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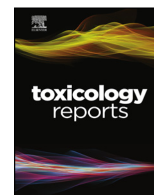

Prenatal alcohol exposure alters p35, CDK5 and GSK3β in the medial frontal cortex and hippocampus of adolescent mice

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ABSTRACT

Fetal alcohol spectrum disorders (FASDs) are the number one cause of preventable mental retardation. An estimated 2–5% of children are diagnosed as having a FASD. While it is known that children prenatally exposed to alcohol experience cognitive deficits and a higher incidence of psychiatric illness later in life, the pathways underlying these abnormalities remain uncertain. GSK3β and CDK5 are protein kinases that are converging points for a vast number of signaling cascades, including those controlling cellular processes critical to learning and memory. We investigated whether levels of GSK3β and CDK5 are affected by moderate prenatal alcohol exposure (PAE), specifically in the hippocampus and medial frontal cortex of the adolescent mouse. In the present work we utilized immunoblotting techniques to demonstrate that moderate PAE increased hippocampal p35 and β-catenin, and decreased total levels of GSK3β, while increasing GSK3β Ser9 and Tyr216 phosphorylation. Interestingly, different alterations were seen in the medial frontal cortex where p35 and CDK5 were decreased and increased total GSK3β was accompanied by reduced Tyr216 of the enzyme. These results suggest that kinase dysregulation during adolescence might be an important contributing factor to the effects of PAE on hippocampal and medial frontal cortical functioning; and by extension, that global modulation of these kinases may produce differing effects depending on brain region.

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1. Introduction

It has been widely recognized that the *in utero* environment can play a profound role in shaping the health and disease susceptibility of the adult [1–3]. During this exquisitely sensitive developmental period, exposure to neurotoxic agents can program lasting and sometimes irreversible damage. Fetal alcohol spectrum disorders (FASDs) generate an array of physiological, behavioral, and intellectual deficits that persist throughout life with particularly pronounced damage occurring in the brain [4]. In a hallmark Center for Disease Control and Prevention report by Streissguth and colleagues [5], greater than 90% of FAS/FASD participants experienced mental health problems. The prevalence of FASD, estimated at 2–5% of children [6], calls for the continued investigation of how this damage transpires and the identification of potential pathways that contribute to perpetuating damage throughout the lifespan. Determination of these pathways could provide novel therapeutic options and potentially decrease the

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later development of comorbid disease states including depression, anxiety disorders, bipolar disorder, and substance abuse [7–12].

Using a mouse model of moderate prenatal alcohol exposure (PAE), we have previously identified disrupted corticosteroid signaling systems in both the medial frontal cortex (mFC) and hippocampus (HPC) of adolescent PAE offspring [13,14]. Corticosteroid signaling includes activation of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) and has been demonstrated to be highly involved in learning and memory processes [15–18], suggesting that dysregulation within brain corticosteroid signaling may contribute to the intellectual impairment after PAE. Curiously, the PAE-induced damage to corticosteroid signaling seems to be specific to discrete brain regions; nuclear levels of GR are increased in the hippocampus with a corresponding increase in trafficking machinery, whereas in the frontal cortex, nuclear accumulation of GR is decreased in conjunction with a decrease in the respective trafficking proteins [13,14]. The signaling cascades that direct GR trafficking alterations in these brain regions after PAE are currently unknown.

Corticosteroid signaling is regulated in part by serine/threonine kinases, including cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase-3β (GSK3β) [19–23]. These kinases orchestrate various cellular functions including neuronal maturation, neurotransmitter release, axon and dendritic organization, neural trafficking, transport, and signal transduction [24–26]; both are implicated in the progression of several major psychiatric illnesses and neurodegenerative diseases [27–29]. Studies have shown that GSK3β and CDK5 are responsive to alcohol [30–32], although the effects generated by a moderate *in vivo* prenatal exposure and the duration of such effects remain unclear. GSK3β and CDK5 can also regulate GR protein stability, subcellular localization, protein interactions, and transcriptional targeting [22,33–35], making these kinases intriguing targets for the investigation of PAE-mediated damage. Based on our previous observations, we sought to investigate the effects of PAE on CDK5 and GSK3β during adolescence. Dysregulated signaling within the GSK3β and CDK5 pathways could be mechanistically related to the GR deficits and could also underlie some of the cognitive and behavioral deficits reported in our PAE model. In the present work, we evaluated CDK5, its activator p35, β-catenin, and GSK3β along with its Ser9 and Tyr216 phosphorylated states in both the mFC and HPC of adolescent mice prenatally exposed to alcohol. To our knowledge, this is the first study to use an *in vivo* model to demonstrate that moderate PAE alters the expression of these critical signaling proteins during the dynamic period of adolescence.

