alpha)) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3beta pathway in Alzheimer mouse model**. *J Biol Chem* 2011, **286**(21):18414-18425.

37. Bhaskar K, Miller M, Chludzinski A, Herrup K, Zagorski M, Lamb BT: **The PI3K-Akt-mTOR pathway regulates Abeta oligomer induced neuronal cell cycle events**. *Mol Neurodegener* 2009, **4**:14.

38. Jiao J, Xue B, Zhang L, Gong Y, Li K, Wang H, Jing L, Xie J, Wang X: **Triptolide inhibits amyloid-beta1-42-induced TNF-alpha and IL-1beta production in cultured rat microglia**. *Journal of neuroimmunology* 2008, **205**(1-2):32-36.

39. Huang HJ, Liang KC, Chang YY, Ke HC, Lin JY, Hsieh-Li HM: **The interaction between acute oligomer Abeta(1-40) and stress severely impaired spatial learning and memory**. *Neurobiol Learn Mem* 2010, **93**(1):8-18.

40. Almeida OF, Conde GL, Crochemore C, Demeneix BA, Fischer D, Hassan AH, Meyer M, Holsboer F, Michaelidis TM: **Subtle shifts in the ratio between pro- and antiapoptotic molecules after activation of corticosteroid receptors decide neuronal fate**. *FASEB J* 2000, **14**(5):779-790.

41. Lai M, Seckl J, Macleod M: **Overexpression of the mineralocorticoid receptor protects against injury in PC12 cells**. *Brain Res Mol Brain Res* 2005, **135**(1-2):276-279.

42. Murphy EK, Spencer RL, Sipe KJ, Herman JP: **Decrements in nuclear glucocorticoid receptor (GR) protein levels and DNA binding in aged rat hippocampus**. *Endocrinology* 2002, **143**(4):1362-1370.

43. Sola S, Amaral JD, Borralho PM, Ramalho RM, Castro RE, Aranha MM, Steer CJ, Rodrigues CM: **Functional modulation of nuclear steroid receptors by tauroursodeoxycholic acid reduces amyloid beta-peptide-induced apoptosis**. *Mol Endocrinol* 2006, **20**(10):2292-2303.

44. Bergann T, Fromm A, Borden SA, Fromm M, Schulzke JD: **Glucocorticoid receptor is indispensable for physiological responses to aldosterone in epithelial Na+ channel induction via the mineralocorticoid receptor in a human colonic cell line**. *Eur J Cell Biol* 2011, **90**(5):432-439.

45. Aguilar D, Strom J, Chen QM: **Glucocorticoid induced leucine zipper inhibits apoptosis of cardiomyocytes by doxorubicin**. *Toxicol Appl Pharmacol* 2014, **276**(1):55-62.

46. Delfino DV, Agostini M, Spinicelli S, Vito P, Riccardi C: **Decrease of Bcl-xL and augmentation of thymocyte apoptosis in GILZ overexpressing transgenic mice**. *Blood* 2004, **104**(13):4134-4141.

47. Kervoelen C, Menoret E, Gomez-Bougie P, Bataille R, Godon C, Marionneau-Lambot S, Moreau P, Pellat-Deceunynck C, Amiot M: **Dexamethasone-induced cell death is restricted to specific molecular subgroups of multiple myeloma**. *Oncotarget* 2015, **6**(29):26922-26934.

48. Grugan KD, Ma C, Singhal S, Krett NL, Rosen ST: **Dual regulation of glucocorticoid-induced leucine zipper (GILZ) by the glucocorticoid receptor and the PI3-**

kinase/AKT pathways in multiple myeloma. *J Steroid Biochem Mol Biol* 2008, **110**(3-5):244-254.

49. Gupta V, Awasthi N, Wagner BJ: **Specific activation of the glucocorticoid receptor and modulation of signal transduction pathways in human lens epithelial cells.** *Invest Ophthalmol Vis Sci* 2007, **48**(4):1724-1734.

50. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM *et al*: **Neuroinflammation in Alzheimer's disease.** *Lancet neurology* 2015, **14**(4):388-405.

51. Latta CH, Brothers HM, Wilcock DM: **Neuroinflammation in Alzheimer's disease; A source of heterogeneity and target for personalized therapy.** *Neuroscience* 2015, **302**:103-111.

52. Gao HM, Hong JS: **Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression.** *Trends in immunology* 2008, **29**(8):357-365.

