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Developmental role of adenosine kinase for the expression of sex-dependent neuropsychiatric behavior

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HIGHLIGHTS

- Perturbation of adenosine kinase expression during development results in behavioral deficits, predominately in male mice.
- There were prominent sex differences in behavior, in line with clinical populations, in adenosine kinase deficient mice.
- Adenosine homeostasis may play a role during development in determining susceptibility to later neuropsychiatric disease.

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ABSTRACT

Deficits in social memory, cognition, and aberrant responses to stimulants are common among persons affected by schizophrenia and other conditions with a presumed developmental etiology. We previously found that expression changes in the adenosine metabolizing enzyme adenosine kinase (ADK) in the adult brain are associated with deficits in various cognitive domains. To distinguish between developmental and adult functions of ADK, we used two transgenic mouse lines with widespread disruption of ADK expression in the adult brain, but differences in the onset of ADK deletion. Specifically, we compared Nestin-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ (ADK$^{\Delta Brain}$) mice with global loss of ADK in the whole brain, beginning in mid-gestation and persisting for life, with Gfa2-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ (ADK$^{\Delta Astro}$) mice that have normal ADK expression throughout development, but lose astrocyte-specific ADK-expression in young adulthood. Because ADK-expression in adulthood is generally confined to astrocytes, adult ADK$^{\Delta Astro}$ mice show a similar expression profile of ADK in key areas of the brain related to neuropsychiatric behavior, compared to adult ADK$^{\Delta Brain}$ mice. We sought to determine a neurodevelopmental role of ADK on the expression of psychiatric behaviors in adult male and female mice. Adult ADK$^{\Delta Brain}$ mice showed significant deficits in social memory in males, significant contextual learning impairments in both sexes, and a hyper-responsiveness to amphetamine in males. In contrast, ADK$^{\Delta Astro}$ mice showed normal social memory and contextual learning but hypo-responsiveness to amphetamine in males. Our results demonstrate a key developmental role of ADK in mediating behaviors in adulthood related to neuropsychiatric disease and support the greater prevalence of these disorders among males.

1. Introduction

The purine ribonucleoside adenosine is a key regulator of network function in the brain, which modulates neuronal excitability and plasticity. It is also a regulator of neuropsychiatric behavior and plays an emerging role in conditions such as schizophrenia (Boison et al., 2012; Shen et al., 2012) and autism spectrum disorder (Masino et al., 2011, 2013). Adenosine can bind to one of four G-protein coupled receptor subtypes (A_1, A_{2A}, A_{2B}, or A_3) which balance inhibitory and stimulatory functions of adenosine in the adult brain (Fredholm, 2010; Fredholm et al., 2011); its role in the developing brain, however, has been under-investigated. There is some support that adenosine receptor expression changes throughout development (Weaver, 1996). However, the key adenosine metabolizing enzyme adenosine kinase (ADK) undergoes major coordinated expression changes during early brain development, resulting in a shift from predominant neuronal expression during prenatal and early postnatal brain development to predominant astrocytic expression beyond postnatal day 14 in the mouse (Kiese et al., 2016;

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Studer et al., 2006). Those coordinated expression changes of ADK in conjunction with the existence of a placental adenosine barrier suggest that strict maintenance of controlled adenosine homeostasis is of crucial importance for brain development (Boison, 2013).

Brain development during gestation is particularly susceptible to damage from environmental sources. Gestating offspring stressed at vulnerable time points are more likely to be diagnosed with schizophrenia, autism, or attention deficit hyperactivity disorder (ADHD) as young adults (Van den Bergh et al., 2017). The neurodevelopmental mechanisms that confer predisposition to adult psychiatric disease are not well understood. Of note, the same biological events known to predispose offspring to adult neuropsychiatric disorders can also perturb adenosine metabolizing enzymes, resulting in net-increases in endogenous adenosine levels (Kowaluk and Jarvis, 2000; Ramakers et al., 2011). Conversely, exposure to caffeine, a non-specific adenosine receptor antagonist, or specific $A_{2A}R$ antagonists during gestation, can interfere with proper brain development in offspring (Sahir et al., 2000; Silva et al., 2013). Additionally, in a genetic model of neurodevelopmental disorders male, but not female, mice showed behavioral impairments and an overexpression of A_{2A} receptors in the striatum (Grissom et al., 2018). Together, this research supports the importance of tight control over the adenosine system, and the detrimental effects that deviation in either direction can have on *in utero* brain development of offspring. Additionally, because human mutations affecting ADK result in severe developmental delay and neurological impairment (Bjursell et al., 2011; Staufner et al., 2016) it is important to distinguish developmental functions of ADK from those in the adult brain. We therefore used a transgenic approach to distinguish the neurobehavioral consequences of gestational ADK deletion versus adult-onset ADK deficiency. We generated two lines of mice that primarily differ in the developmental timing and cell-specificity of ADK elimination: Nestin-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ (ADK$^{\Delta Brain}$) and Gfa2-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ (ADK$^{\Delta Astro}$) mice. ADK$^{\Delta Brain}$ mice permanently lose ADK expression in all cells of the brain after gestational day (GD) 12. In contrast, ADK$^{\Delta Astro}$ mice maintain normal ADK expression throughout development but lose their astrocyte-specific ADK expression after parturition, but before adulthood. We assessed 8 week old male and female mice for sex differences in psychiatrically-relevant behavioral aberrations in social memory, amphetamine-induced psychomotor responses, and contextual fear learning. We hypothesized that ADK$^{\Delta Brain}$ mice, regardless of sex, would display a more severe phenotype than ADK$^{\Delta Astro}$ mice indicating a neurodevelopmental role of ADK.

2. Materials & methods

All methods were approved by the Institutional Animal Care and Use Committee at Legacy Research Institute and were consistent with the NIH guidelines for the Care and Use of Laboratory Animals.

2.1. Transgenic mice

All mice were in an identical C57BL/6 background and were bred in our in house animal facility. They had ad libitum access to food and water and were maintained on a 12/12 light/dark cycle, with lights on at 0700. ADK floxed female mice (Xu et al., 2017) were bred with male Nestin-Cre (Giusti et al., 2014) or Gfa2-Cre (Matos et al., 2015) mice to generate both Cre(−/−) and Cre(±) *Adk$^{fl/fl}$* offspring as littermates. Mice were genotyped at weaning. Because ADK$^{\Delta Brain}$ mice develop stress induced seizures at around 12 weeks of age (Sandau et al., 2016) we began behavioral testing at 8 weeks prior to the onset of epilepsy. During handling and behavioral testing we paid attention to potentially confounding seizure activity. During those tests we did not detect any seizure activity irrespective of genotype.

Female mice were cycled prior to behavioral testing by vaginal cytology (Caligioni, 2009). Female mice were tested at estrous cycle phases that were not proestrous when estrogen and progesterone levels peak, in order to minimize the effects of steroid hormones in influencing social and cognitive behaviors. Additionally, we included Cre(+/−)-control mice for both ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ mice to control for off-target effects of Cre expression (Giusti et al., 2014).

2.2. Social memory

Social memory is impaired in several models of schizophrenia (Becker and Grecksch, 2000; Piskorowski et al., 2016). In this task, an experimental mouse is allowed to socially interact with the same stimulus mouse on two occasions. Decreased time spent interacting with the stimulus mouse at the second interaction is an index of memory for the stimulus mouse. For this task, a new unused cage was placed in an open field arena with a camera mounted above the testing arena. A cylindrical mesh cage was placed to one side of the cage, leaving room for a mouse to explore on all sides of the mesh housing. The experimental mouse was allowed to habituate to the empty area for five minutes. Then a novel stimulus mouse was placed in the mesh housing and the experimental mouse was allowed to investigate for five minutes. There was a one hour inter-trial interval where the experimental mouse was returned to its homecage. The stimulus mouse and experimental mouse were age- and sex-matched. Between trials, the experimental cage was changed to a clean unused cage and the same stimulus mouse was placed under the mesh housing. The experimental mouse was allowed another five minutes to explore the stimulus mouse. Time spent with nose on the mesh investigating the stimulus mouse was recorded as the primary dependent measure. Tracking utilized the Multiple Body Points module in Ethovision (Noldus, Wageningen, The Netherlands). Between trials meshed cages were cleaned with 0.5% acetic acid and 70% isopropyl alcohol.