2. Materials and methods

2.1. Prenatal alcohol exposure

All procedures were performed in accordance with the University of New Mexico Institutional Animal Care and Use Committee (IACUC) guidelines. The limited-access PAE was conducted as described previously [36]. C57BL/6J (Jackson Laboratory, Bar Harbor, ME) mice were maintained on a reverse light/dark cycle (lights off at 0800 h and on at 2000 h) with food and water *ad libitum* in a temperature controlled facility. Females were assigned to either a PAE group (10% (w/v) ethanol in 0.066% saccharin) or a 0.066% saccharin control group (SAC). Water bottles were removed and females were provided with access to the respective solution from 1000 h to 1400 h daily. Drinking began prior to pregnancy and was maintained until parturition, at which point alcohol was withdrawn using a step-down procedure over 6 days. This model of drinking produces average blood alcohol concentrations of ∼90 mg/dL at the end of the four hour drinking period [36,37].

2.2. Sample collection

PAE and SAC offspring were weaned at ∼postnatal day (PND) 23 and housed with same-sex littermates. At PND 40–50, male offspring were sacrificed by decapitation and the HPC and mFC were rapidly dissected on ice. The mFC area was defined as the cingulate, infralimbic and prelimbic regions combined [13]. Tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C until tissue processing.

2.3. Tissue homogenization

The HPC and mFC were homogenized in a buffer containing 20 mM Tris–HCl, pH7.4, 1 mM EDTA, 320 mM sucrose, 20 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 10 mM sodium fluoride, 200 μM sodium orthovanadate, and protease inhibitor cocktail (1:1000, Sigma–Aldrich, St. Louis, MO). The homogenate was centrifuged at 1000× *g* for 6 min at 4 °C. The supernatant was collected and the pellet was washed in the homogenization buffer and centrifuged again. The second supernatant was collected and combined with the first, producing a “post-nuclear lysate” which was used in the following protein analysis.

2.4. Immunoblotting

Target proteins were assessed in the HPC and mFC using standard Western immunoblotting techniques as described in [13]. An $N = 6–7$ PAE and SAC samples were run on the same blot for each protein. Tissue homogenate was diluted in NuPAGE® LDS Sample Buffer (#NP0007, Invitrogen™, Grand Island, NY), heated for 10 min at 70°, loaded into 4–12% Bis–Tris gels (#NP0336, Invitrogen™) and electrophoresed for 1.5 h at 165 V (15 μg total protein for GSK3β and β-catenin, 20 μg for CDK5 and p35, 10 μg for pGSK3β Ser9 and pGSK3β Tyr216). For CDK5, β-catenin, GSK3β, and p35, protein was transferred to PVDF membranes (#162-0177, Bio-Rad Laboratories, Hercules, CA) for 1 h at 40 V. Membranes were blocked in Tropix I-BLOCK (#T2015, Applied Biosystems, Grand Island, NY) for 1 h. Blots were then washed in TBST and incubated overnight at 4 °C with primary antibody diluted in I-BLOCK (GSK3β: Santa Cruz Biotechnology, Santa Cruz, CA, #sc-7291, 1:1000, p35: Santa Cruz Biotechnology, #sc-820, 1:1000, CDK5: Santa Cruz Biotechnology, #sc-173, 1:1000, β-catenin: Santa Cruz Biotechnology, #sc-7963,

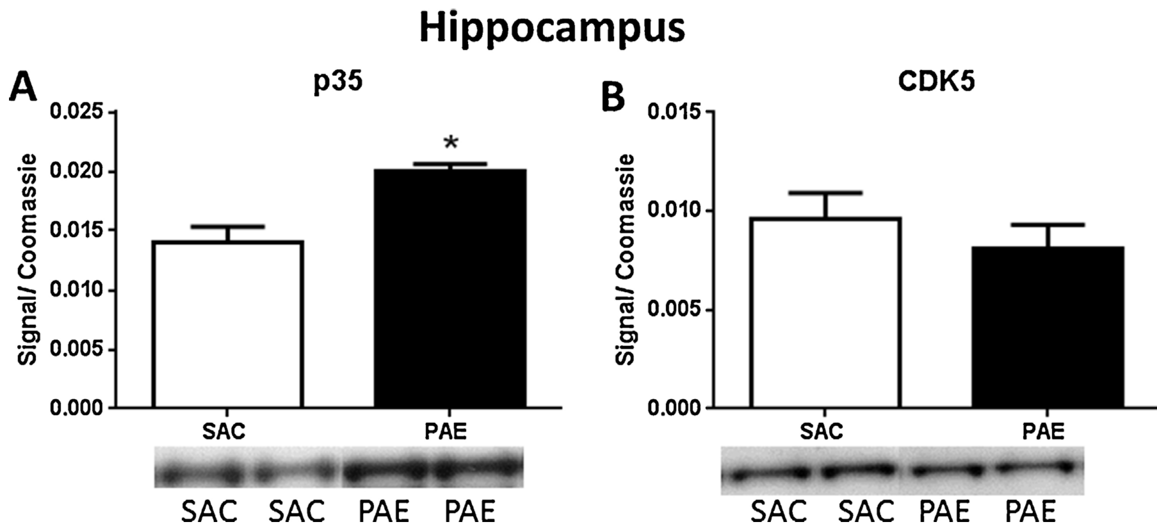

Fig. 1. Prenatal alcohol exposure alters p35 but not CDK5 in the hippocampus. (A) p35 was significantly increased (*$p = .0027$) in PAE ($N = 6$) compared to saccharin controls ($N = 7$). B). CDK5 was unchanged ($p > .05$) between PAE ($N = 7$) and saccharin controls ($N = 7$). Representative Western blot images are shown below each figure. All data are mean ± SEM.

