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Short communication

Glycogen synthase kinase-3beta affects size of dentate gyrus and species-typical behavioral tasks in transgenic and knockout mice

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HIGHLIGHTS

- Glycogen synthase kinase-3 (GSK-3) is implicated in several brain disorders.
- Neuron-specific GSK-3beta transgenic mice and knock-out mice were investigated.
- Both strains of mice show smaller dentate gyrus.
- Adult neurogenesis is reduced in transgenic GSK-3beta mice, but not in knock-out mice.
- Mice show deficits in species-typical behavior – nest building, digging, marble burying.
- Adult brain neurogenesis may contribute to species-typical behaviors.

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ABSTRACT

Glycogen synthase kinase-3 (GSK-3), a multifunctional serine-threonine kinase, is an important regulator in numerous signaling pathways and processes including adult brain neurogenesis. GSK-3 (mal)functioning was implicated in many diseases, in particular neurological and behavioral disorders. We investigated the impact of altered levels of the GSK-3β isoform on hippocampal size, number of doublecortin-positive cells, and hippocampal-dependent behaviors. Both GSK-3β transgenic mice (GSK-3β[S9A] mice) and GSK-3β neuron-specific knockout (GSK-3$\beta^{n-/-}$) mice, showed reduced size of the dentate gyrus (DG) and were impaired in three hippocampal-dependent, species-typical behavioral tasks: digging, marble burying and nest building. We further demonstrate that the number of differentiating, doublecortin-positive new neurons is reduced in GSK-3β[S9A] mice, but not in GSK-3$\beta^{n-/-}$ mice. We conclude that GSK-3β activity must be critically controlled to allow wild type-like volume of the dentate gyrus and for normal execution of hippocampal-dependent, species-typical behavior.

Most species express two GSK-3 isoforms – alpha and beta – which are both widely expressed, also in the nervous system, where GSK-3 activity is a key element of several different signaling pathways, including those activated by Wnt/β-catenin, Sonic Hedgehog, Notch, growth factor/RTK/Akt, DISC1, dopamine receptors, and G protein-coupled receptor signals. These pathways are crucial for brain development, especially neural progenitor homeostasis/self-renewal, neurogenesis, neuronal migration, neural differentiation, and eventually synaptic development (reviewed in [1–3]). GSK-3 acts upon over a 100 substrates and interacts with many partners. Malfunction of such interactions is anticipated to cause several diseases, and contribute to behavioral defects: Alzheimer's disease (AD), autism spectrum disorders, bipolar disorder, depression, ALS, schizophrenia, among others (reviewed in [1,2,4,5]). GSK-3β is studied especially intensely because of its role in the connection between amyloid and tau pathogenesis in AD [6]. Of note, AD and most of the behavioral disorders mentioned above are associated with a smaller or degenerated hippocampal formation [7,8]. Throughout adulthood, GSK-3 continues to regulate numerous processes including neurogenesis in the DG of the hippocampal formation, acting mainly as an inhibitor of the canonical Wnt signaling pathway, which promotes adult neurogenesis [5]. This latter process along with its functional significance is studied intensely in behavioral models of learning and memory (e.g., [9,10]) as well as depression and anxiety [11]. Apart from adulthood, however, changes during the early developmental period are also known to affect the DG size and/or function in these phenomena [12–15].

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Much of our knowledge regarding GSK-3β functioning comes from genetically modified mice. GSK-3β[S9A] transgenic mice express the constitutively active GSK-3β[S9A] transgene, driven by the Thy-1 gene promoter, which limits GSK-3β expression to post-natal neurons. These mice showed an overall reduction of brain size including the hippocampal formation [16]. Conversely, an increased overall brain weight was observed in GSK-3α knockout mice [1]. Here we confirm that GSK-3β[S9A] mice have a smaller DG than wild-type mice.