2.3. Amphetamine-induced psychomotor responses

Baseline measurements were first acquired by placing mice in an open field chamber and allowing them to freely explore for 60 min. Mice were then administered amphetamine (2.5 mg/kg, Sigma-Aldrich, St. Louis MO, USA) before being placed back into their open field chamber. Activity was recorded for a further two and a half hours. Activity was analyzed by Ethovision software (Noldus, Wageningen, The Netherlands). The maze was cleaned between trials with 70% isopropyl alcohol.

2.4. Contextual fear

The contextual learning paradigm took place over three days in specialized chambers that automated recording and analysis of freezing behavior (Med Associates, Fairfax, VT). On day one, mice were habituated to the testing chamber for seven minutes. On day two mice were returned to their original chamber and allowed to explore for three minutes. After that a five second (80 db) tone was given followed by a one second (0.5 mA) footshock. The tone/shock was given a total of three times with a 150 s intertrial interval. On day three, mice were returned to their original testing chamber and freezing behavior was measured for three minutes. Because we previously published that the presence alone of Cre does not affect contextual learning outcomes in mice (Sandau et al., 2016), we did not include Cre(+)-control mice in this behavioral test.

2.5. Western blotting

Gestational tissue was obtained from pregnant ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ dams at several, and approximately similar, gestational time points. Rapid cervical dislocation was performed on dams, and fetal tissue immediately extracted, frozen in liquid nitrogen and stored at −80 °C. Early gestation tissues were later carefully thawed and the whole rostral ("head") region was removed for analysis, while the

caudal region was saved for genotyping. For late gestation, P0 and P14 tissues, the skull was cut and pure brain tissue was extracted and used for analysis. The precise gestational age was determined by Theiler stage (Bolon and Ward, 2015). Postnatal tissue was obtained from isoflurane-anesthetized mice followed by rapid cervical dislocation. Brain regions, prefrontal cortex (PFC), hippocampus, and striatum, were immediately dissected out and frozen in liquid nitrogen prior to storing at −80 °C until needed.

For Western blotting, tissues were homogenized with RIPA buffer containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). 20 μg of protein was loaded into 10% gels using the Criterion XT system (Biorad, Hercules, CA, USA) which was then transferred to PVDF membrane. Blots were probed for ADK (1:4,500, Bethyl Laboratories, TX, USA) and alpha-tubulin (1:2,500, Abcam, MA, USA) in 3% milk overnight at 4 °C then tagged with anti-rabbit HRP secondary (1:20,000) and developed using chemiluminescence (Pico West, Pearce, IL, USA). Results were scanned and analyzed using Bio-Rad Touch imager and accompanying Image Lab software.

2.6. Immunohistochemistry

Immunohistochemistry (IHC) was performed as previously published (Sandau et al., 2016). In brief, adult mice were anesthetized with isofluorane until unresponsive. Mice were perfused with 0.9% saline, followed by 4% paraformaldehyde. Sections were cut on a cryostat at 40 μm. Tissues were saved in cryoprotectant antifreeze at −20 degrees Celsius until used for staining. DAB staining was performed for ADK (1:1,000, Bethyl Laboratories, TX, USA). Samples were imaged using a Leica confocal microscope.

2.7. Statistical analysis

Behavioral analysis used three-way repeated measure ANOVAs to detect differences between mouse genotypes, sex and time/trial in social memory and amphetamine challenge. We note in the results whether sphericity is assumed for each behavior. Two-way ANOVAs were used to analyze differences in genotype and sex in social memory discrimination and contextual fear learning. Western blot results for adult ADK levels in prefrontal cortex, hippocampus and striatum were analyzed between each brain region as a two-way ANOVA. Unplanned post hoc comparisons used Tukey multiple comparison tests and Bonferoni corrections for multiple contrast comparisons. GraphPad Prism 7.0 and SPSS 17.0 were used for statistical analysis and graphing of data.

3. Results

3.1. Reduction of ADK expression in $ADK^{\Delta Brain}$ and $ADK^{\Delta Astro}$ mice

We used immunohistochemistry and Western blot analysis, analyzed by two-way ANOVAs, to determine the timing and extent of ADK reduction in our mutant animals. In gestational brain tissue (Fig. 1A), we observed a significant loss of ADK expression in $ADK^{\Delta Brain}$ mice as early as GD16. There was a significant effect for ADK to vary by age group in $ADK^{\Delta Brain}$ mice ($F_{2,20} = 5.6$, $p < 0.05$; Fig. 1A) and genotype ($F_{1,20} = 17.6$, $p < 0.001$), but no interaction. Post hoc tests show that at GD16 (adjusted p-value = 0.01) and P0 (adjusted p-value = 0.011) there is significantly less ADK expression in the brains of $ADK^{\Delta Brain}$ mice compared to their wildtype counterparts. In $ADK^{\Delta Astro}$ there was a significant main effect for age ($F_{3,25} = 6.1$, $p < 0.01$; Fig. 1B). Post hoc tests show that PND14 mice (of both genotypes) have less ADK than mice at GD8 (adjusted p-value = 0.002). We also looked specifically at both strains at PND0 to confirm that gestational loss of ADK is $ADK^{\Delta Brain}$ - specific. There was a main effect for genotype ($F_{1,13} = 4.7$, $p = 0.05$; Fig. 1C). Post hoc comparison confirms that there is a significant difference between $ADK^{\Delta Brain}$ and $ADK^{\Delta Astro}$ transgenic mice in brain ADK levels at PND0 (adjusted p-value = 0.039), where $ADK^{\Delta Astro}$ mice retain normal levels of ADK compared to $ADK^{\Delta Brain}$ mice.

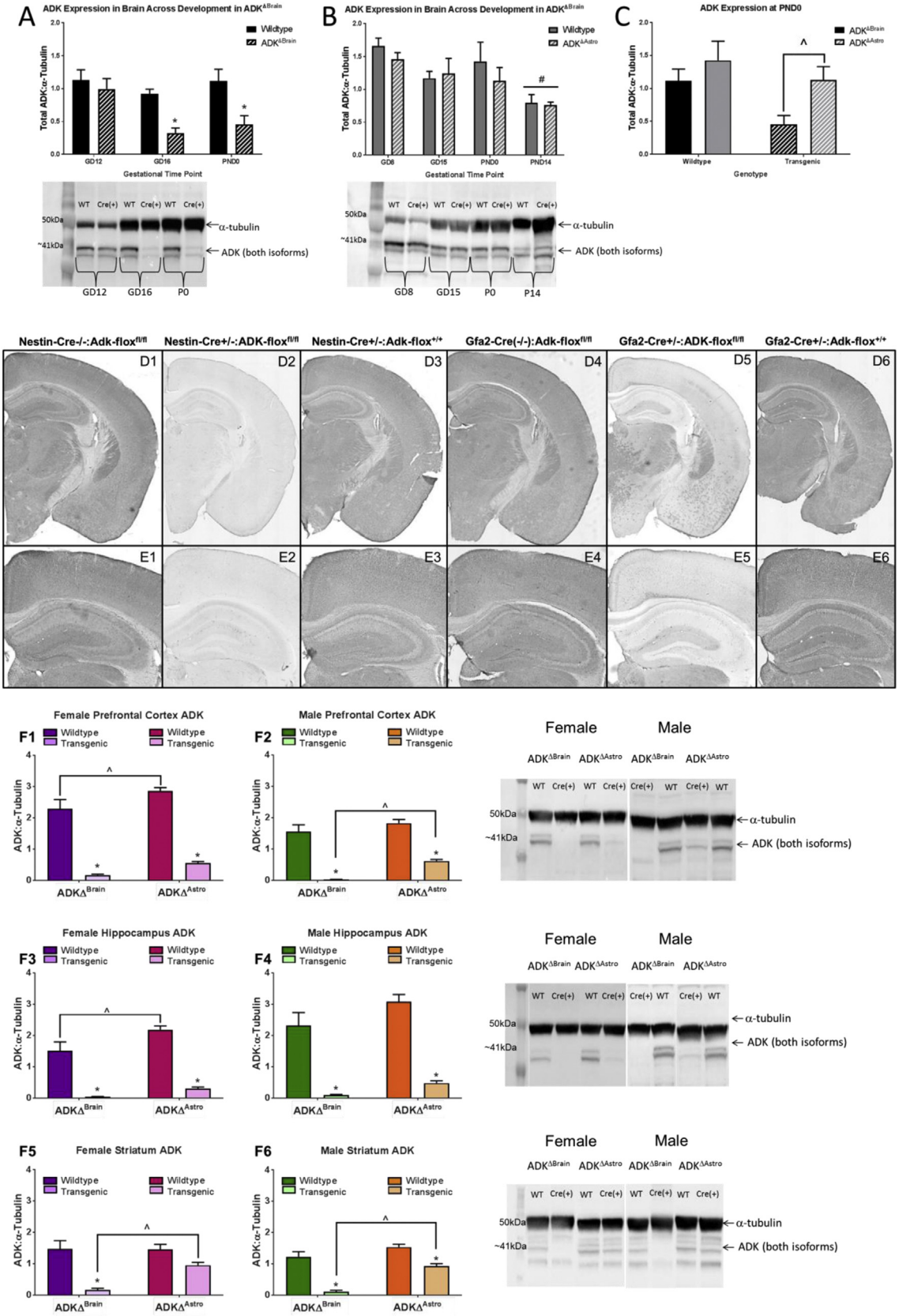
In adult brains, both Western blot analysis and immunohistochemistry showed genotype- and age-dependent reductions in ADK expression (Fig. 1D–F6), whereas Nissl staining did not reveal any gross abnormalities in any genotype (data not shown). DAB staining (Fig. 1, D1-E6) confirms the absence of ADK staining in the $ADK^{\Delta Brain}$ brain (C2, D2), while $ADK^{\Delta Astro}$ mice show widespread reduction of ADK expression in hippocampus and dorsal cortex, with residual ADK expression in thalamus and striatal areas (D5, E5).