1:1000). Membranes were incubated in goat anti-rabbit or goat anti-mouse secondary antibody solution (#31460, Pierce, Rockford, IL 1:30,000), detected with Western Lightning Plus-ECL developing solution (#NEL105001EA; Perkin Elmer, Waltham, MA) and exposed to x-ray film (#F-BX57, Phenix Research Products, Candler, NC). Immunoreactivities were quantified using Quantity One 1-D Analysis Software (Bio-Rad Laboratories). For both phosphoisoforms of GSK3β, proteins were transferred to Immobilon-FL membranes and blocked with Odyssey™ Blocking Buffer (#927-40000, LI-COR Biosciences, Lincoln, NE). Primary antibodies (GSK3β 216: #GM1321, ECM Biosciences, Versailles, KY, 1:1000: GSK3β Ser9, #D85E12, Cell Signaling, Danvers, MA 1:1000) were detected using IRDye 680RD goat anti-mouse (#926-68070, LI-COR Biosciences, 1:10,000) or 800CW goat anti-rabbit (#926-32211, LI-COR Biosciences, 1:10,000). Blots were scanned using two-channel infrared direct detection (Odyssey Imaging System, LI-COR Biosciences) and quantified using Image Studio (version 3.1 LI-COR Biosciences).

2.5. Coomassie staining

Coomassie staining was performed as a within-lane loading control as described in our previous publications [13,14]. After immunodetection, membranes were stained with Coomassie Brilliant Blue R-250 (Bio-Rad; #161-0400) and quantified using Quantity One Software (Bio-Rad Laboratories). The target protein signal for each lane was corrected against the Coomassie stain for each lane to account for any possible loading discrepancies.

2.6. Statistical analysis

All data were analyzed by Student's t-test using Graphpad PRISM® Software vs. 6.03. Statistical significance was set at $p < .05$ and sample size was defined as the number of animals sampled in each condition, each from a different litter

3. Results

3.1. Prenatal alcohol exposure alters p35 but not CDK5 protein in the hippocampus

To determine the effect of PAE on the protein expression of both CDK5 and its activator, p35, we performed Western immunoblotting on hippocampal tissue. CDK5 activity has been documented to be correlated with levels of expression of p35 [38]. A significant increase in p35 was observed in PAE offspring compared to SAC controls (Fig. 1); $t(11) = 3.85$, $p = .0027$. CDK5 protein was unaltered in PAE compared to SAC controls (Fig. 1); $t(12) = 0.8495$, $p > .05$.

3.2. Prenatal alcohol exposure alters both p35 and CDK5 in the medial frontal cortex

Next, we examined whether PAE would have the same effect on CDK5 and p35 in the mFC as in the HPC. CDK5 and its activator have been demonstrated to play important roles in both of these regions [39,29]. We performed Western immunoblotting for CDK5 and p35 protein in mFC samples from both PAE and SAC controls. We found that p35 was decreased in PAE mFC compared to SAC controls (Fig. 2); $t(12) = 3.064$, $p = .0098$, showing an opposite finding from the HPC. Interestingly, CDK5 protein was also decreased in the mFC in PAE compared to SAC controls (Fig. 2); $t(11) = 4.263$, $p = .0013$.

3.3. Prenatal alcohol exposure decreases GSK3β in the hippocampus andaltersGSK3β phosphorylation

GSK3β is a constitutively active enzyme that is controlled primarily through inhibitory phosphorylation.