53. Bales KR, Du Y, Holtzman D, Cordell B, Paul SM: **Neuroinflammation and Alzheimer's disease: critical roles for cytokine/Abeta-induced glial activation, NF-kappaB, and apolipoprotein E.** *Neurobiology of aging* 2000, **21**(3):427-432; discussion 451-423.

54. Clementi ME, Pezzotti M, Orsini F, Sampaolese B, Mezzogori D, Grassi C, Giardina B, Misiti F: **Alzheimer's amyloid beta-peptide (1-42) induces cell death in human neuroblastoma via bax/bcl-2 ratio increase: an intriguing role for methionine 35.** *Biochem Biophys Res Commun* 2006, **342**(1):206-213.

55. Kitamura Y, Shimohama S, Kamoshima W, Ota T, Matsuoka Y, Nomura Y, Smith MA, Perry G, Whitehouse PJ, Taniguchi T: **Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease.** *Brain research* 1998, **780**(2):260-269.
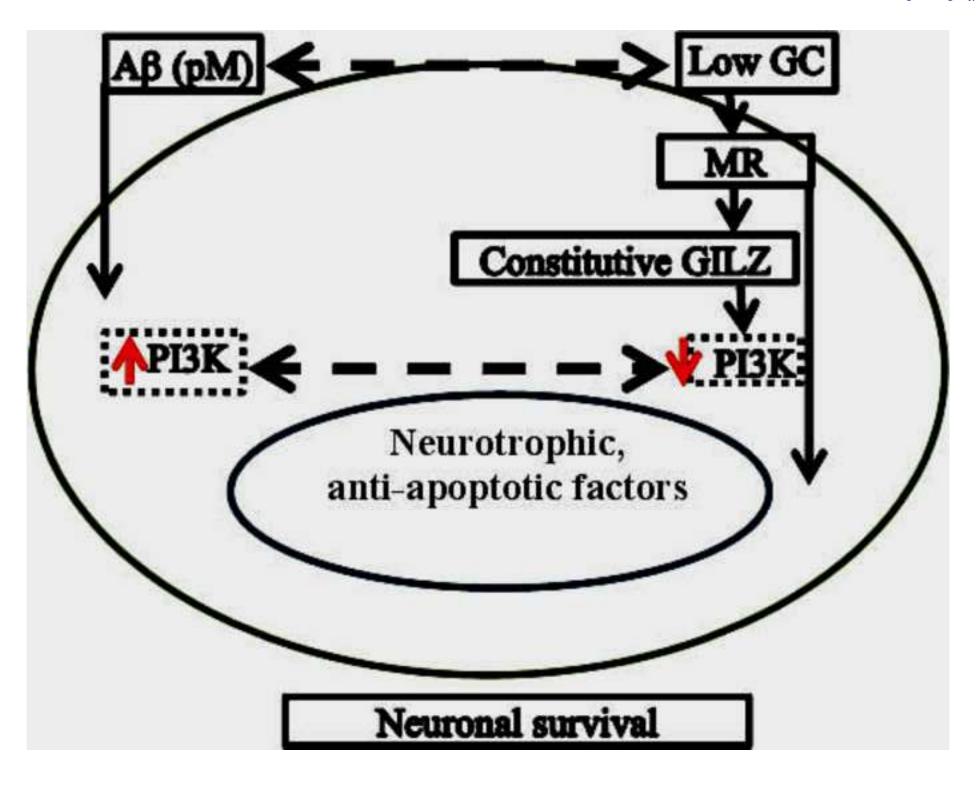
56. MacGibbon GA, Lawlor PA, Sirimanne ES, Walton MR, Connor B, Young D, Williams C, Gluckman P, Faull RL, Hughes P *et al*: **Bax expression in mammalian neurons undergoing apoptosis, and in Alzheimer's disease hippocampus**. *Brain research* 1997, **750**(1-2):223-234.

57. Dursun E, Gezen-Ak D, Hanagasi H, Bilgic B, Lohmann E, Ertan S, Atasoy IL, Alaylioglu M, Araz OS, Onal B *et al*: **The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease**. *Journal of neuroimmunology* 2015, **283**:50-57.

58. Tischner D, Reichardt HM: **Glucocorticoids in the control of neuroinflammation**. *Mol Cell Endocrinol* 2007, **275**(1-2):62-70.

59. McKay LI, Cidlowski JA: **Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways**. *Endocr Rev* 1999, **20**(4):435-459.