Available GSK-3β models are still being actively characterized, and only incomplete and sometimes contradictory data are available, illustrating the complexity of the problem. In general, animal models for neuro-developmental disorders show altered levels and activity of GSK-3 in the brain (summarized in [2,4]), while inhibition of GSK-3 activity reduces the pathological symptoms, e.g., of bipolar disorder [5]. However, heterozygous GSK-3β (+/−) mice display multiple neurobehavioral abnormalities: reduced immobility in forced-swimming test, increased anxiety, reduced movement and exploration, and impaired memory-formation and consolidation [1,2].

Increased activity of GSK-3β in the GSK-3β[S9A] mice causes minor psychomotor deficits [16], hyperactivity and behavioral correlates of mania, i.e., hypophagia, increased locomotor activity, increased acoustic startle response with decreased habituation as confirmed in the open field task [17]. GSK-3α/β knock-in mice, in which both GSK isoforms are constitutively active, displayed normal sociability behavior but impaired social preference and anxiety-related behavior during social interaction [18].

Induced knockout of GSK-3β in GSK-3$\beta^{n-/-}$ mice, that we analyzed, was also controlled by the Thy-1 gene promoter and therefore was also limited to post-natal neurons. We measured the size of the DG in GSK-3$\beta^{n-/-}$ mice and observed, in both genotypes, the smaller hippocampal DG. We went on to study the effects of increased and decreased GSK-3β activity on the number of maturing neurons and on 3 types of hippocampal-dependent species-typical behaviors: digging, marble burying and nest building [19]. These tests were chosen for their simplicity, minimal equipment requirements, ethological features and lack of strong stressful stimuli.

In total, 71 mice in the age bracket of 3–4 months were analyzed or tested in this study: GSK-3β[S9A] mice ($n = 17$) with wild-type (WT, $n = 18$) littermates as controls, and GSK-3$\beta^{n-/-}$ mice ($n = 17$) with GSK-3$\beta^{loxP/loxP}$ littermates, not expressing Cre recombinase, as controls ($n = 19$). Mice were kept under a natural light/dark cycle in Plexiglas cages with water and food provided ad libitum. The GSK-3β[S9A] mice (line 5) were heterozygous for the transgene, maintained in the FVB/N genetic background [16,20]. GSK-3$\beta^{n-/-}$ mice were obtained by crossing mice with floxed GSK-3β genes with Thy-1-Cre recombinase transgenic mice as described [21], maintained in a mixed FVB × C57BL/6 genetic background. All efforts were made to minimize the number of animals and their discomfort. The experiments were carried out in accordance with the EC Directives 86/609 and 2010/63, approved and supervised by the KULeuven Ethical Commission for Animal Welfare.

The volume of the DG of both mouse strains (GSK-3β[S9A], $n = 5$; WT, $n = 5$; GSK-3$\beta^{n-/-}$, $n = 6$; GSK-3$\beta^{loxP/loxP}$, $n = 6$) was measured using Cavalieri's method [22]. The density of immature neurons was estimated by immunohistochemical staining for doublecortin (DCX) in GSK-3β[S9A] and WT-mice ($n = 6$ each) as well as in GSK-3$\beta^{n-/-}$ mice ($n = 5$) and GSK-3$\beta^{loxP/loxP}$ control mice ($n = 6$). The mice were perfused transcardiacally with ice-cold saline for 2 min, and the brains were removed and fixed in 4% PFA overnight. Vibratome coronal brain sections (40 μm) were processed for standard immunohistochemistry. The primary antibody was goat polyclonal anti-DCX (Santa-Cruz, 1:200) followed by biotinylated rabbit anti-goat as the secondary antibody (Vector Laboratories, 1:5000). Sections were counterstained with hematoxylin, dehydrated by passage through a graded series of alcohol-solutions, and delipidated in xylene before mounting in DepeX for microscopic analysis. DCX-stained cells were counted in both hippocampi on 3–4 sections per animal randomly selected (−2.30 to −3.28 from bregma), and the average density of DCX+ cells was calculated for statistical analysis.