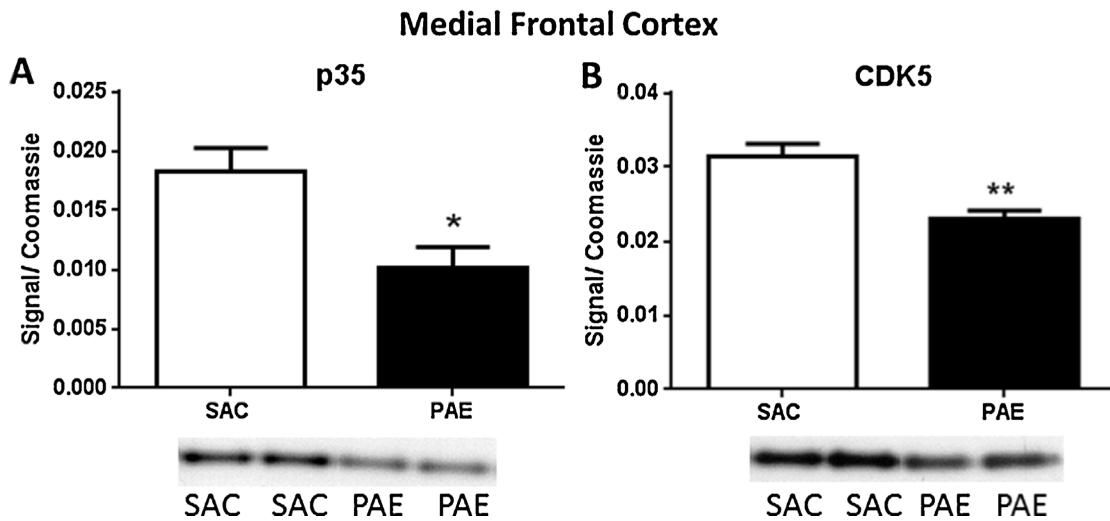

Fig. 2. Prenatal alcohol exposure decreases both p35 and CDK5 protein in the medial frontal cortex. (A) p35 is significantly decreased (* $p = .0098$) in PAE ($N = 7$) compared to saccharin controls ($N = 7$). (B) CDK5 protein is also significantly decreased (** $p = .0013$) in PAE ($N = 7$) compared to saccharin controls ($N = 6$). Representative Western blot images are shown below. All data are expressed as mean ± SEM.

Phosphorylation at the Tyr216 site is necessary for optimal activity, while phosphorylation on the Ser9 residue inactivates the kinase [40]. GSK3β has also been reported to be phosphorylated at Thr43 and Ser389 which are suggested to increase Ser9 phosphorylation [41,42]. To determine the effect of PAE on GSK3β in the HPC, we measured total GSK3β and levels of phosphorylation at both Ser9 and Tyr216. Western immunoblotting showed a significant decrease in the total (both unphosphorylated and phosphorylated isoforms) levels of GSK3β (Fig. 3A); $t(12) = 3.220$, $p = .0074$. Analysis of phosphorylated GSK3β isoforms revealed an increase in Ser9 phosphorylation (Fig. 3B), $p = .038$. As this comparison resulted in a significant Levene's test [$F(12) = 5.4$, $p = .04$], a Mann–Whitney test was used for the post hoc test. There was also an increase in phospho-Tyr216 GSK3β in PAE mice (Fig. 3C), $t(12) = 2.22$, $p = .046$.

3.4. Prenatal alcohol exposure increases GSK3β in the medial frontal cortex and increases Ser9 but not Tyr216 phosphorylation

We also investigated whether PAE would have a similar effect on GSK3β and its phosphorylation in the mFC. PAE significantly increased total GSK3β expression in the mFC compared to SA/C controls (Fig. 4A); $t(12) = 3.593$, $p = .0037$, showing an opposite finding from the HPC. There was no change in Ser9 phosphorylation levels (Fig. 4B), $t(12) = 1.20$, $p > .05$, while the level of phospho-Tyr216 was significantly decreased (Fig. 4C), $t(11) = 3.22$, $p = .008$.

3.5. Prenatal alcohol exposure increases β-catenin in the hippocampus but not medial frontal cortex

Both CDK5 and GSK3β play regulatory roles in β-catenin signaling. CDK5 can bind to β-catenin and regulate its interaction with scaffolding molecules [43–45], while increased GSK3β can target β-catenin for degradation [46]. Thus, we measured β-catenin levels in both the HPC and mFC to determine if the PAE-induced changes in p35/CDK5 and GSK3β would affect this particular substrate. In the HPC of PAE mice, we observed a significant increase in β-catenin compared to SAC controls (Fig. 5); $t(11) = 2.480$, $p = .0306$, although there was no significant change in the mFC $t(12) = 0.1099$, $p > .05$.

4. Discussion

Alterations in GSK3β levels and activity have been associated with neurogenesis, mood disorders, and learning and memory [31]. While not a part of the present study, previous work with our PAE model has found deficits in neurogenesis [47] increased depressive behavior [48] and learning deficits [36]. Although the existing literature suggests that CDK5 and GSK3β contribute to behavioral and cognitive functioning, their roles in PAE-associated impairments in these processes are unknown. Furthermore, the possibility of regional specificity of PAE-induced changes in CDK5 and GSK3β is not fully understood. In the present work, we began to address these issues utilizing an *in vivo* model of moderate PAE to identify alterations in CDK5 and GSK3β in the adolescent HPC and mFC. It is noteworthy that we assessed effects manifested during adolescence, as developmentally significant time points, such as adolescence, are frequently neglected in studies that employ *in vivo* animal models of PAE.