IHC results were confirmed with Western blots (Fig. 1, F1-F6). There were significant main effects for strain and genotype for female PFC ($F_{1,20} = 8.76$, $p < 0.01$; $F_{1,20} = 194.4$, $p < 0.0001$). Tukey corrected post hoc comparisons showed that both $ADK^{\Delta Brain}$ (adjusted p-value = 0.0001) and $ADK^{\Delta Astro}$ (adjusted p-value = 0.0001) transgenic mice had significantly less ADK than their wildtype counterparts, and the main effect of strain was driven by $ADK^{\Delta Astro}$ wildtype females having more ADK than wildtype $ADK^{\Delta Brain}$ females (adjusted p-value = 0.042). In male PFC there was a significant main effect for strain ($F_{1,20} = 11.31$, $p < 0.01$) and genotype ($F_{1,20} = 112.7$, $p < 0.0001$). Post hoc tests showed that both $ADK^{\Delta Brain}$ (adjusted p-value = 0.0001) and $ADK^{\Delta Astro}$ (adjusted p-value = 0.0001) transgenic male mice had significantly less ADK in the PFC compared to wildtypes; additionally, $ADK^{\Delta Astro}$ transgenic males had significantly more ADK in the PFC than $ADK^{\Delta Brain}$ transgenic males (adjusted p-value = 0.019). In the female hippocampus, there was a significant main effect of strain ($F_{1,20} = 9.03$, $p < 0.01$) and genotype ($F_{1,20} = 115.2$, $p < 0.0001$). Post hoc results are identical to the female PFC where both $ADK^{\Delta Brain}$ (adjusted p-value = 0.0001) and $ADK^{\Delta Astro}$ (adjusted p-value = 0.0001) transgenic mice had significantly less ADK than their wildtype counterparts, while $ADK^{\Delta Astro}$ wildtype females have more ADK than wildtype $ADK^{\Delta Brain}$ females (adjusted p-value = 0.029). In the male hippocampus there was a significant main effect of strain ($F_{1,20} = 5.83$, $p < 0.05$) and genotype ($F_{1,20} = 104.5$, $p < 0.0001$). Multiple comparisons showed that transgenic $ADK^{\Delta Brain}$ (adjusted p-value = 0.0001) and $ADK^{\Delta Astro}$ (adjusted p-value = 0.0001) mice have less ADK than their wildtype counterparts; however, after correcting for multiple comparisons there were no specific differences in the groups due to strain. There were no significant interactions in female or male PFC or hippocampus. In female striatum there was a significant interaction ($F_{1,20} = 2.71$, $p < 0.05$), main effect for strain ($F1,20 = 5.5$, $p < 0.05$) and genotype ($F1,20 = 30.0$, $p < 0.0001$). Multiple comparison tests showed that, in females, only $ADK^{\Delta Brain}$ transgenics had a significant decrease in ADK compared to wildtype counterparts (adjusted p-value = 0.0001) and that $ADK^{\Delta Astro}$ transgenic mice also had significantly more ADK than $ADK^{\Delta Brain}$ (adjusted p-value = 0.016) transgenic females. In the male striatum there was a significant interaction ($F_{1,19} = 5.79$, $p < 0.05$), main effect for genotype ($F1,19 = 65.2$, $p < 0.0001$) and strain ($F1,19 = 28.4$, $p < 0.0001$). Both $ADK^{\Delta Brain}$ (adjusted p-value = 0.0001) and $ADK^{\Delta Astro}$ (adjusted p-value = 0.003) transgenic mice had significantly less ADK in their striatums than their wildtype counterparts; additionally, $ADK^{\Delta Astro}$ transgenics had more ADK than $ADK^{\Delta Brain}$ transgenics (adjusted p-value = 0.0002).

3.2. $ADK^{\Delta Brain}$ mice have deficits in social memory

Social cognition deficits are a common feature of neurodevelopmental psychiatric disorders like schizophrenia and autism (Insel and Fernald, 2004). As such, we used the social memory task to determine whether gestational disruption of ADK in the brains of developing offspring would result in similar behavioral profiles, in our mice, as in clinical populations.

Examining social interaction time between experimental mice and a stimulus mouse, we found in $ADK^{\Delta Brain}$ mice, with sphericity assumed, there was a significant effect of trial ($F_{1,44} = 139.4$, $p < 0.0001$; Fig. 2A), a trial by sex interaction ($F_{1,44} = 14.7$, $p < 0.0001$), and a



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trial by genotype interaction ($F_{2,44} = 3.9$, $p < 0.05$). There were no main effects for sex or genotype, nor was there a three-way interaction for time. Specific contrasts, with Bonferroni corrections (alpha adjusted to 0.02), showed that in Trial 1, females were more social than their male counterparts, a difference that disappeared in Trial 2 ($p = 0.005$); additionally, male transgenic, unlike all other genotypes, did not

Fig. 1. ADK expression in the brain during development. Expression of total ADK varies across development in ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ mice. **A**: ADK deletion in the brains of ADK$^{\Delta Brain}$ mice begins in mid-gestation between GD12 and GD16 resulting in a significant decrease in total ADK levels by GD16 and continuing to P0 (n = 3–8/group). **B**: ADK deletion in astrocytes of ADK$^{\Delta Astro}$ mice does not begin until later in post-natal development, these results confirm that these mice retain normal ADK levels until at least P14 (n = 3–9/group). By adulthood, the ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ phenotypes are fully established. # indicates $p < 0.05$ compared to GD8. **C**: ADK expression data from PND0 was compared across strains and genotypes to highlight that in transgenic mice, the ADK$^{\Delta Brain}$ mice have a significant decrease in ADK compared to their ADK$^{\Delta Astro}$ transgenic counterparts at PND0. Panels **D-F** show DAB staining for ADK across the genotypes in adult mice (age ~10 weeks). Staining for ADK is noticeably absent in Nestin-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ [**C2, D2**]. Gfa2-Cre$^{+/-}$:ADK-flox$^{fl/fl}$ [ADK$^{\Delta Astro}$] also show a decrease in global ADK levels (**C5, D5**) throughout the brain, but retain some ADK staining which is well observed in the hippocampus (**D5**) and thalamus areas (**D5**). ADK deletion was confirmed in adults with Western blotting (**F1-F6**) across three brain regions (n = 6/group) Male and female ADK$^{\Delta Brain}$ mice have negligible remaining levels of total ADK levels in the hippocampus, prefrontal cortex, and the striatum compared to respective wildtypes. ADK$^{\Delta Astro}$ male and female mice also have less ADK than wildtypes, except for in female striatum (**F5**). There were additional genotype effects were wildtype ADK$^{\Delta Astro}$ females have more ADK than female wildtype ADK$^{\Delta Brain}$ mice in the PFC and hippocampus (**F1, F3**). Conversely, transgenic ADK$^{\Delta Astro}$ mice have more ADK than transgenic ADK$^{\Delta Brain}$ mice in the male cortex and striatum, and female striatum. * indicates significant difference due to genotype. ^ indicates significant difference due to strain. # indicates significant difference due to age.