60. Riccardi C: **[GILZ (glucocorticoid-induced leucine zipper), a mediator of the anti-inflammatory and immunosuppressive activity of glucocorticoids]**. *Ann Ig* 2010, **22**(1 Suppl 1):53-59.

61. Srinivasan M, Janardhanam S: **Novel p65 Binding Glucocorticoid-induced Leucine Zipper Peptide Suppresses Experimental Autoimmune Encephalomyelitis.** . *Journal of Biological Chemistry* 2011, **286**(52):44799-44810.

62. Cannarile L, Cuzzocrea S, Santucci L, Agostini M, Mazzon E, Esposito E, Muia C, Coppo M, Di Paola R, Riccardi C: **Glucocorticoid-induced leucine zipper is protective in Th1-mediated models of colitis**. *Gastroenterology* 2009, **136**(2):530-541.

63. Mazzon E, Bruscoli S, Galuppo M, Biagioli M, Sorcini D, Bereshchenko O, Fiorucci C, Migliorati G, Bramanti P, Riccardi C: **Glucocorticoid-induced leucine zipper (GILZ)**
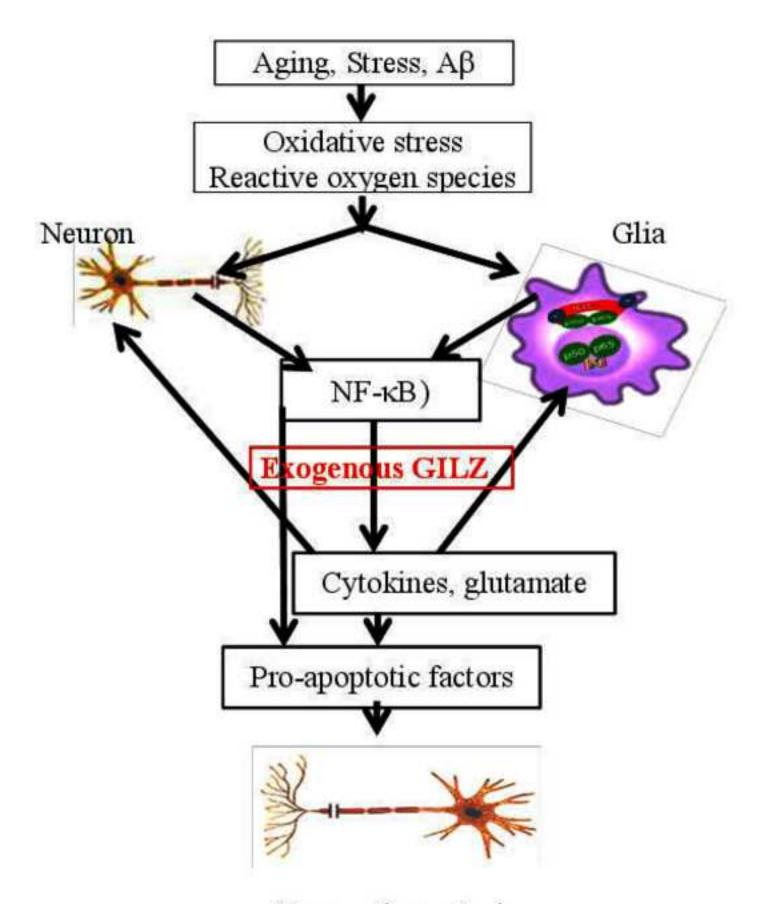
controls inflammation and tissue damage after spinal cord injury. *CNS Neurosci Ther* 2014, **20**(11):973-981.

64.   Srinivasan M: **Glucocoriticoid induced leucine zipper as therapeutic agents in multiple sclerosis**. In: *Patent application publication.* Edited by office USPI; 2014.

65.   Srinivasan M, Blackburn C, Lahiri D: **Functional characterization of a competitive peptide antagonist of p65 in human macrophage like cells suggests a therapeutic potential for chronic inflammation**. *Drug Des Devel Ther* 2014(Accepted (In press)).

66.   Faigle R, Song H: **Signaling mechanisms regulating adult neural stem cells and neurogenesis**. *Biochimica et biophysica acta* 2013, **1830**(2):2435-2448.

67.   Zhang Y, Hu W: **NFkappaB signaling regulates embryonic and adult neurogenesis**. *Front Biol (Beijing)* 2012, **7**(4).