Three species-dependent behavioral tests were evaluated blindly. Hippocampal-dependent digging paradigm was performed as described [23]. Mice (GSK-3β[S9A], $n = 5$; WT, $n = 7$; GSK-3$\beta^{n-/-}$, $n = 5$; GSK-3$\beta^{loxP/loxP}$, $n = 8$) were placed in a clean 35 cm × 20 cm × 18 cm cage with a 5 cm thick standard wooden bedding. After each animal testing the bedding was flattened and firmed for reuse. Total test duration was 180 s, and the latency to start digging, the number of digging bouts and the total duration of digging were recorded. Digging was defined as coordinated movements of fore- or hindlimbs that displace the bedding.

Marble burying, another hippocampal-dependent test [23], was performed as described [24]. Mice (GSK-3β[S9A], $n = 5$; WT, $n = 8$; GSK-3$\beta^{n-/-}$, $n = 5$; GSK-3$\beta^{loxP/loxP}$, $n = 8$) were placed in a clean 35 cm × 20 cm × 18 cm cage with 12 glass marbles, 13 mm in diameter placed in two rows in a regular pattern on the standard bedding surface: evenly spaced about 6 cm apart. Total test duration was 30 min and the number of marbles buried within the bedding to at least 2/3 of their diameter was counted.

The nest building ability of the mice, the third hippocampal-dependent task [25], was measured as described [26]. All mice (GSK-3β[S9A], $n = 5$; WT, $n = 8$; GSK-3$\beta^{n-/-}$, $n = 5$; GSK-3$\beta^{loxP/loxP}$, $n = 8$) were housed individually for at least 1 week in 13 cm × 15 cm × 22 cm cages with standard bedding. A standard piece of paper towel (23 cm × 23 cm) was provided 3 h prior to inspection, and the nests were assessed using the following scoring system: 0, no nest; 1, primitive flat nest, pad-shaped, flat paper tissue which slightly elevates a mouse above the bedding; 2, more complex nest including warping and biting the paper towel; 3, complex accurate cup-shaped nests with shredded paper interwoven to form the walls of the cup; 4, complex hooded nest with walls forming a ceiling so the nest becomes a hollow sphere with one opening. The scoring was confirmed by an independent observer with high inter-rater reliability; Cohen's kappa coefficient was 0.946.

Statistical analysis was performed using a dedicated software package (STATISTICA data analysis software system, version 7.1.; StatSoft, Inc., 2005). In cases when the data did not meet the assumptions for ANOVA, the results were transformed using Box-Cox transformation, as advised by the statistical software package (JMP 8.0.1.) which tests for normality and equality of variance. Also, non-parametrical Mann–Whitney tests were used. The minimal level of significance was set at $p < 0.05$.

Changing the level of GSK-3β in either direction in transgenic mice resulted in a smaller size of the DG: in GSK-3β[S9A] mice the structure was 15.1% smaller than in WT controls ($p < 0.01$, Mann–Whitney), which paralleled previous data [16]. Surprisingly, inactivating the GSK-3β gene also resulted in 11.3% reduction of DG size in GSK-3$\beta^{n-/-}$ animals vs. their appropriate controls ($p = 0.05$, Mann–Whitney) (Fig. 1A and B). We tested the hypothesis that both reductions are related to impaired neurogenesis and we investigated the number of new, immature DCX-positive neuronal precursors. Reduction in their number could reflect disturbed development or be a sign of disturbed microenvironment, e.g., reduced concentration of growth factors (or both), which could contribute to the changes in DG volume. However, only in case of GSK-3β[S9A] mice a reduction in the density of DCX-positive cells in DG granule layer was evident (39.4%; $p < 0.01$, Mann–Whitney, Fig. 1C and D), while in GSK-3$\beta^{n-/-}$ animals, no effect on the number of DCX+ cells in the DG was observed. The difference in