significantly vary in their social interaction times between Trial 1 and Trial 2 ($p = 0.29$). For social discrimination index, there was a significant main effect of sex ($F_{1,44} = 14.7$, $p < 0.001$; Fig. 2B) and genotype ($F_{2,44} = 3.9$, $p < 0.05$). The interaction between sex and genotype for discrimination index was not significant. Post hoc tests corrected for multiple comparisons show that the sex difference was driven by female transgenic mice being significantly different from male transgenic mice (adjusted p-value = 0.012), while the genotype main effect was driven by wildtype males varying significantly from their transgenic counterparts (adjusted p-value = 0.019).

In ADK$^{\Delta Astro}$ mice for social memory in a repeated measures ANOVA there was an effect of Trial ($F_{1,54} = 84.9$, $p < 0.0001$), but there were no differences due to sex ($F_{1,54} = 3.9$, $p > 0.05$) or genotype ($F_{2,54} = 1.5$, $p > 0.05$), nor were any interactions present: all sexes and genotypes retained a social memory for their encounter in Trial 1 as evidenced by the significant decrease in social time in Trial 2. This was supported by the discrimination index where there were no significant differences between the sexes ($F_{1,54} = 0.4$, $p > 0.05$) or genotypes ($F_{2,54} = 0.02$, $p > 0.05$), nor an interaction ($F_{2,54} = 0.1$, $p > 0.05$).

3.3. ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ mice have opposite responses to amphetamine

Amphetamine challenge provides details into the brain's dopaminergic functioning: hyperfunction is indicative of schizophrenia-like phenotypes (Pezze et al., 2014), while hypofunction is related to attention deficit/hyperactivity disorder (ADHD) (Del Campo et al., 2011; Fone and Nutt, 2005; Trinh et al., 2003) or depression (Subbaiah, 2017). We observed that gestational loss of ADK produces

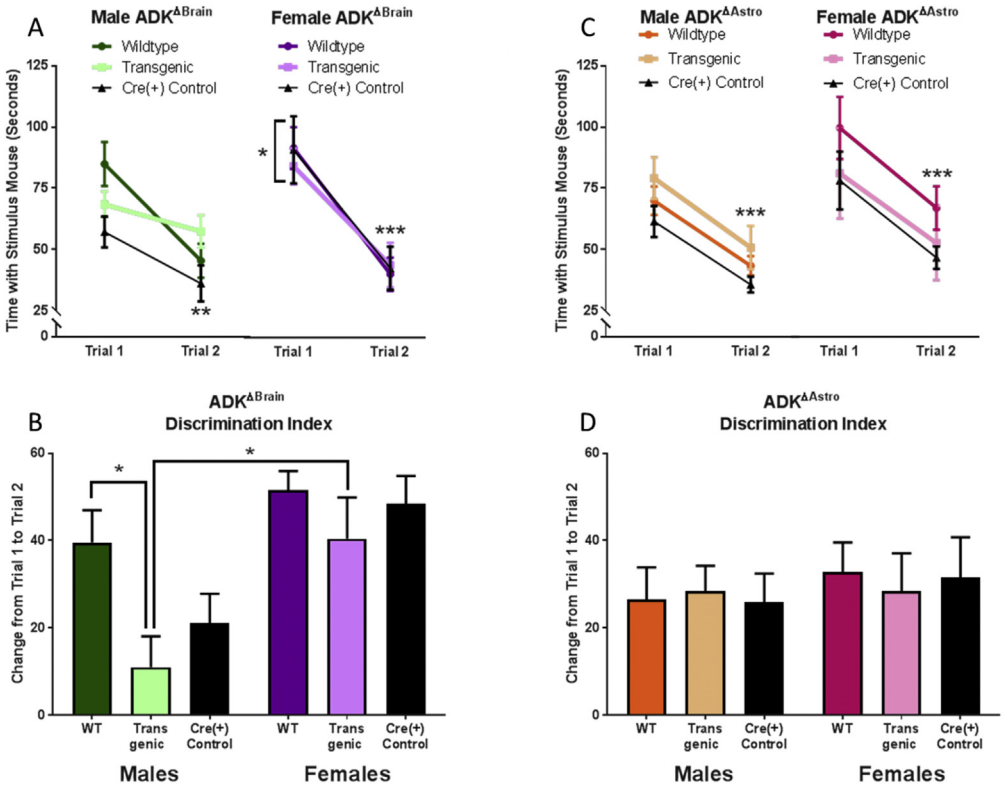

Fig. 2. Social memory performance. Loss of ADK during development produces a deficit in social memory (**A**) in male ADK$^{\Delta Brain}$ mice (**), who do not differ in the time spent investigating a stimulus mouse between Trial 1 and Trial 2. This deficit is not present in female transgenic mice, where all genotypes spend significantly less time with the stimulus mouse in Trial 2 (***). Additionally, in Trial 1 female mice spent more time interacting with the stimulus mouse than male mice (*). (**B**) Male ADK$^{\Delta Brain}$ transgenic mice had significantly lower discrimination scores than their male wildtype and female transgenic counterparts (*). (**C**) Both male and female ADK$^{\Delta Astro}$ mice spent significantly less time exploring a stimulus mouse in Trial 2 versus Trial 1 (***). (**D**) Similarly, there was no differences due to sex or genotype in discrimination index for ADK$^{\Delta Astro}$ mice. Repeated measures 3-way ANOVAs and 2-way ANOVAs were used to analyze the social memory and social discrimination data, respectively. Group n's: ADK$^{\Delta Astro}$ males (WT n = 14, transgenic n = 10, Cre(+)-Control n = 8); ADK$^{\Delta Astro}$ females (WT n = 11, transgenic n = 8, Cre(+)-Control n = 9); ADK$^{\Delta Brain}$ males (WT n = 8, transgenic n = 10, Cre(+)-Control (n = 8); female ADK$^{\Delta Brain}$ (WT n = 8, transgenic n = 9, Cre (+)-Control n = 7).