These findings demonstrate that moderate PAE produces region-specific alterations in p35, CDK5, β-catenin, and GSK3β and its phosphorylation in adolescent male offspring. In the HPC, the p35 activator of CDK5 was increased although no change in total CDK5 was noted (Fig. 1). Work by Ou and colleagues determined that CDK5 activity correlates with p35 expression instead of CDK5 expression [38] and, thus, the increased p35 levels in the PAE mouse HPC could produce aberrantly high levels of CDK5 activity in this brain region. Our p35 finding is consistent with a

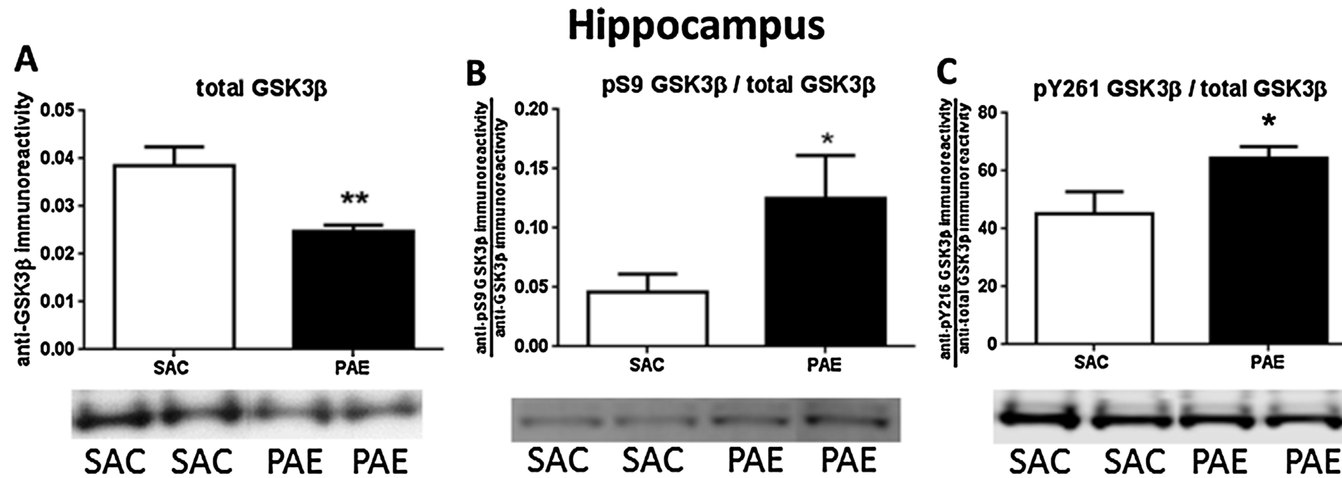

Fig. 3. Prenatal alcohol exposure alters GSK3β total protein as well as Ser9 and Tyr216 phosphorylation in the hippocampus. (A) Total GSK3β protein was significantly decreased (**$p = .0074$) in prenatal alcohol exposure (PAE, $N = 7$) compared to saccharin controls (SAC, $N = 7$) mouse hippocampus. (B) Phosphorylation of GSK3β Ser9 was significantly increased (*$p = .038$) in PAE ($N = 7$) compared to SAC ($N = 7$). (C) Phosphorylation of GSK3β Tyr216 was also increased (*$p = .046$) in PAE ($N = 7$) compared to SAC ($N = 7$). Representative Western images are shown below for each. All data are expressed as mean ± SEM.

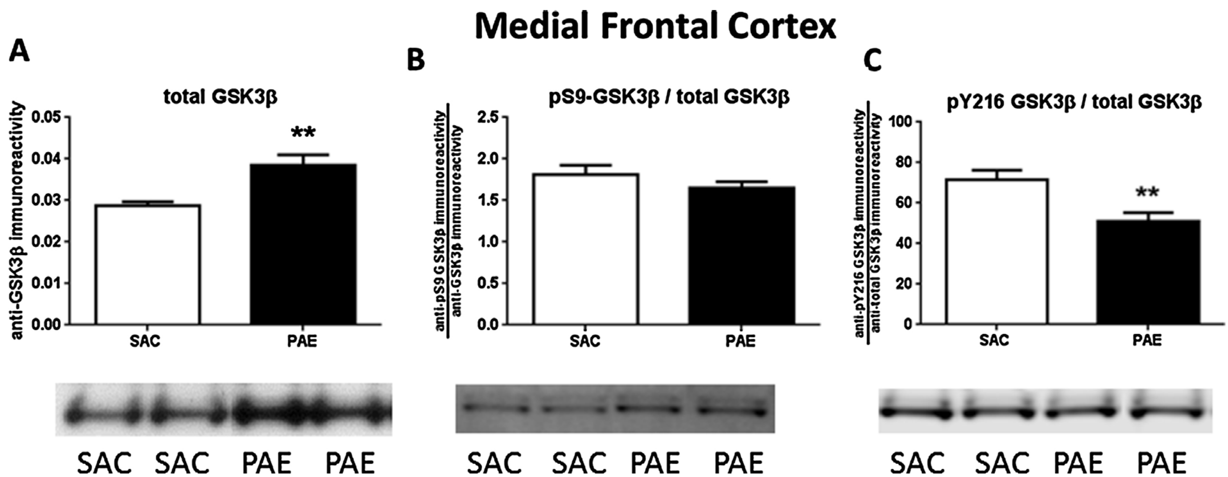

Fig. 4. Prenatal alcohol exposure increases GSK3β total protein and decreases GSK3β Tyr216phosphorylation in the medial frontal cortex. (A) Total GSK3β protein is significantly increased (**$p = .0037$) in prenatal alcohol exposure (PAE, $N = 7$) compared to saccharin controls (SAC, $N = 7$) mouse medial frontal cortex. (B) Phosphorylation of GSK3β at the Ser9 was not different between PAE ($N = 7$) and SAC ($N = 7$) mice. (C) Phosphorylation of GSK3β Tyr216 residue was decreased (**$p = .008$, >.05) in PAE ($N = 6$) compared to SAC ($N = 7$) mice. Representative Western blot images are shown below for each. All data are expressed as mean ± SEM.