68.   Saaltink DJ, Vreugdenhil E: **Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition?** *Cellular and molecular life sciences : CMLS* 2014, **71**(13):2499-2515.

69.   Bortolotto V, Cuccurazzu B, Canonico PL, Grilli M: **NF-kappaB mediated regulation of adult hippocampal neurogenesis: relevance to mood disorders and antidepressant activity**. *Biomed Res Int* 2014, **2014**:612798.

70.   Pizzi M, Goffi F, Boroni F, Benarese M, Perkins SE, Liou HC, Spano P: **Opposing roles for NF-kappa B/Rel factors p65 and c-Rel in the modulation of neuron survival elicited by glutamate and interleukin-1beta**. *J Biol Chem* 2002, **277**(23):20717-20723.

71.   Egeland M, Zunszain PA, Pariante CM: **Molecular mechanisms in the regulation of adult neurogenesis during stress**. *Nat Rev Neurosci* 2015, **16**(4):189-200.

72.   Shi X, Shi W, Li Q, Song B, Wan M, Bai S, Cao X: **A glucocorticoid-induced leucine-zipper protein, GILZ, inhibits adipogenesis of mesenchymal cells**. *EMBO Rep* 2003, **4**(4):374-380.

73. Zhang W, Yang N, Shi XM: **Regulation of mesenchymal stem cell osteogenic differentiation by glucocorticoid-induced leucine zipper (GILZ)**. *J Biol Chem* 2008, **283**(8):4723-4729.

74. Morales-Garcia JA, Luna-Medina R, Alfaro-Cervello C, Cortes-Canteli M, Santos A, Garcia-Verdugo JM, Perez-Castillo A: **Peroxisome proliferator-activated receptor gamma ligands regulate neural stem cell proliferation and differentiation in vitro and in vivo**. *Glia* 2011, **59**(2):293-307.

75. Pulido-Salgado M, Vidal-Taboada JM, Saura J: **C/EBPbeta and C/EBPdelta transcription factors: Basic biology and roles in the CNS**. *Prog Neurobiol* 2015, **132**:1-33.

76. Ko CY, Chang WC, Wang JM: **Biological roles of CCAAT/Enhancer-binding protein delta during inflammation**. *J Biomed Sci* 2015, **22**:6.

77. Wu Z, Bucher NL, Farmer SR: **Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids**. *Mol Cell Biol* 1996, **16**(8):4128-4136.

78. Ramos RA, Nishio Y, Maiyar AC, Simon KE, Ridder CC, Ge Y, Firestone GL: **Glucocorticoid-stimulated CCAAT/enhancer-binding protein alpha expression is required for steroid-induced G1 cell cycle arrest of minimal-deviation rat hepatoma cells**. *Mol Cell Biol* 1996, **16**(10):5288-5301.

79. Mandrekar-Colucci S, Sauerbeck A, Popovich PG, McTigue DM: **PPAR agonists as therapeutics for CNS trauma and neurological diseases**. *ASN Neuro* 2013, **5**(5):e00129.

80. Pan G, Cao J, Yang N, Ding K, Fan C, Xiong WC, Hamrick M, Isales CM, Shi XM: **Role of glucocorticoid-induced leucine zipper (GILZ) in bone acquisition**. *J Biol Chem* 2014, **289**(28):19373-19382.

81. Boeckx C, Benitez-Burraco A: **Osteogenesis and neurogenesis: a robust link also for language evolution**. *Front Cell Neurosci* 2015, **9**:291.

82. Jeong JH, Jin JS, Kim HN, Kang SM, Liu JC, Lengner CJ, Otto F, Mundlos S, Stein JL, van Wijnen AJ *et al*: **Expression of Runx2 transcription factor in non-skeletal tissues, sperm and brain**. *J Cell Physiol* 2008, **217**(2):511-517.

83. Vladimirova V, Waha A, Luckerath K, Pesheva P, Probstmeier R: **Runx2 is expressed in human glioma cells and mediates the expression of galectin-3**. *J Neurosci Res* 2008, **86**(11):2450-2461.

84. Yao Z, Li Y, Yin X, Dong Y, Xing L, Boyce BF: **NF-kappaB RelB negatively regulates osteoblast differentiation and bone formation**. *J Bone Miner Res* 2014, **29**(4):866-877.

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Figure 1

Figure 2

Neuronal apoptosis

Figure 3

Figure 3