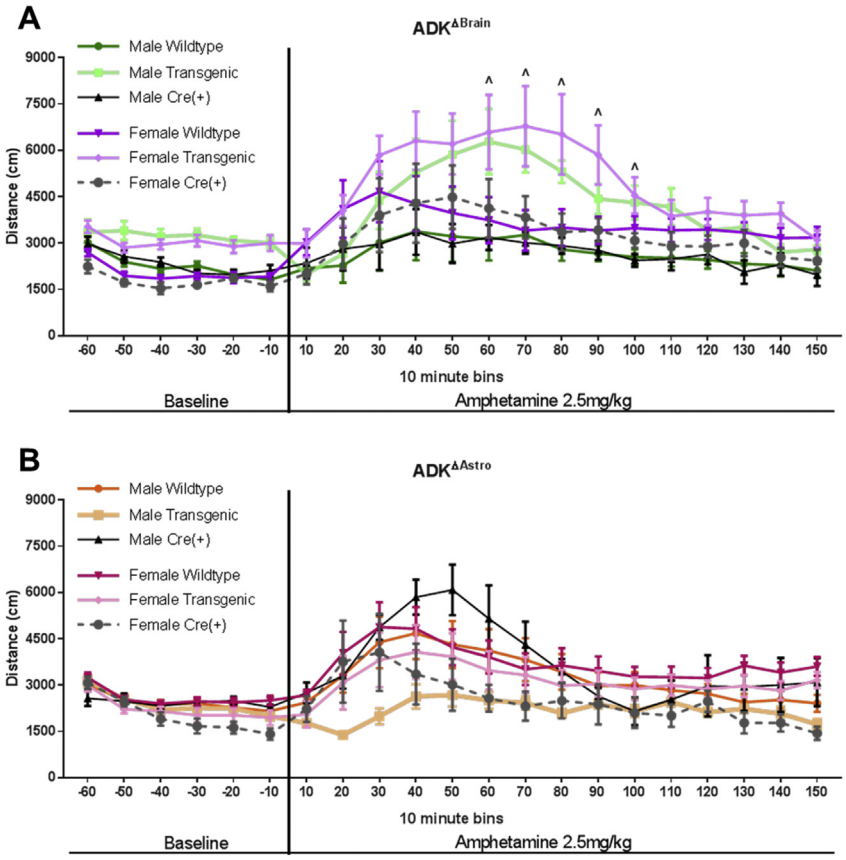

Fig. 3. Amphetamine-induced psychomotor responses. Loss of ADK during development results in a significantly greater response to a 2.5 mg/kg administration of amphetamine in both male and female (**A**) $ADK^{\Delta Brain}$ mice. Adult loss of ADK in $ADK^{\Delta Astro}$ does not significantly alter amphetamine responses compared to wildtype and Cre(+) control mice. ^ indicates that transgenic mice are significantly different from wildtype/Cre(+) at that time point, $p < 0.05$. Repeated measures 3-way ANOVAs were used to analyze these data. Group n's: male $ADK^{\Delta Brain}$ (WT = 16, transgenic = 8, Cre(+)-Control = 6); male $ADK^{\Delta Astro}$ (WT = 19, transgenic = 14, Cre(+)-Control = 4); female $ADK^{\Delta Brain}$ (WT = 14, transgenic = 11, Cre(+)-Control = 4); and female $ADK^{\Delta Astro}$ (WT = 16, transgenic = 13, Cre(+)-Control = 5).

schizophrenia-like amphetamine hyper-responsiveness in both male and female mice. This affect was absent in the $ADK^{\Delta Astro}$ mice.

A repeated measures 3-way ANOVA was used to determine group differences. Sphericity was not assumed, and our within-subjects results showed a main effect of time ($F_{2.4,1100} = 14.9$, $p < 0.0001$; Fig. 3A) with no interactions between time and either genotype or sex present. We also found a significant main effect for genotype ($F_{2,55} = 9.8$, $p < 0.0001$). No effects or interactions were found with sex. Using specific contrasts with Bonferoni corrections (alpha adjusted to 0.00625) we identified that transgenic $ADK^{\Delta Brain}$ mice were significantly different from both wildtype and Cre(+) control mice, specifically at time points 60 (p-value = 0.003), 70 (p-value = 0.001), 80 (p-value = 0.002), 90 (p-value = 0.002), and 100 (p-value = 0.005) minutes distance traveled was significantly increased for transgenic mice above other genotypes.

The same analysis was conducted for $ADK^{\Delta Astro}$ mice where sphericity was not assumed. Only a significant effect of time was present ($F_{2.4,1440} = 14.0$, $p < 0.0001$; Fig. 3B). There were no significant main effects of age or genotype, nor were any of the interactions significant.

3.4. $ADK^{\Delta Brain}$ mice have cognitive deficits in contextual fear learning

Contextual fear is dependent on the hippocampus and learning deficits are a common symptom in schizophrenia. Previously we published that $ADK^{\Delta Brain}$ male mice had learning impairments and poor in contextual memory (Sandau et al., 2016). We extended those findings to show that female $ADK^{\Delta Brain}$ mice have a similar deficit. In associative learning there was a main effect of genotype ($F_{1,36} = 17.1$, $p < 0.001$; Fig. 4A), but no effects of sex ($F_{1,36} = 1.3$, $p > 0.05$) or interaction ($F_{1,36} = 0.01$, $p > 0.05$), where both male (adjusted p-value = 0.003) and female (adjusted p-value = 0.03) transgenic mice spent significantly less time freezing compared to wildtype mice. The same effects were found in contextual memory recall. There was a main effect of genotype ($F_{1,31} = 18.0$, $p < 0.001$), but no effect of sex ($F_{1,31} = 0.8$, $p > 0.05$) or interaction ($F_{1,31} = 0.004$, $p > 0.05$), again transgenic mice, in both males (adjusted p-value = 0.007) females (adjusted p-value = 0.016), spent significantly less time freezing than their wildtype counterparts.

For $ADK^{\Delta Astro}$ mice in associative learning there was a significant interaction ($F_{1,29} = 6.8$, $p < 0.05$; Fig. 4C) and a main effect for sex ($F_{1,29} = 15.5$, < 0.001), but no effect of genotype ($F_{1,29} = 2.4$, $p > 0.05$). Post hoc comparisons show that transgenic female mice spent significantly more time freezing than female wildtypes (adjusted p-value = 0.037), male wildtypes (adjusted p-value = 0.004) and male transgenics (adjusted p-value = 0.001). This increase in associative learning did not, however, translate into significant differences in contextual memory. Unlike $ADK^{\Delta Brain}$ mice, there were no main effects for genotype ($F_{1,28} = 4.1$, $p > 0.05$; Fig. 4D), sex ($F_{1,28} = 0.2$, $p > 0.05$) or an interaction ($F_{1,28} = 0.2$, $p > 0.05$) present in the $ADK^{\Delta Astro}$ mice for contextual memory recall.

4. Discussion

ADK can affect behavior both directly through regulation of extracellular adenosine levels in the adult brain or indirectly through developmental influences. Comparing a mouse model with gestational ADK deletion to a line with adult onset ADK deletion allowed us to mechanistically dissect both possibilities. The fetal brain is protected from the maternal adenosine system through a placental adenosine barrier; while in the adult brain ADK regulates the availability of extracellular adenosine and hence the level of adenosine receptor activation (Boison, 2013). The role of extracellular adenosine in the control of behavioral outcomes in the adult brain is well established and regionally restricted adenosine augmentation via cell grafts or gene therapy has been explored as a therapeutic strategy for