report by Li and colleagues [49] that shows PAE increases p35 protein in the HPC of adult rats. This study also found that PAE increases CDK5 in the HPC; although we did not find CDK5 protein to be altered in the HPC in our mouse model, the difference between the studies' results may be due to differences in alcohol exposure levels and routes of administration, with their 25% (w/v) intragastric gavage being much higher than our 10% (w/v) voluntary drinking paradigm. Our study shows that even moderate amounts of ethanol possess the neurotoxic potential to alter p35 expression much later in life. Total GSK3β protein was decreased by PAE in the HPC (Fig. 3). In conjunction with this, increased phosphorylation at the inhibitory Ser9 residue of GSK3β was observed, while phosphorylation of the activating Tyr216 residue was also increased. The effect of these changes on GSK3β catalytic activity was not determined in our studies but is it likely that it is reduced, as total enzyme levels were reduced and GSK3β is a constitutively active kinase that is primarily regulated by inhibition at Ser9 [50]. Taken together, these findings show that regulation of CDK5 and GSK3β is shifted in favor of decreased GSK3β and increased p35 in the PAE adolescent HPC.

In contrast to the HPC protein changes, both p35 and CDK5 were decreased in the mFC (Fig. 2). Decreases in these protein levels show that PAE is altering protein content in a direction consistent with decreased CDK5 activity. Total GSK3β protein was increased and Tyr216 phosphorylation was decreased (Fig. 4). The effect of these changes on GSK3β activity was not measured in these studies but it is likely that the increased GSK3β levels are associated

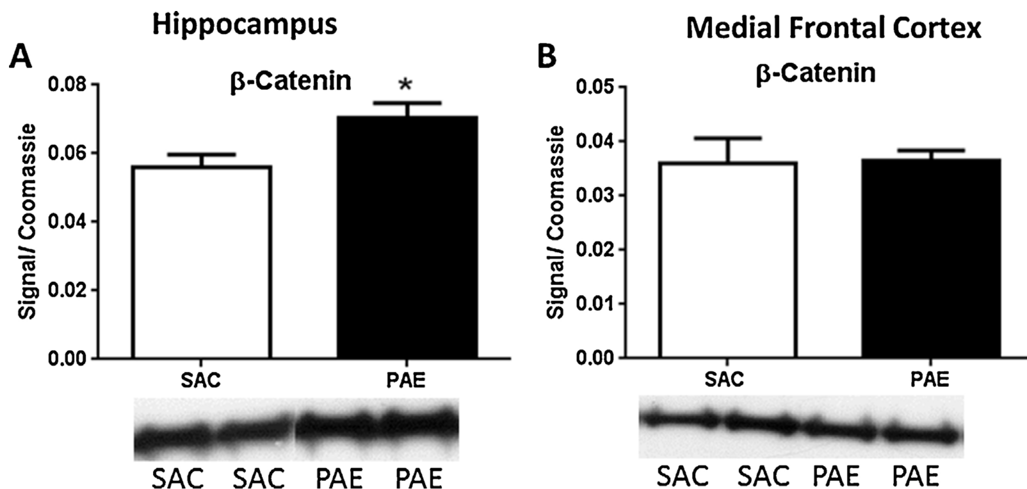

Fig. 5. Prenatal alcohol exposure increases β-catenin in the hippocampus but not medial frontal cortex. (A) β-Catenin is significantly increased in the HPC (*$p = .0306$) in PAE ($N = 7$) compared to saccharin controls ($N = 6$). (B) β-Catenin is unchanged in the mFC ($p > .05$) between PAE ($N = 7$) and saccharin controls ($N = 7$). Representative Western blot images are shown below for each. All data is expressed as mean ± SEM.