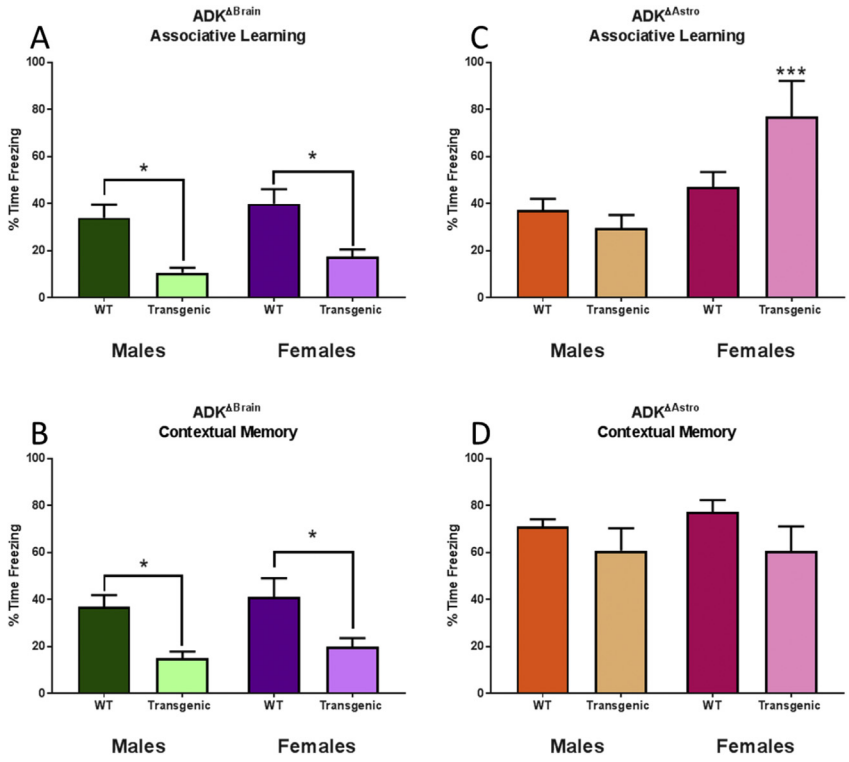

Fig. 4. Contextual fear learning. A consistent extension of previous findings shows that both male and female ADK$^{\Delta Brain}$ mice have cognitive deficits in both associative learning (**A**) and spatially-dependent task contextual fear memory (**B**). In ADK$^{\Delta Astro}$ mice, female transgenic mice had significantly increased freezing during associative learning (**C**), but there were no sex or genotype differences in contextual memory recall (**D**). * indicates significantly different from wildtype, $p < 0.05$. *** indicates significantly different from WT males, transgenic males, and WT females, $p < 0.5$. Two-way ANOVAs were used to analyze these data. Group n's: male ADK$^{\Delta Brain}$ (WT = 10, transgenic = 9); male ADK$^{\Delta Astro}$ (WT = 11, transgenic = 7); female ADK$^{\Delta Brain}$ (WT = 8, transgenic = 8); and female ADK$^{\Delta Astro}$ (WT = 9, transgenic = 5).

neuropsychiatric conditions including schizophrenia (Shen et al., 2012; Theofilas et al., 2011).

Our results demonstrate for the first time a key role for ADK in regulating neuropsychiatric development. Disruption of adenosine metabolism, during mid-to late-gestation, resulted in significant social, psychomotor, and cognitive impairments, which are commonly found as endophenotypes of severe psychiatric conditions. These behaviors are mediated by distinct brain regions, and thus provide clues as to how ADK affects the development of specific brain areas. We became interested in social memory as a task that could be related to a variety of neuropsychiatric disorders and because it is primarily regulated by very distinct regions like CA2 of the hippocampus, the forebrain, and olfaction (Jacobs and Tsien, 2017; Smith et al., 2016). The altered social memory responses reported here suggest that ADK deletion during development may interfere with specific systems involved in this behavior, such as oxytocin or vasopressin, likely through increased activation of the A_1 receptor (A_1R), but in males only. A_1R agonists have long been explored for their neuroprotective effects; they work both pre- and post-synaptically to inhibit glutamate release and hyperpolarize NMDA currents (Fredholm and Dunwiddie, 1988). A_1Rs are expressed pervasively throughout the adult brain; but its expression in the rodent brain does not occur until GD14 (Weaver, 1996), which coincides with the timing of ADK deletion in ADK$^{\Delta Brain}$ mice. By deleting ADK by GD16, we potentially augment the level of adenosine available in the developing brain to bind to the newly expressed A_1Rs. Although A_1R actions may be beneficial in adulthood, during development, increased activation of A_1Rs would result in deficient NMDAR activation and glutamate release. Antagonism of NMDARs during brain development impairs neuronal growth and survival, damages several brain regions and produces schizophrenia-like behaviors (du Bois and Huang, 2007). Although we did not observe any previous studies examining a role for adenosine in social memory, our results align with increased A_1R activation producing social deficits. Vasopressin action in CA2 of the hippocampus is vital for social memory (Smith et al., 2016), and ATP-derived adenosine can inhibit vasopressin release from hypothalamic neurons via its A_1 receptor (Song and Sladek, 2005), suggesting that A_1R activation could also suppress or interfere with vasopressin actions in the CA2 during development resulting in a social memory deficit. Additionally, the absence of a social memory deficit in the ADK$^{\Delta Astro}$ mice, with normal ADK expression throughout gestation, supports our hypothesis that manipulation of ADK in development may play a key role in neuropsychiatric diseases like schizophrenia and other diseases characterized by social deficits.

Much to the detriment of understanding neuropsychiatric disorder etiology, sex differences are rarely examined in mechanistic studies. Here, we observed that female ADK$^{\Delta Brain}$ mice did not suffer from social memory deficits like their male counterparts. Although we cannot determine the precise mechanism driving their protection against developing this phenotype, estrogen is a potent neuroprotectant, and also regulates social memory behavior (Acevedo-Rodriguez et al., 2015; McCarthy, 1995; Miller and Caldwell, 2015). Overall, men are more likely to experience social-based behavioral deficits, both in the context of schizophrenia and also with autism diagnosis (Abel et al., 2010; Ferri et al., 2018). The absence of this effect in female ADK$^{\Delta Brain}$ mice supports estrogen's power effects to mediate social learning (Ervin et al., 2015). Congruent with others, our female ADK$^{\Delta Brain}$ mice showed a main effect for increased social interaction in Trial 1, and maintained a strong memory overall in Trial 2 testing. Additionally, the oxytocin and vasopressin systems integral for social interaction and memory are heavily controlled by nuclear estrogen receptor activation in males (Ervin et al., 2015), while estrogen's requirement for social behavior in females is well-validated (Tang et al., 2005). Not only does estrogen exert beneficial effects in the hippocampus via growth hormones (Harte-Hargrove et al., 2013), but the use of non-feminizing selective estrogen receptor modulators has been proposed as a treatment for men with schizophrenia, due to the prominent sex differences in severity and presentation of schizophrenia in men compared to women (Kulkarni et al., 2013). We hypothesize that higher levels of estrogen in female ADK$^{\Delta Brain}$ mice prevents the loss of social memory that would otherwise be caused by the loss of ADK and resulting perturbation of the oxytocin and vasopressin systems. These data support increased susceptibility to social and psychotic-like behaviors in males and show how adenosine signaling may mediate these effects different in males versus females by either developmental or adult-based mechanisms.

Testing ADK$^{\Delta Brain}$ and ADK$^{\Delta Astro}$ mice in psychomotor behavior yielded surprising results. All strains experienced a significant change in motor behavior, as indicated by the main effects of time, due to amphetamine administration. Of interest is the effect of amphetamine to significantly increase motor behavior in ADK$^{\Delta Brain}$ transgenic mice even above the increase seen in wildtypes and controls. Of interest, is the effect of amphetamine to amplify activity in the ADK$^{\Delta Brain}$ mice and the absence of an additive effect in the ADK$^{\Delta Astro}$ male mice. Both A_1Rs and A_{2A}Rs are expressed at high levels in the medium spiny neurons of the striatum, which utilize adenosine and dopamine to mediate locomotor behavior (Ferre et al., 1997; Fuxe et al., 1998). Adenosine and dopamine have a well-established antagonistic relationship via their receptors, whereby adenosine binding to its A_1 or A_{2A} receptor will decrease the binding affinity of dopamine to its D_1 and D_2 receptor, respectively (Franco et al., 2000). The cause of hyperlocomotion in ADK$^{\Delta Brain}$ mice is less straightforward. Adenosine receptors undergo desensitization, whereby prolonged agonist activation results in the uncoupling of the receptor from the G-protein, and the eventual internalization and lysosomal degradation of the receptor (Sheth et al., 2014). Although future work will need to characterize adenosine receptor changes across all brain regions, we have confirmed that ADK$^{\Delta Brain}$ mice have elevated levels of adenosine, as well as, decreased levels of A_1R in the hippocampus (Sandau et al., 2016). This result is indicative of desensitization stemming from lifelong elevated adenosine levels in the brain. If similar desensitization occurs in the striatum with A_1Rs and/or A_2ARs, we would expect less dimerization with, and thus less antagonism of, dopamine receptors within the striatum, allowing a hyper-responsiveness to amphetamine administration in ADK$^{\Delta Brain}$ mice compared to wildtypes. With neuronal ADK intact, adenosine levels may not be as chronically elevated and, thus, the same level of internalization would not occur in the ADK$^{\Delta Astro}$ mice. This mechanism is supported by the literature, but is currently speculative. Further studies will need to expand the characterization of these mice to include any changes in levels of adenosine receptors throughout the brain and how these resulting changes may cause adaptive activity at adenosine receptor complexes with dopamine receptors. Additionally, these mice may be prove a useful tool in understanding addiction and drug sensitization mechanisms within the striatum and adenosine's potential role in those behaviors.