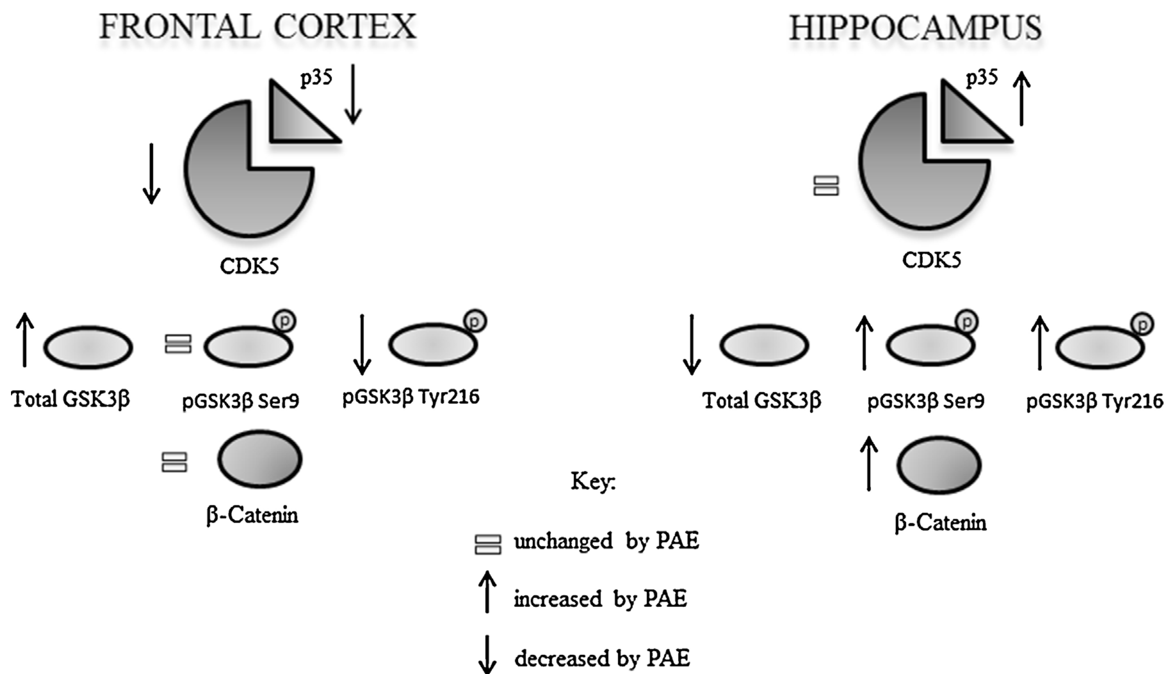

Fig. 6. Summary of findings: prenatal alcohol exposure differentially affects p35, CDK5, β-catenin, total GSK3β, GSK3β Ser9 phosphorylation, and GSK3β Tyr216 phosphorylation in the hippocampus and medial frontal cortex of adolescent male offspring.

with a relative increase in the amount of GSK3β activity present in the cell, as inhibitory regulation was not altered; however, the reduction in Tyr216 phosphorylation may, in part, compensate for the increase in total GSK3β protein. Liu and colleagues [51] have reported that acute treatment (two doses of 2.5 g ethanol/kg, separated by 2 h) of 7-day-old mice with ethanol was associated with reduced GSK3β Ser9 phosphorylation with little effect on the level of total GSK3β or Tyr216 phosphorylation of the enzyme in the cerebral cortex. Acute alcohol treatment paradigms like that in the Liu study have been demonstrated to increase cell death through increasing GSK3b. Differences between our results and those of these investigators are likely due to differences in the treatment paradigm (exposure throughout gestation vs. acute postnatal treatment), the age of testing of the animals (adolescence vs. postnatal day 7) and the tissue assayed (isolated medial frontal cortex vs. whole cerebral cortex). The rise in total protein levels of GSK3β occurs in conjunction with a decrease in p35 and CDK5 levels in the mFC, which is opposite from the decrease in total GSK3β and increase in p35 in the HPC (Fig. 6). Interestingly, CDK5 and GSK3β have been shown to inversely regulate each other. Deleting the p35 gene, the predominant CDK5 activator [52], increases GSK3β and redirects GSK3β gene targeting [25]. Other studies have also described the cross-talk between CDK5 and GSK3 [53–55]. Our results demonstrate that protein regulation is shifted toward p35 and away from GSK3β expression in the HPC, but toward GSK3β expression and away from p35/CDK5 in the mFC (Fig. 6). This could have important implications for therapeutic interventions that utilize global up/down regulation of target proteins since our findings identify regional specificity in PAE alterations; pharmacological inhibition of one of these kinases might also redirect the targeting of the other kinase.

Both CDK5 and GSK3β can modulate β-catenin signaling; this regulation is complex, however, and depends on other kinases to prime the substrate as well as scaffolding proteins to successfully link the kinase with the substrate [56]. β-Catenin performs various important cellular functions that range from cytoskeletal structuring to intracellular signaling [57]. Bath application for 96 h of 20 and 100 mM ethanol to neural stem cells derived from human fetal brains produced a decrease in the expression of β-catenin [32]. Our results of increased β-catenin in the HPC of adolescent PAE mice are interesting in light of the Vangipuram and Lyman study in that the observed increase could reflect a compensatory upregulation of β-catenin that occurs following PAE. The increase in HPC β-catenin in contrast with unchanged β-catenin in the mFC (Fig. 5) might imply that the effects of PAE on adolescent β-catenin are subject to additional regulatory mechanisms that differ between these brain regions, including scaffolding proteins such as presenilin and axin [44,46]. More work should be done to determine the developmental changes of β-catenin in response to PAE.

It is of particular interest that PAE-associated changes differ in the HPC and the mFC; that is; PAE produced differential regulation of the target proteins depending on the brain region examined. GSK3β total protein was decreased in the HPC and increased in the mFC, while p35 was increased in the HPC and decreased in the mFC (Fig. 6). This parallels our previous finding of decreased nuclear GR in the mFC [13] and increased nuclear GR in the HPC [14]. It is unclear as to why PAE differentially regulates these proteins in the HPC and mFC, but may be related to the

distinct function of these brain structures in cognitive processes. The GR is known to play important roles in both of the regions, although GR signaling differs regionally. Work by Kitchener and colleagues determined that GR binding to glucocorticoid response element sites is longer lasting and of higher amplitude in the HPC than in the frontal cortex [58]. Similarly, prenatal stress has been shown to increase GSK3 protein in the frontal cortex but not in the HPC, and the authors conclude that GSK3β could be a target of the maladaptive glucocorticoid action on frontal cortex neurons [59]. Because GSK3β and CDK5 play roles in the regulation of GR signaling [20,22,60], we speculate that these changes in PAE could be functionally related. Future work from our laboratory will aim to elucidate this relationship.

One interpretation of these findings is that PAE alters the developmental trajectories compared to age-matched controls. Adolescence is a period of rapid brain development in which subtle variances may affect later brain structure and function [61,62]. Beurel and colleagues present the interesting finding that GSK3β has specific developmental regulation that greatly differs between the adolescent and adult brain. Using C57BL/6J mice, they demonstrate that there are large increases in HPC GSK3β levels between PND1 and the juvenile period followed by decreased levels in adolescence and an even further decrease once adult levels are reached by eight weeks [63]. Their work also showed that Ser9 phosphorylation is higher in the juvenile mouse and decreases steadily until adult levels are reached; however, treatment with lithium or fluoxetine did not show the robust increase in Ser9 phosphorylation for adolescents that it does for adult mice, proving that these mood stabilizers do not exert the same effects on adolescent GSK3β as in adults [63]. The PAE mice have increased Ser9 phosphorylation of GSK3β compared to controls in the HPC, indicating that this kinase is being regulated differently than age-matched controls in this brain region. The changes that we observe in GSK3β phosphorylation and total GSK3β levels could be indicative of differing developmental trajectories between PAE and control brains. The findings of Beurel et al. [63] reveal that many of these kinases undergo a great deal of fluctuation during different developmental points. Thus, while several studies indicate that reductions in GSK3β are neuroprotective, it is difficult to draw this conclusion in all brain regions and at all developmental time points. Relative to the present study where ethanol exposure occurs prenatally, GSK3 deletion results in a hyperproliferation of neural progenitors which, in turn, results in a suppression of post-mitotic neurons and a dysregulation of signaling pathways critical for brain development, including β-catenin [64]. This suggests that GSK3 beta activity is necessary for normal brain processes and that it's important that it is at the right level of activity at the right time.

Abnormalities in both CDK5 and GSK3 are well documented in numerous mood disorders as well as addiction [65–69]. Work by Famy and colleagues [7] found that individuals prenatally exposed to alcohol have a higher risk of developing psychopathology including alcohol/drug dependence, major depression, and other psychiatric disorders. Multiple signaling pathways converge upon both GSK3β and CDK5 and changes to these kinases could have far-reaching downstream effects, especially during adolescent brain development. As 75% of mental illness cases manifest by age 25, adolescence represents a critical developmental window in this process [70]. More work should be done to determine the effects of altered GSK3β and CDK5 protein in the PAE adolescent brain. Prenatal insults such as PAE that change kinase balance during this time could potentially be related to later development of psychiatric illness and neurodegenerative diseases.

5. Conclusions

The findings from this work are an important first step in determining the age-dependent regulation of CDK5 and GSK3β by moderate PAE. CDK5 and GSK3β are currently drug targets in a number of psychiatric and neurodegenerative illnesses [71,72] and understanding how these kinases change during different developmental stages will provide important information with respect to PAE-related deficits. The adolescent to adult transition is a time period that is still inadequately understood in the PAE field and might have important consequences for the development of and susceptibility to later disease.

Author contributions

Conception and design: A.M. Allan, K.K. Caldwell, L.A. Cunningham and S.L. Goggin. Development of methodology: A.M. Allan, K.K. Caldwell and S.L. Goggin. Acquisition of data: S.L. Goggin. Analysis and interpretation of data: A.M. Allan, K.K. Caldwell and S.L. Goggin. Writing, review, and/or revision of the manuscript: A.M. Allan, K.K. Caldwell and S.L. Goggin. Administrative, technical, or material support: C.R. Tyler, M. Labrecque, Michael Riblett. Study supervision: A.M. Allan and K.K. Caldwell.

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Conflicts of interest

All authors declare no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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