Females were not buffered against cognitive deficits produced by developmental loss of ADK. Both male and female ADK$^{\Delta Brain}$ mice demonstrated significant impairments in contextual learning. Both male and female A_{2A}R knockout mice show cognitive deficits in object recognition (Moscoso-Castro et al., 2016). We previously showed that male ADK$^{\Delta Brain}$ mice have significant cognitive deficits in contextual fear learning, a dorsal hippocampus-dependent task (Sandau et al., 2016). The cognitive impairment was attributed to their enhanced LTP, A_{2A}R and BDNF signaling (Sandau et al., 2016); these mechanisms individually can affect learning (Cunha et al., 2009; Gimenez-Llort et al., 2007), so collectively they result in severe memory deficits. We have now replicated and extended that finding to show that female ADK$^{\Delta Brain}$ mice also have a contextual learning deficit, and any neuroprotection afforded them by estrogen, is not sufficient to overcome the three-fold assault on cognition produced by the critical loss of ADK during gestational development. An additional sex difference was observed in ADK$^{\Delta Astro}$ mice in associative learning. Female transgenic ADK$^{\Delta Astro}$ mice had significantly enhanced percent time freezing compared to all other sex/genotype groups. The associative learning measure is the freezing behavior following each tone/shock pairing. Since the boosted associative learning in female transgenic ADK$^{\Delta Astro}$ mice did not translate into improved contextual memory, it cannot be fully determined, yet, whether they have enhanced learning capabilities or if loss of ADK in their astrocytes has altered their sensing processes. Regardless, this was a prominent sex difference observed in the ADK$^{\Delta Astro}$ mice that underscores ADK's potential role in learning processes and highlights that ADK's effects can be influenced, enhanced, or nullified by differences in female hormones (often attributed to estrogen).

5. Conclusions

Our study is among the first to analyze sex differences in ADK-related physiology and to show a susceptibility to neuropsychiatric disorders stemming from developmental perturbation of ADK. Our findings run parallel with clinical observations of schizophrenia, autism, and ADHD, whereby males are more likely to express social deficits and be diagnosed with ADHD, while female subjects remain more resilient against many of these disorders.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2018.08.025.

References

Abel, K.M., Drake, R., Goldstein, J.M., 2010. Sex differences in schizophrenia. Int. Rev. Psychiatr. 22, 417–428.
Acevedo-Rodriguez, A., Mani, S.K., Handa, R.J., 2015. Oxytocin and estrogen receptor beta in the brain: an overview. Front. Endocrinol. 6, 160.
Becker, A., Grecksch, G., 2000. Social memory is impaired in neonatally ibotenic acid lesioned rats. Behav. Brain Res. 109, 137–140.
Bjursell, M.K., Blom, H.J., Cayuela, J.A., Engvall, M.L., Lesko, N., Balasubramaniam, S., Brandberg, G., Halldin, M., Falkenberg, M., Jakobs, C., Smith, D., Struys, E., von Dobeln, U., Gustafsson, C.M., Lundeberg, J., Wedell, A., 2011. Adenosine kinase deficiency disrupts the methionine cycle and causes hypermethioninemia, encephalopathy, and abnormal liver function. Am. J. Hum. Genet. 89, 507–515.
Boison, D., 2013. Adenosine kinase: exploitation for therapeutic gain. Pharmacol. Rev. 65, 906–943.
Boison, D., Singer, P., Shen, H.Y., Feldon, J., Yee, B.K., 2012. Adenosine hypothesis of schizophrenia - opportunities for pharmacotherapy. Neuropharmacology 62, 1527–1543.
Bolon, B., Ward, J.M., 2015. Anatomy and physiolgy of the developing mouse and placenta. In: Bolon, B. (Ed.), Pathology of the Developing Mouse: a Systematic Approach. CRC Press, Boca Raton, FL, USA, pp. 39–98.
Caligioni, C.S., 2009. Assessing reproductive status/stages in mice. Curr Protoc Neurosci. https://doi.org/10.1002/0471142301.nsa04is48. Appendix 4, Appendix 4I, PMID:19575469.
Cunha, C., Angelucci, A., D'Antoni, A., Dobrossy, M.D., Dunnett, S.B., Berardi, N., Brambilla, R., 2009. Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments. Neurobiol. Dis. 33, 358–368.
Del Campo, N., Chamberlain, S.R., Sahakian, B.J., Robbins, T.W., 2011. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biol. Psychiatr. 69, e145–157.
du Bois, T.M., Huang, X.F., 2007. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain Res. Rev. 53, 260–270.
Ervin, K.S., Lymer, J.M., Matta, R., Clipperton-Allen, A.E., Kavaliers, M., Choleris, E., 2015. Estrogen involvement in social behavior in rodents: rapid and long-term actions. Horm. Behav. 74, 53–76.
Ferre, S., Fredholm, B.B., Morelli, M., Popoli, P., Fuxe, K., 1997. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci. 20, 482–487.
Ferri, S.L., Abel, T., Brodkin, E.S., 2018. Sex differences in autism spectrum disorder: a review. Curr. Psychiatr. Rep. 20, 9.
Fone, K.C., Nutt, D.J., 2005. Stimulants: use and abuse in the treatment of attention deficit hyperactivity disorder. Curr. Opin. Pharmacol. 5, 87–93.
Franco, R., Ferre, S., Agnati, L., Torvinen, M., Gines, S., Hillion, J., Casado, V., Lledo, P., Zoli, M., Lluis, C., Fuxe, K., 2000. Evidence for adenosine/dopamine receptor interactions: indications for heteromerization. Neuropsychopharmacology 23, S50–S59.
Fredholm, B.B., 2010. Adenosine receptors as drug targets. Exp. Cell Res. 316, 1284–1288.
Fredholm, B.B., Dunwiddie, T.V., 1988. How does adenosine inhibit transmitter release? Trends Pharmacol. Sci. 9, 130–134.
Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Linden, J., Muller, C.E., 2011. International union of basic and clinical pharmacology. Lxxxi. Nomenclature and classification of adenosine receptors–an update. Pharmacol. Rev. 63, 1–34.
Fuxe, K., Ferre, S., Zoli, M., Agnati, L.F., 1998. Integrated events in central dopamine transmission as analyzed at multiple levels. Evidence for intramembrane adenosine A2A/dopamine D2 and adenosine A1/dopamine D1 receptor interactions in the basal ganglia. Brain Res Brain Res Rev 26, 258–273.

Gimenez-Llort, L., Schiffmann, S.N., Shmidt, T., Canela, L., Camon, L., Wassholm, M., Canals, M., Terasmaa, A., Fernandez-Teruel, A., Tobena, A., Popova, E., Ferre, S., Agnati, L., Ciruela, F., Martinez, E., Scheel-Kruger, J., Lluis, C., Franco, R., Fuxe, K., Bader, M., 2007. Working memory deficits in transgenic rats overexpressing human adenosine A2A receptors in the brain. Neurobiol. Learn. Mem. 87, 42–56.

Giusti, S.A., Vercelli, C.A., Vogl, A.M., Kolarz, A.W., Pino, N.S., Deussing, J.M., Refojo, D., 2014. Behavioral phenotyping of Nestin-Cre mice: implications for genetic mouse models of psychiatric disorders. J. Psychiatr. Res. 55, 87–95.

Grissom, N.M., McKee, S.E., Schoch, H., Bowman, N., Havekes, R., O'Brien, W.T., Mahrt, E., Siegel, S., Commons, K., Portfors, C., Nickl-Jockschat, T., Reyes, T.M., Abel, T., 2018. Male-specific deficits in natural reward learning in a mouse model of neurodevelopmental disorders. Mol. Psychiatr. 23, 544–555.

Harte-Hargrove, L.C., Maclusky, N.J., Scharfman, H.E., 2013. Brain-derived neurotrophic factor-estrogen interactions in the hippocampal mossy fiber pathway: implications for normal brain function and disease. Neuroscience 239, 46–66.

Insel, T.R., Fernald, R.D., 2004. How the brain processes social information: searching for the social brain. Annu. Rev. Neurosci. 27, 697–722.

Jacobs, S., Tsien, J.Z., 2017. Adult forebrain NMDA receptors gate social motivation and social memory. Neurobiol. Learn. Mem. 138, 164–172.

Kiese, K., Jablonski, J., Boison, D., Kobow, K., 2016. Dynamic regulation of the adenosine kinase gene during early postnatal brain development and maturation. Front Mol. Neurosci Manuscript ID: 213651.

Kowaluk, E.A., Jarvis, M.F., 2000. Therapeutic potential of adenosine kinase inhibitors. Expet Opin. Invest. Drugs 9, 551–564.

Kulkarni, J., Gavrilidis, E., Worsley, R., Van Rheenen, T., Hayes, E., 2013. The role of estrogen in the treatment of men with schizophrenia. Int. J. Endocrinol. Metabol. 11, 129–136.

Masino, S.A., Kawamura Jr., M., Cote, J.L., Williams, R.B., Ruskin, D.N., 2013. Adenosine and autism: a spectrum of opportunities. Neuropharmacology 68, 116–121.

Masino, S.A., Kawamura Jr., M., Plotkin, L.M., Svedova, J., DiMario Jr., F.J., Eigsti, I.M., 2011. The relationship between the neuromodulator adenosine and behavioral symptoms of autism. Neurosci. Lett. 500, 1–5.

Matos, M., Shen, H.Y., Augusto, E., Wang, Y., Wei, C.J., Wang, Y.T., Agostinho, P., Boison, D., Cunha, R.A., Chen, J.F., 2015. Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. Biol. Psychiatr. https://doi.org/10.1016/j.biopsych.2015.02.026. [Epub ahead of print].

McCarthy, M.M., 1995. Estrogen modulation of oxytocin and its relation to behavior. Adv. Exp. Med. Biol. 395, 235–245.

Miller, T.V., Caldwell, H.K., 2015. Oxytocin during development: possible organizational effects on behavior. Front. Endocrinol. 6, 76.

Moscoso-Castro, M., Gracia-Rubio, I., Ciruela, F., Valverde, O., 2016. Genetic blockade of adenosine A2A receptors induces cognitive impairments and anatomical changes related to psychotic symptoms in mice. Eur. Neuropsychopharmacol 26, 1227–1240.

Pezze, M., McGarrity, S., Mason, R., Fone, K.C., Bast, T., 2014. Too little and too much: hypoactivation and disinhibition of medial prefrontal cortex cause attentional deficits. J. Neurosci. 34, 7931–7946.

Piskorowski, R.A., Nasrallah, K., Diamantopoulou, A., Mukai, J., Hassan, S.I., Siegelbaum, S.A., Gogos, J.A., Chevaleyre, V., 2016. Age-dependent specific changes in area CA2 of the Hippocampus and social memory deficit in a mouse model of the 22q11.2 deletion syndrome. Neuron 89, 163–176.

Ramakers, B.P., Riksen, N.P., van der Hoeven, J.G., Smits, P., Pickkers, P., 2011. Modulation of innate immunity by adenosine receptor stimulation. Shock 36, 208–215.

Sahir, N., Bahi, N., Evrard, P., Gressens, P., 2000. Caffeine induces in vivo premature appearance of telencephalic vesicles. Brain Res Dev Brain Res 121, 213–217.

Sandau, U.S., Colino-Oliveira, M., Jones, A., Saleumvong, B., Coffman, S.Q., Liu, L., Miranda-Lourenco, C., Palminha, C., Batalha, V.L., Xu, Y., Huo, Y., Diogenes, M.J., Sebastiao, A.M., Boison, D., 2016. Adenosine kinase deficiency in the brain results in maladaptive synaptic plasticity. J. Neurosci. 36, 12117–12128.

Shen, H.Y., Singer, P., Lytle, N., Wei, C.J., Lan, J.Q., Williams-Karnesky, R.L., Chen, J.F., Yee, B.K., Boison, D., 2012. Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia. J. Clin. Invest. 122, 2567–2577.

Sheth, S., Brito, R., Mukherjea, D., Rybak, L.P., Ramkumar, V., 2014. Adenosine receptors: expression, function and regulation. Int. J. Mol. Sci. 15, 2024–2052.

Silva, C.G., Metin, C., Fazeli, W., Machado, N.J., Darmopil, S., Launay, P.S., Ghestem, A., Nesa, M.P., Bassot, E., Szabo, E., Baqi, Y., Muller, C.E., Tome, A.R., Ivanov, A., Isbrandt, D., Zilberter, Y., Cunha, R.A., Esclapez, M., Bernard, C., 2013. Adenosine receptor antagonists including caffeine alter fetal brain development in mice. Sci. Transl. Med. 5 197ra104.

Smith, A.S., Williams Avram, S.K., Cymerblit-Sabba, A., Song, J., Young, W.S., 2016. Targeted activation of the hippocampal CA2 area strongly enhances social memory. Mol. Psychiatr. 21 (8), 1137–1144.

Song, Z., Sladek, C.D., 2005. Does conversion of ATP to adenosine terminate ATP-stimulated vasopressin release from hypothalamo-neurohypophyseal explants? Brain Res. 1047, 105–111.

Staufner, C., Lindner, M., Dionisi-Vici, C., Freisinger, P., Dobbelaere, D., Douillard, C., Makhseed, N., Straub, B.K., Kahrizi, K., Ballhausen, D., la Marca, G., Kolker, S., Haas, D., Hoffmann, G.F., Grunert, S.C., Blom, H.J., 2016. Adenosine kinase deficiency: expanding the clinical spectrum and evaluating therapeutic options. J. Inherit. Metab. Dis. 39, 273–283.

Studer, F.E., Fedele, D.E., Marowsky, A., Schwerdel, C., Wernli, K., Vogt, K., Fritschy, J.M., Boison, D., 2006. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. Neuroscience 142, 125–137.

Subbaiah, M.A.M., 2017. Triple reuptake inhibitors as potential therapeutics for depression and other disorders: design paradigm and developmental challenges. J. Med. Chem. 61 (6), 2133–2165.

Tang, A.C., Nakazawa, M., Romeo, R.D., Reeb, B.C., Sisti, H., McEwen, B.S., 2005. Effects of long-term estrogen replacement on social investigation and social memory in ovariectomized C57BL/6 mice. Horm. Behav. 47, 350–357.

Theofilas, P., Brar, S., Stewart, K.-A., Shen, H.-Y., Sandau, U.S., Poulsen, D.J., Boison, D., 2011. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 52, 589–601 PMCID: PMC3075862.

Trinh, J.V., Nehrenberg, D.L., Jacobsen, J.P., Caron, M.G., Wetsel, W.C., 2003. Differential psychostimulant-induced activation of neural circuits in dopamine transporter knockout and wild type mice. Neuroscience 118, 297–310.

Van den Bergh, B.R.H., van den Heuvel, M.I., Lahti, M., Braeken, M., de Rooij, S.R., Entringer, S., Hoyer, D., Roseboom, T., Raikkonen, K., King, S., Schwab, M., 2017. Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. Neurosci. Biobehav. Rev S0149-7634(16)30734-5.

Weaver, D.R., 1996. A1-adenosine receptor gene expression in fetal rat brain. Brain Res Dev Brain Res 94, 205–223.

Xu, Y., Wang, Y., Yan, S., Zhou, Y., Yang, Q., Pan, Y., Zeng, X., An, X., Liu, Z., Wang, L., Xu, J., Cao, Y., Fulton, D.J., Weintraub, N.L., Bagi, Z., Hoda, M.N., Wang, X., Li, Q., Hong, M., Jiang, X., Boison, D., Weber, C., Wu, C., Huo, Y., 2017. Intracellular adenosine regulates epigenetic programming in endothelial cells to promote angiogenesis. EMBO Mol. Med. 9, 1263–1278.